Light signaling in *Arabis alpina*, a perennial relative of *Arabidopsis thaliana*

Inaugural-Dissertation

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Panpan Jiang

(Aus Henan; China)

Köln, 2017

Berichterstatter:	Prof. Dr. Ute Höcker
	Prof. Dr. Maria Albani
Prüfungsvorsitzender:	Prof. Dr. Wolfgang Werr
Tag der mündlichen Prüfung:	24.April. 2017

Table of Contents

Abbreviations

35S	35S promoter of Cauliflower Mosaic virus
°C	degree Celsius
A	Adenine
B	Blue light
bHLH	basic helix loop helix
bp	basepair
BR	Brassinosteroids
C-terminal	carboxy-terminal
cDNA	complementary DNA
Col	Clolumbia, ecotype of *Arabidopsis thaliana*
d	day(s)
Dk	darkenss
et al.	et alii (and others)
FR	far-red light
FRc	continuous far-red light
G	green light
GA	Gibberellin acid
h	hour(s)
HA	human influenza hemagglutinin
IAA	indole-3-acetic acid
kb	kilo basepair
LDP	long day plant
min	minute
µmol	micro mol
mRNA	messenger-ribonucleic-acid
N-terminal	amino-terminal
ORF	open reading frame
PCR	polymerase chain reaction
Pr	red light absorbing phytochrome confromation
Pfr	far-red light absorbing phytochrome confromation
R, Rc	red light, continuous red light
RT-PCR	reverse transcription PCR
SAS	shade avoidance syndrome
SDP	short day plant
UV	ultra violet
Wc	continuous white light

Abstract

Light is one of the most important environmental factors affecting plant development throughout the whole life cycle. Arabidopsis SPA and COP1 proteins act as central repressors of photomorphogenesis. However, previous studies on the functions of SPA and COP1 proteins are limited to only a few species and not in perennial species. In the present study, I examined the functionality of COP1 and SPA1 proteins from the new perennial model species *Arabis alpina* in Arabidopsis. The open reading frame of *COP1* and *SPA1* in the wild type *A. alpina* Paj were amplified and expressed in Arabidopsis *cop1* and *spa* mutants, respectively. The analyses in the transgenic plants suggested conserved basic mechanisms of AaCOP1 and AaSPA1 during evolution between perennials and annuals in Brassicaceae family. The light regulation of *SPA* (*SPA1/2/3/4*) transcripts in *A. alpina* Paj was similar as in Arabidopsis. In the accession Wca, however, *AaSPA2* mRNA abundance was increased by light, suggesting a different light regulation mechanism on Wca *SPA2* in comparison to the wild type Paj.

Natural variation in photoperiodic flowering has been studied in Arabidopsis, the loss- or gain-of-function of phytochromes (*PHYA*, *PHYB*, *PHYC*, *PHYD*) and cryptochromes (*CRY2*) could lead to reduced photoperiod responses in natural accessions of Arabidopsis. In the present study, natural variation of photoperiodic flowering in different *A. alpina* accessions was investigated. The accession Wca acts as a photoperiod-insensitive plant. A time course experiment was conducted to analyze the expression of the floral meristem identity genes *AaLFY* and *AaAP1*, which could support the observed photoperiod-insensitivity in Wca. Furthermore, Wca did not respond to different fluence rates of red light, suggesting a possibly dysfunctional phyB in Wca. On the other hand, the distinct flowering behavior between the long-day-plant Dor and the photoperiod-insensitive Wca could not be correlated to the diurnal oscillation of *AaCO* and *AaFT*. Moreover, the decrease of *AaFT* expression in the SD/LD shift experiment suggests additional factors might be involved in the floral transition in Wca.

Arabidopsis responds to low R:FR ratio with elongated hypocotyl and petiole length at seedlings stage, and with the elongation of petiole in the rosette leaves, inhibition of leaf blade expansion and accelerated flowering in adult plants. The responses of *A. alpina* Paj to low R:FR appear to be age-dependent: as Paj displayed tolerance to shade in two-week-old seedlings but exhibited elongation of stem in eight-week-old adult plants. The expression of auxin biosynthesis genes *YUC*s in *A. alpina* could be correlated to the age-dependent SAS phenotype observed.

Zusammenfassung

Licht ist einer der wichtigsten Umweltfaktoren für Pflanzen und beeinflusst ihr Wachstum während des gesamten Lebenszyklus. In Arabidopsis agieren SPA und COP1 Proteine als zentraler Repressor von Photomorphogenese. Nichtsdestotrotz beschränken sich bisherige Funktionsstudien von SPA und COP1 Proteinen auf nur wenige Spezies und schließen keine mehrjährigen Arten ein. In vorliegender Arbeit habe ich die Funktionalität von COP1 und SPA1 Proteinen aus der mehrjährigen Modellpflanze *Arabis alpina* in Arabidopsis untersucht. Die offenen Leserahmen von *COP1* und *SPA1* vom *A. alpina* Wildtyp Paj wurden amplifiziert und jeweils in Arabidopsis *cop1* und *spa* Mutanten exprimiert.Die Untersuchung der transgenen Pflanzen deutet auf konservierte Grundmechanismen von AaCOP1 und AaSPA1 während der Evolution von mehr- und einjährigen Pflanzen in der Familie der Brassicacaen hin. Die Lichtregulation von *SPA* (*SPA1/2/3/4*) Transkripten in *A. alpina* Paj ähnelte der von Arabidopsis. In der Akzession Wca war die mRNA Menge von *AaSPA2* durch Licht erhöht, was auf unterschiedliche Mechanismen der Lichtregulation von Wca *SPA2* im Vergleich zum Wildtyp Paj hindeutet.

Die natürliche Variation photoperiodischer Blühinduktion wurde bereits in Arabidopsis untersucht. In natürlichen Arabidopsisakzessionen führen Loss-of-function-Mutationen oder Gain-of-function-Mutationen von Phytochromen (*PHYA*, *PHYB*, *PHYC*, *PHYD*) und Cryptochromen (*CRY2*) zu verminderten photoperiodischen Antworten. In dieser Arbeit wurde die natürliche Variation photoperiodischer Blühinduktion in verschiedenen *A.alpina* Akzessionen untersucht. Die Akzession Wca verhält sich wie eine photoperioden-unempfindliche Pflanze. Ein Zeitverlaufsexperiment wurde durchgeführt um die Expression der Blütenmeristemsidentitätsgene *AaLFY* und *AaAP1* zu untersuchen, was die beobachtete Photoperioden-unempfindlichkeit untermauern könnte. Außerdem sprach Wca nicht auf verschiedene Fluenzraten von Rotlicht an, was möglicherweise auf ein dysfunktionelles phyB in Wca hindeutet. Das unterschiedliche Blühverhalten der Langtagspflanze Dor und der photoperioden-unempfindlichen Akzession Wca konnte nicht mit der tageszyklischen Oszillation von *AaCO* und *AaFT* korreliert werden. Außerdem legt die Abnahme der AaFT Expression im Kurztag/Langtag shift Experiment nahe, dass weitere Faktoren im Übergang zur Blüte involviert sein könnten.

Als Keimling reagiert Arabidopsis auf ein geringes Rotlicht-zu-Dunkelrotlichtverhältnis mit einer Verlängerung des Hypokotyls und der Petiolen. Adulte Pflanzen zeigen verlängerte Petiolen der Rosettenblätter, Unterdrückung der Blattspreitenausdehnung und beschleunigtes Blühen. In *A. aplina* scheint die Reaktion auf ein gerines Rotlicht-zu-Dunkelrotlichtverhältnis abhängig vom Alter zu sein: Paj zeigte Toleranz gegenüber Schatten in zwei Wochen alten Keimlingen, wies

aber verlängerte Stengel in acht Wochen alten Pflanzen auf. Die Expression der Auxinbiosynthesegene *YUC*s konnte in *A. alpina* mit dem altersabhängigen Phänotyp der Schattenvermeidungsantwort korreliert werden.

I. Introduction

I.1 Photoreceptors and light signal transduction in Arabidopsis

As sessile organisms, plants monitor the living environment constantly and make adaptations for their best survival to ensure reproduction. One of the most important environmental factors affecting plant development throughout the whole life cycle is light (Casal et al., 2005), which is not only utilized by plants as a source of energy, but also as a source of information signal. From germination to seedling and adult development to the onset of reproduction, plants accurately mediate the development changes in response to light (Neff et al., 1998, 2000).

I.1.1 Light perception and photomorphogenesis in Arabidopsis

In the model plant *Arabidopsis thaliana (*Arabidopsis*)*, the initiation of light signal transduction is mediated by five distinct classes of photoreceptors which perceive the quality, quantity and direction of light (Chen et al, 2004; Franklin and Quail, 2010; Möglich et al., 2010; Ziegler & Möglich 2015). These photoreceptors include phytochromes which detects the red (R) and far-red (FR) wavelengths (Clack et al., 1994; Franklin and Quail, 2010; Li et al., 2015), cryptochromes and phototropins that perceive ultraviolet A (UV-A) and blue light (Briggs and Christie, 2002; Huala et al., 1997; Lin, 2002), the ZEITLUPE protein family that absorbs the blue light (B) (Somers et al., 2000), and the ULTRAVIOLET RESISTANCE LOCUS 8 (UVR8) receptor that contributes to the UV-B light perception (Rizzini et al., 2011). The effect of light on plant growth and development involving major photoreceptors is illustrated in Figure I-1. Particularly, light has a dramatic effect on the morphogenesis at seedling stage. This is displayed by seedlings grown in darkness with elongated hypocotyls, closed apical hooks, folded and pale cotyledons. This skotomorphogenesis is terminated once light is perceived. Thereafter, seedlings undergo photomorphogenesis in the light by exhibiting de-etiolation, or inhibition of hypocotyl elongation, expanded cotyledons with developing green chloroplasts and open apical hooks (Von Arnim and Deng, 1996; McNellis and Deng, 1995). The detection of R and FR light, the R:FR ratio in particular, initiates shade avoidance responses (introduced in detail in section I.1.4).

Phytochromes are so far the most well-characterized photoreceptors. They exist as dimers of two identical -120 kDa polypeptides, with each monomer attaching to a liner tetrapyrrole chromophore, phytochromobilin (Franklin et al, 2005). There are two isoforms of phytochromes: the inactive Pr form which accumulates in dark-grown tissues, and the Pfr form which is biologically active and photo-converted from Pr upon R-absorption. Pfr can be photo-converted

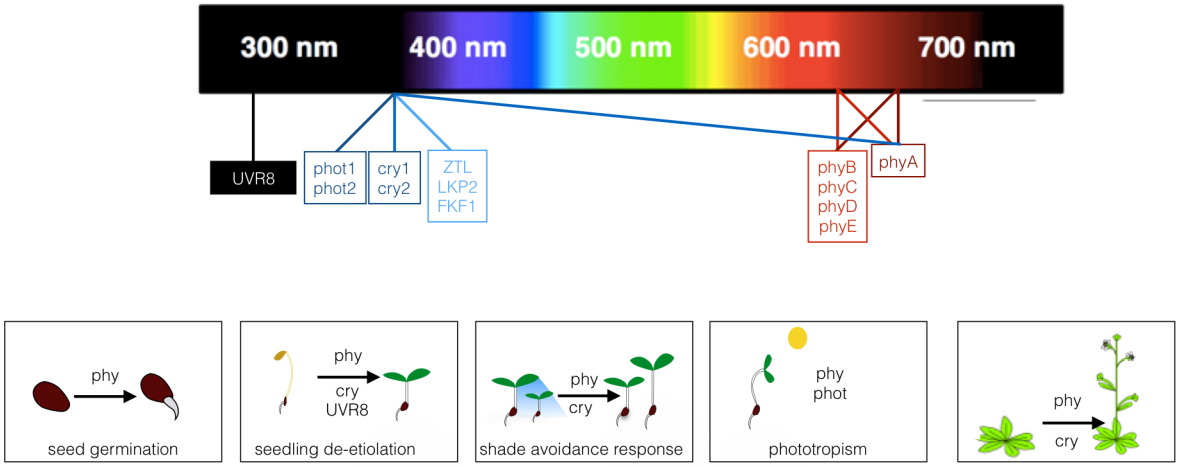


Figure I-1. Light regulates different developmental stages of a plant from germination to flowering.

Different wavelengths of light are perceived by different classes of photoreceptors, thereby to regulate different stages of plant development. phyA perceives mainly continuous far-red light, but also red and blue light. phyB-E perceive red light. Cryptochromes, together with phototropins and the Zeitlupe perceive blue light. UVR8 is a recently identified receptor for UV-B light. Seed germination is exclusively regulated by the phytochromes. However, starting from seed de-etiolation, which is regulated by phytochromes, cryptochromes and UVR8, different photoreceptors function together to regulate development. Shade avoidance responses are regulated by both phytochromes and cryptochromes. Phytochromes together with phototropins mediate the phototropism, and photoperiodic flowering is regulated by both cryptochromes, ZTLs and phytochromes.

back to Pr form by FR light or the light-independent dark reversion (Rockwell et al., 2006), which results in an equilibrium of the two forms under most irradiation conditions. The five phytochromes are subdivided into two groups: the photo-liable phyA which accumulates in darkness and in FR falls into phytochrome type I, while *phyB*, *phyC*, *phyD* and *phyE* encode the type II phytochromes which are more stable in the light (Sharrock et al., 2002; Sharrock and Quail, 1989; Jang et al., 2010). The type II phytochromes are translocated to the nucleus from the cytosol upon R absorption (Kircher et al., 1999, 2002). Subsequently, in the nucleus they mediate the low fluence responses (LFR) which are R/FR reversible (Nagy and Schäfer, 2002). Furthermore, in low R:FR conditions, the inactive Pr form of phyB contributes to neighbor detection, as the *phyB* mutant displays a constitutive shade avoidance phenotype in open sunlight conditions (Halliday et al., 1994). Very recently, phyB is proved to be a temperature sensor in plants (Legris et al., 2016; Jung et al., 2016) in addition to its photoreceptor functions. By contrast, the type I phyA mediates the very low fluence response (VLFR), which initiates the de-etiolation between soil and above-ground environments (Botto et al., 1996; Shinomura et al.,

1996), as well as the high irradiance in response to FR light (HIR) (Casal et al., 2000; Franklin et al., 2007). The light-activated nuclear localization of the Pfr form of phyA is facilitated by FAR-RED ELONGATED HYPOCOTYL1 (FHY1) and its homologue FHY1-LIKE (FHL) (Hiltbrunner et al., 2006; Genoud et al., 2008).

In Arabidopsis, three cryptochromes-encoding genes exist: *CRY1*, *CRY2* and *CRY3*, yet only the former two have been shown to encode functional chromoproteins (Yu et al., 2010). cry1 and cry2 share highly conserved N-terminal PHR (photolyase homologous region) domain which mediates photon absorption by binding a chromophore, and a poorly conserved C-terminal domain which may act as an effector domain. Despite the distinctive C-terminal domain, the two cryptochromes have many overlapping functions, including in hypocotyl growth inhibition (Ahmad et al., 1998; Lin et al., 1998) and function of circadian clock. cry2 has an additional role in the photoperiodic regulation of flowering, by stabilizing the function of CO protein (Liu et al., 2008a). Both cry1 and cry2 are nuclear-localized proteins and have considerable indirect effect on the transcriptional regulation of nuclear genes to convey divergent functions in plant development (Jiao et al., 2003; Kleine et al., 2007; Ohgishi et al., 2004). Furthermore, cry2 responses are observed preferentially at low-blue light irradiance whereas cry1 is stable in bright light and mediates both low and high light responses (Ahmad & Cashmere 1993; Lin et al., 1998; Yu et al., 2007).

Several aspects of plant development are cooperatively mediated by phytochromes and cryptochromes. Although seed germination is exclusively regulated by phytochromes, seedling de-etiolation and stomata differentiation are promoted by phyA, phyB and cryptochromes in FR, R and B, respectively. (Botto et al., 1996; Shinomura et al., 1996; Somers et al., 1991; Ahmad and Cashmore, 1993; Kang et al., 2009). phyB performs a predominant role in the inhibition of shade avoidance response, with redundant roles identified for phyD and phyE in Arabidopsis (Franklin and Quail 2010). Recently, the inhibition of shade avoidance response was reported to involve additionally cryptochromes (Keuskamp et al., 2011; Pedmale et al., 2015; Pierik et al., 2009; de Wit et al., 2016; Fraser et al., 2016), which is introduced in detail in section I.1.4. Furthermore, the transition to flowering requires positive regulation by cry1, cry2 and phyA, and repression by phyB, showing their antagonistic roles in the regulation of flowering time (Mockler et al., 2003; Valverde et al., 2004).

I.1.2 The COP1/SPA complex is a central repressor of transcription factors

In Arabidopsis, more than 20% of the expressed genes are light regulated, involving a variety of transcription factors (Tepperman et al., 2001; Jiao et al., 2005). These transcription factors can

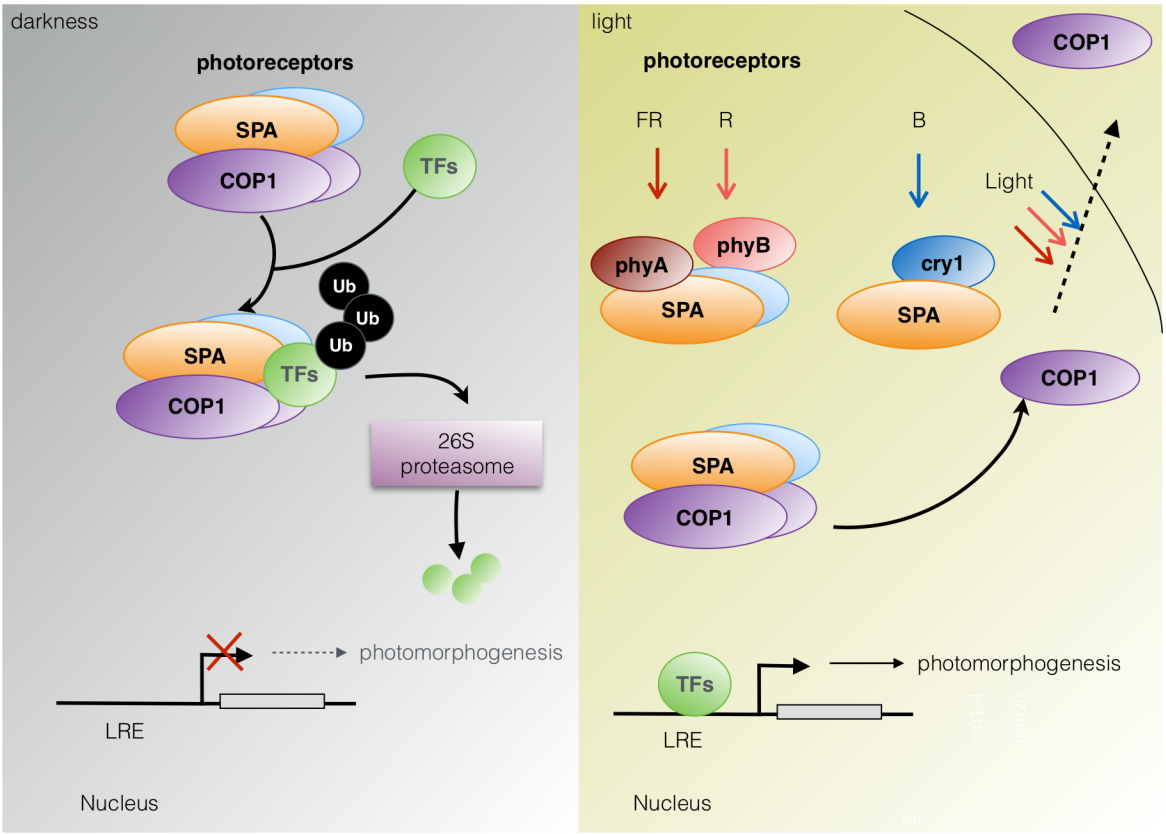


Figure I-2. The COP1/SPA complex is a repressor in light signaling.

(A) In darkness, the COP1/SPA complex is responsible for the proteasome-mediated degradation of transcription factors (TFs), which leads to skotomorphogenesis in dark-grown seedlings. Two COP1 and two SPAs from the SPA family form a tetrameric complex and interact with the substrate transcription factors. Ub, ubiquitin.
(B) Under light, the formation of COP1/SPA complex is inhibited by photoreceptors. phyA and phyB disrupt the COP1/SPA complex by interacting with the SPA proteins. Under blue light, cry1 directly binds to SPA1 and sequesters it from COP1. Therefore, the COP1/SPA complex is disrupted for its E3 function. Visible light also promotes the nuclear export of COP1 for long-term repression. As a result, the transcription factors accumulate to target and promote photomorphogenesis.

be directly targeted by photoreceptors to affect the expression of downstream genes which consist of light-responsive elements (LREs) in the promoter region (Jiao et al., 2007; Castillon et al., 2007). A well-known example are the transcription factors PHYTOCHROME INTERACTING FACTORS (PIFs) which are important for shade avoidance responses as well as to maintain skotomorphogenesis in seedlings (introduced in detail in I.1.5). Photoreceptors can in addition indirectly regulate expression of genes post-translationally. Analysis of mutants that display constitutive photomorphogenic development in darkness revealed a group of involved genes —

the *CONSTITUTIVE PHOTOMORPHOGENESIS (COP)/DE-ETIOLATED (DET)/FUSCA(FUS)* gene group (Wei and Deng, 1996; Kwok et al., 1996).

COP1 is the most well-characterized member of the COP/DET/FUS group that acts as part of the E3 ubiquitin ligases to target transcription factors for degradation (Deng et al., 1991; Lau and Deng 2012). In Arabidopsis, *COP1* encodes a 76 kDa protein that contains a C-terminal WD40-repeat domain, a central coiled-coil domain which serve as protein-protein interaction domains, a N-terminal RING-finger domain which is essential for the E3-ubiquitin ligase function, a nuclear localization sequence (NLS) and a cytoplasmic localization sequence (Deng et al., 1992; Torii et al., 1998). Furthermore, screening for suppressors of a weak *phyA* mutant revealed multiple mutants of the suppressor of *phyA-105-1* (*spa1*) and *spa1-like* (*spa*) which display constitutive photomorphogenic phenotypes similar as the *cop1* mutants (Hoecker et al., 1998 and 1999; Laubinger et al., 2004; Laubinger and Hoecker, 2003). The four SPA proteins consist highly identical coiled-coil domain and the WD-40 repeat domain, which they share with the COP1 protein, and a more diverse N-terminal domain (Hoecker et al., 1999; Laubinger et al., 2004). The SPAs interact with COP1 and with each other via the coiled-coil domain, and appears to form a tetrameric complex consisting of two COP1 proteins and two homo- or hetero-dimerized SPA proteins (Hoecker and Quail, 2001; Laubinger and Hoecker, 2003; Saijo et al., 2003; Laubinger et al., 2004; Zhu et al., 2008).

In darkness, where the photoreceptors are inactive, the COP1/SPA complex targets the positive regulators of light responsive genes for degradation (Figure I-2). In the presence of light, the COP1/SPA complex interacts with phyA, phyB and cryptochromes. The repressive effect of photoreceptors on the COP1/SPA complex allows the accumulation of the photomorphogenesis-promoting transcription factors, resulting in photomorphogenic development (Figure I-2) (Jang et al., 2010; Seo et al., 2004; Wang et al., 2001; Yang et al., 2001). cry1 disrupts the COP1/SPA complex by directly binding to SPA1 (Lian et al., 2011; Liu et al., 2011), whereas the binding of cry2 to SPA1 represses COP1 activity by strengthening cry2-COP1 interaction (Zuo et al., 2011). PhyA and phyB also disrupt the COP1/SPA complex by direct interaction with SPA proteins (Sheerin et al., 2015; Zheng et al., 2013). At the seedling stage, the substrates of the COP1/SPA complex include the well-characterized the bZIP transcription factor *LONG HYPOCOTYL 5* (*HY5*), the MYB transcription factor *LONG AFTER FAR-RED LIGHT 1* (*LAF1*) and the atypical bHLH factor *LONG HYPOCOTYL IN FAR-RED 1* (*HFR1*) (Osterlund et al., 2000; Saijo et al., 2003; Seo et al., 2003; Jang et al., 2005; Yang et al., 2005). HY5 is rapidly up-regulated by light and subsequently binds to the LREs of genes to activate or repress their expression (Li et al., 2010; Lee et al., 2007). So far only a subset of the phenotypes of *cop1* and multiple *spa* mutants can be explained by the known targets, thus novel targets of COP1/SPA complex remain to be uncovered. Moreover, the COP1/SPA complex regulates shade

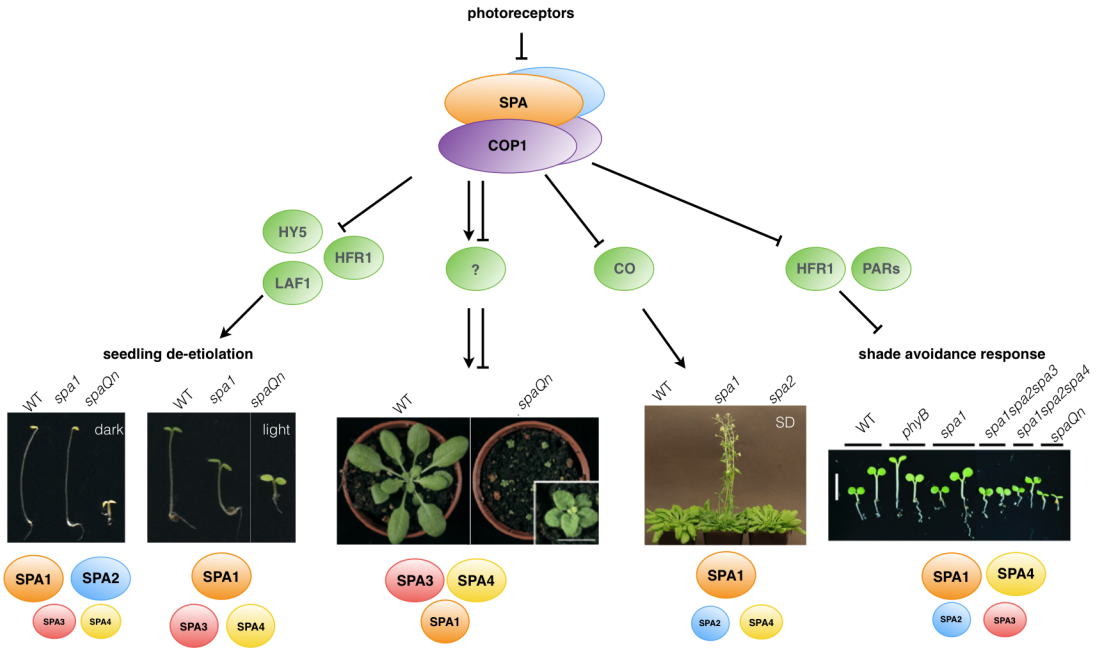


Figure I-3. The regulation of plant development processes by SPAs in Arabidopsis.

The COP1/SPA complex regulates seedling development, adult plant size, photoperiodic flowering and shade avoidance. The four SPA proteins play different roles in these processes. SPA1 and SPA2 play dominant role in suppressing photomorphogenesis in dark-grown seedlings, whereas SPA3 and SPA4 are major regulators of vegetative growth in adult plant. SPA1 predominantly suppresses flowering under short-day conditions. SPA1 and SPA4 play major roles in regulating the shade avoidance responses. Arrows represent positive regulation, perpendicular lines represent negative regulation. (Photographs modified from Hoecker 2005; Laubinger et al., 2006; Rolauffs et al., 2012)

avoidance response through PIFs and the BBX family genes (introduced in section I.1.5), and photoperiod flowering by repressing the activity of CO in late afternoon (introduced in section I. 3.1).

I.1.3 Roles of SPA proteins in light-regulated Arabidopsis plant development

In Arabidopsis, four SPAs are present (SPA1, SPA2, SPA3, SPA4), of which SPA1 was the first being identified as suppressor of a weak *phyA* mutation (Hoecker et al., 1998). Based on their sequence similarity, the four SPAs can be divided into two subgroups. Originating from gene duplication during evolution (Simillion et al., 2002), the subgroup SPA1 and SPA2 contain longer N-terminal extension and NLS which are absent in the subgroup SPA3 and SPA4 (Laubinger and Hoecker, 2003; Laubinger et al., 2004). The *spa quadruple* (*spaQn*) mutant displays a

constitutive photomorphogenesis seedling phenotype, as well as a dwarfed adult phenotype, similar as the *cop1* mutant (Laubinger et al., 2004). The *spa* double and triple mutant lack, to different degrees, the constitutive photomorphogenesis of *spaQn*, and further analysis indicate the four *SPA*s have redundant as well as distinct functions in various stages of plant development. In dark-grown seedlings, *SPA1* and *SPA2* both are sufficient to suppress the photomorphgenesis; whereas in light-grown seedlings, *SPA1* functions predominantly to repress the overstimulation of photomorphogenesis, with minor roles played by *SPA3* and *SPA4*. (Laubinger et al., 2004; Laubinger et al., 2006; Fittinghoff et al., 2006; Balcerowicz et al., 2011). Among all the *spa* single mutants, the *spa1* single mutant exhibits distinctive increased photomorphogenesis in B, R and FR (Hoecker et al., 1998, 1999; Baumgardt et al., 2002) and early-flowering phenotype under SD condition (Laubinger et al., 2006). During vegetative plant growth, *SPA3* and *SPA4* play a major role in regulating leaf size, in concert with *SPA1*, whereas SPA2 has nearly no function (Laubinger et al., 2004; Fittinghoff et al., 2006).

The different functions of four SPAs in plant development are also reflected on their regulatory level. Light up-regulates the transcript levels of *SPA1*, *SPA3* and *SPA4*, but not *SPA2*. The diverged function of light-regulation on different SPA proteins during evolution was studied in detail, particularly between SPA1 and SPA2 (Balcerowicz et al., 2011; Chen et al., 2015, 2016; Holtkotte et al., 2016). The chimeric constructs, which were generated to express *SPA1* and *SPA2* open-reading-frames (ORFs) under the control of *SPA1* or *SPA2* 5' and 3' regulatory sequences, confirmed that only the protein sequence of SPA1 or SPA2 determines their distinct functionality in the light (Balcerowicz et al., 2011). The SPA2 protein is degraded under light (Balcerowicz et al., 2011). The rapid light-induced degradation of SPA2 protein requires phytochromes in red, far-red and blue light as well as COP1 for ubiquitination (Chen et al., 2015). SPA1 and SPA2 protein both interact with cry1, but SPA2 fails to associate with cry2, which is likely due to the sub-functionalization of the N-terminal domains between SPA1 and SPA2 (Chen et al., 2016). Furthermore, Holtkotte et al., (2016) showed that the kinase-like domain at the N-terminal of SPA1 is important for its activity in darkness, but not in light.

I.1.4 Light signaling in the shade avoidance responses in Arabidopsis

When shaded by competitors, which represent a threat due to limited light resources, plants initiate escape responses by elongation growth, termed as shade avoidance syndrome (SAS). Rapid SAS responses consists of leaf hyponasty and stem elongation, which elevate leaves above the canopy or towards canopy gaps, facilitating light capture (Gommers et al., 2012; Casal 2012). Red, blue and UV-B wavebands are depleted when sunlight transmits through living vegetation, resulting in enriched green and far-red wavebands thus reduced ratios of blue to green light (B:G) and red to far-red light (R:FR). The sensing of encroaching vegetation

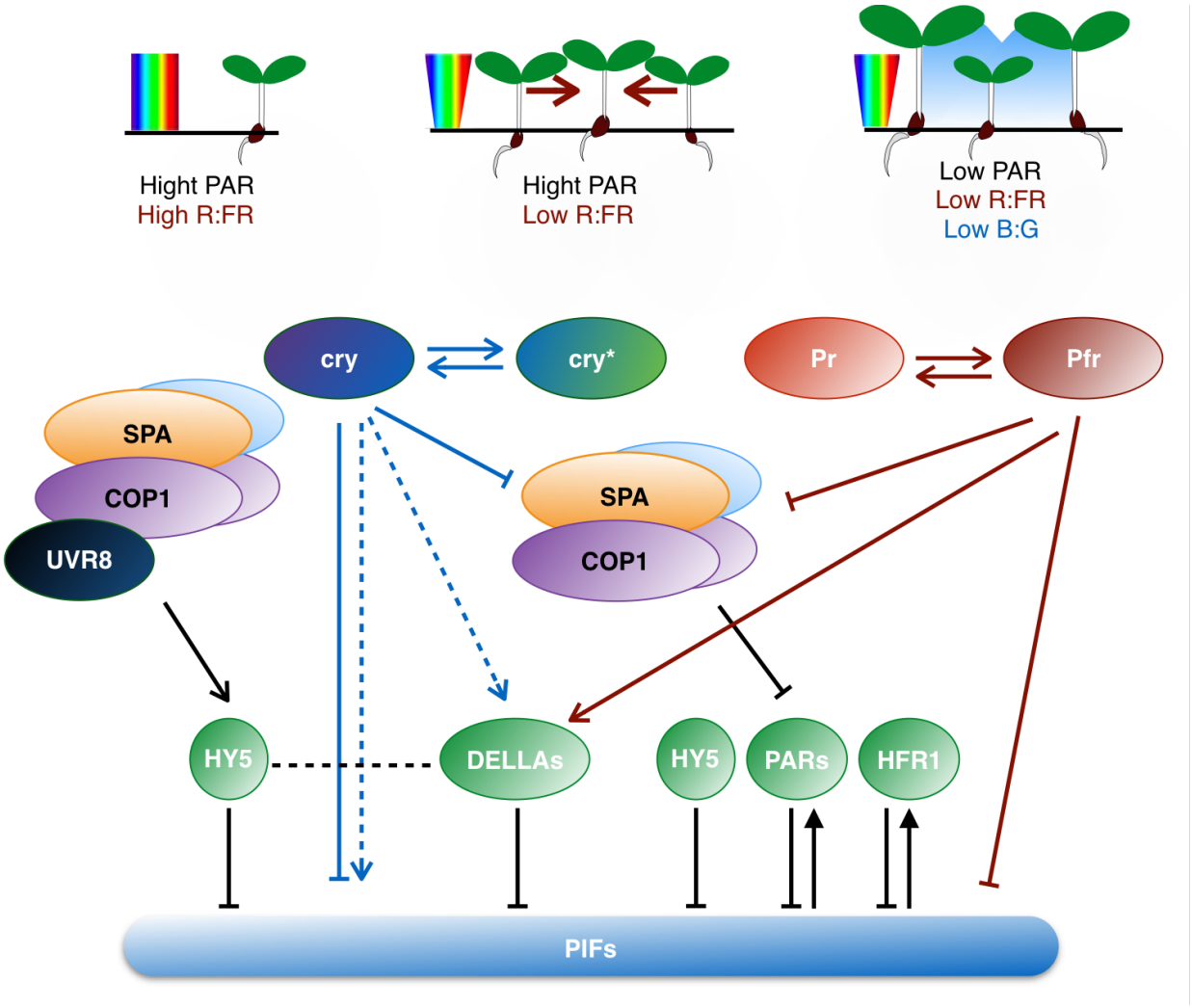


Figure I-4. Two different strategies to cope with shade (A) and schematic representation of the photoreceptors-PIFs signaling pathways in shade avoidance (B).

(A) Plants cope with vegetation shade which causes a reduction of R:FR ratio, but unaltered PAR, or canopy shade which causes a reduction of R:FR and B:G ratio and low PAR.
(B)UV-B triggers PIF degradation likely via UVR8 which binds to the COP1/SPA complex to promote the accumulation of HY5 and to limit PIF activity. cry is activated by UV/B light and converted to inactive state by G light (cry*). In low B light, cry form a complex with PIF4 and PIF5 to repress shade avoidance. Conversation of phy to the active Pfr form is promoted in R. Active phy and cry disrupt the COP1/SPA complex which target HY5, PARs and HFR1 for degradation, thus activating PIFs. PIFs positively regulate PARs and HFR1. DELLAs inhibit PIFs function, and is likely stabilized by cry in low B light, Pfr in low R:FR and indirectly via HY5 in UV-B. Solid arrows represent positive regulation, perpendicular lines represent negative regulation. Dotted lines indicate hypothesized regulation. Figure based on Fraser et al., 2016; Casal 2013; Gommers et al., 2012.

involves the well-studied phytochromes for lowered R:FR (Franklin and Quail 2010; Lorrain et al., 2008; Martínez-García et al., 2014); recent studies also revealed important roles of blue and

UV-B photoreceptors on the signaling network in controlling shade avoidance (Keller et al., 2011; Pedmale et al., 2015; Ma et al., 2016; Hayes et al., 2014).

I.1.5 The perception of shade by photoreceptors and regulation of downstream integrators

In Arabidopsis, phyB functions predominantly in SAS inhibition, with redundant roles by phyD and phyE (Franklin and Quail 2010). In high R:FR, phyB accumulates in active Pfr form and is translocated to the nucleus to bind the basic Helix-Loop-Helix (bHLH) transcription factors PIFs (Leiva and Monte 2014). The binding of phyB Pfr to PIFs initiates the degradation of PIFs through ubiquitination (Ni et al., 2014). In the shaded condition with low R:FR, phyB Pfr is converted to the inactive Pr form, which releases the suppression on PIFs, thus allowing the accumulation of PIFs at target downstream genes to promote stem elongation (Hornitschek et al., 2012).

The PIFs (PIF4 ,PIF5, PIF7), as the positive regulators of SAS, promote hypocotyl elongation in part by up-regulating the homeodomain-leucine zipper transcription factor, *ARABIDOPSIS THALIANA HOMEOBOX 2* (*ATHB2*) and *ATHB4* which are important for light-hormone interaction (Sorin et al., 2009), and the transcription of the cell-wall modification enzymes XYLOGLUCAN ENDOTRANSGLUCOSYLASE / HYDROLASES / XYLOGLUCAN ENDOTRANS- GLUCOSYLASE/HYDROLASES – RELATED PROTEINS (XTH/XTR) (Eklöf and Brumer, 2010; Figure I-4). Among these, *ATHB2* and *XTR7* are established target gens by the PIFs and strongly up-regulated by low R:FR (de Lucas et al., 2008; Lorrain et al., 2008; Sasidharan et al., 2010). Moreover, the PIFs promote hypocotyl elongation in part by activating the transcription of auxin biosynthesis YUCCA enzymes (Hornitschek et al., 2012; Li et al., 2012). The low R:FR-induced auxin biosynthesis is introduced in next section.

The low R:FR induced reduction of phyB Pfr may indirectly enhance PIF activity via negative regulators such as *HFR1, ELONGATED HYPOCOTYL 5 (HY5)* and *PHYTOCHROME RAPID REGULATED (PARs)*, which are mediated for degradation by the increased COP1/SPA1 complex (Rolauffs et al., 2012; Fraser et al., 2016). The bZIP transcription factor HY5 inhibits PIF transcript abundance via direct physical interaction with PIF (Chen et al., 2013; Delker et al., 2014). The bHLH factors *HFR1*, *PAR1* and *PAR2* physically interact with PIF4 and PIF5 and repress their function (Hornitschek et al., 2009). The *hfr1* mutant showed exaggerated hypocotyl elongation in response to low R:FR, and over-expression of *HFR1* led to diminished elongation response (Hornitschek et al., 2009; Galstyan et al., 2011). The transcript levels of *HFR1* are elevated swiftly upon onset of low R:FR and last for days in prolonged shade conditions (Sessa et al., 2005). PAR1 and PAR2 repress PIF function similar as HFR1 by forming heterodimers

that prevent binding PIFs to promoters of target genes (Galstyan et al., 2011). In a similar manner, *PIF3-LIKE 1* (*PIL1*) is also quickly up-regulated by low R:FR condition and represses function of PIFs. However, *PIL1* might involve a more complex function as both positive and negative roles were assigned in different studies (Roig-Villanova et al., 2006,2007; Lorrain et al., 2008; Salter et al., 2003).

Alongside the decreased R:FR ratio in canopy shade, plants also perceive a reduction of UV and blue light. Very recently, Pedmale et al. (2015) first reported that cryptochromes perceive limiting blue light by binding to PIF4 and PIF5 to form a regulatory complex and regulate cell wall modifying enzymes. Subsequently, the blue-light inhibition of hypocotyl elongation via PIFs was further investigated by Ma et al. (2015). Therefore, low blue light or low B:G mediates shade avoidance response through cryptochromes, and PIFs integrate multiple light signals by binding to different photoreceptors. In addition, UVR8 binds to COP1 and positively regulate the expression of *HY5* (Brown and Jenkins 2008). UV-B light perceived by UVR8 negatively regulate PIF function, thereby inhibiting expression of auxin biosynthesis genes and hypocotyl elongation (Hayes et al.,2014). Taken together, the mechanistic detail of how SAS are regulated are well studied yet still limited, involving multiple photoreceptors and key integrator PIF transcription factors.

I.1.6 Light regulation of the auxin action in the SAS

In Arabidopsis, Indole-3-acetic acid (IAA) is the main active auxin and mainly synthesized from tryptophan (Tao et al., 2008). This process of synthesizing IAA involves two key groups of enzymes TRYPTOPHAN AMINOTRANSFERASE 1 (TAA1) and YUCCA, in which TAA1 produces IPA from tryptophan and YUC metabolizes IPA to IAA (Stepanova et al., 2011; Won et al., 2011). IAA is produced primarily in the cotyledons, young leaves and meristems; the auxin can be transported to other tissues either via passive distribution through the phloem and to all sink tissues, or actively via the polar auxin transport (PAT) (Gao et al., 2002). The active transport requires the auxin efflux regulator *PIN-FORMED 3* (*PIN3*) to direct auxin to the hypocotyl epidermis (Keuskamp et al., 2010).

The auxin pathway can be regulated by light at all levels. phyB, which predominantly regulates SAS, was shown to negatively regulate auxin biosynthesis by inhibiting the TAA1-dependent auxin production (Tao et al., 2008). Both TAA1 and YUCCA are important in increasing free auxin levels rapidly in response to shade (Tao et al., 2008). However, the expression of TAA1 is not responsive to simulated shade treatment (Tao et al., 2008). In Arabdopsis, YUCCA consists of 11 members with largely redundant functions (Cheng et al., 2006, 2007; Zhao et al., 2001). Low R:FR induces auxin biosynthesis by up-regulating the transcript levels of YUCCA enzymes which catalyzes the rate-limiting step in auxin-biosynthesis (Hornitschek et al., 2012). These

includes *YUC1* and *YUC4* which are expressed in the aerial part of seedling and important for low R:FR induced hypocotyl elongation (Won et al., 2011), as well as *YUC2*, *YUC5*, *YUC8*, and *YUC9* which are essential for phytochrome-mediated elongation (Tao et al., 2008). Low R:FR also promotes hypocotyl elongation by increasing the expression and relocalisation of PIN3, the auxin efflux regulator (Keuskamp et al., 2010). The PIFs mediates the sensitivity of plants to auxin levels when plants are under limited resources e.g. canopy shade, thus making plant responses more efficient (Hersch et al., 2014).

I.2 Flowering control in Arabidopsis and other annual plants

At a certain time point in the life cycle of plants, a transition from vegetative to reproductive development occurs. This transition is rarely reversible, ensuring successful pollination and seed setting, thus successful reproduction. Plants respond to changing environment to initiate the developmental transition. In the past decades, physiological analyses have attributed the flowering responses to discrete environmental cues, such as day length and winter temperatures. Efforts aiming to dissect the molecular mechanisms underlying floral transition, made impressive progress in the model species *Arabiodpsis thaliana*. Until recently, five major genetic pathways controlling flowering were identified, including environmental induction through photoperiod, vernalization, gibberellins, autonomous pathways, and aging pathway by sequentially operating miRNAs (typically miR156 and miR172) (Mouradov et al., 2002, Boss et al. 2004, Fernando and Coupland 2012, Khan et al. 2014). In addition, environmental factors such as light quality, light intensity, ambient temperature and nutrient state also affect flowering time.

I.2.1 Photoperiod pathway

Plants sense changes in day length, or photoperiod as one of the most important cues for floral transition. Classical physiological studies trying to identify how plants are able to recognize the optimal conditions for flowering can be dated back to 1920, when Garner and Allard first proposed ‘photoperiodism’ and suggested the mechanism of photoperiod flowering could be tied to the sensing of duration of light in a given day. In addition, they grouped plants to three different groups by flowering responses: long-day plants (LDP) which flower as day length increases in late spring, short-day plants (SDP) which flower as day length wanes as autumn begins, and day-neutral plants (DNP) which flower regardless of the photoperiod (Garner and Allard, 1920). It took decades for scientists to answer critical questions such as where in the plant the day length is sensed and how the signal is transmitted throughout the organism. Chailakhyan (1968) determined that a mobile signal originates from the leaf to induce flowering,

and it´s not until the discovery of the FT protein as a mobile signal in the Arabidopsis that a key candidate was identified (e.g. Koornneef et al., 1991; Corbesier et al. 2007). Recent advances in studying the LDP Arabidopsis revealed that the core of the day-length measurement mechanism lies in the circadian regulation of *CONSTANS* (*CO*) expression and subsequent activation of *FT* gene (Hayama and Coupland, 2004; Imaizumi and Kay, 2006). As our understanding of the photoperiod sensing mechanism expanded, the conserved functions of CO and FT, and similar regulatory networks were also identified and found in other plant species such as rice, barley, tomato and potato (Taoka et al, 2011; Yan et al., 2006; Lifschitz et al, 2006; Navarro et al, 2011).

The *co* mutant was isolated by mutagenic screens and interested researchers with its 'day-neutral' phenotype (Putterill et al., 1995), as the *co* mutant flowers much later than the wild type plants in inductive LD conditions, but displayed similar flowering time in SD conditions. Moreover, over-expression of *CO* resulted in flowering regardless of the external photoperiod (Simon et al., 1996). The *ft* mutant flowers late under LD conditions, and is only slightly affected under SD (Koornneef et al., 1991). Further genetic studies on the late flowering *ft* mutant revealed that *CO* and *FT* are components of the same regulatory pathway (Koornneef et al., 1991). Therefore, the *CO-FT* module are key players to initiate flowering under inductive conditions. Many factors have been shown to regulate CO and FT through a variety of mechanisms (André and Coupland, 2012).

Further studies showed circadian clock control the transcriptional and post translational regulation of CO to regulate the photoperiodic response (Figure I-5). The transcription of *CO* is controlled by many circadian clock proteins, such as CCA1, LHY and PRRs. The abundant clock protein CCA1 and LHY in the morning directly or indirectly up-regulate the gene expression of *CYCLING DOF FACTORS* (*CDFs*), which transcriptionally repress *CO*. In long day conditions, the repression of *CO* transcripts by CDF proteins is released by the FKF1-GI complex which degrades the CDFs in the late afternoon. Maintaining the expression of *CO* to late afternoon allows for the subsequent activation of *FT* in long days, enabling the photoperiodic flowering response (Figure I-5). In short day conditions, the FKF1 and GI proteins are out of phase because the GI–FKF1 complex only rises around 10 hours after dawn (Sawa et al., 2007), which results in accumulation of *CO* transcripts only in dark period. Furthermore, once the CO repression by the CDF proteins is relieved, the FBH proteins, which are bHLH transcription factors, directly binds to the promoter of *CO* and subsequently induce *CO* accumulation in late afternoon or in the dark both under LD or SD conditions (Ito et al., 2012).

Post-translational regulation of CO is also essential for the photoperiodic flowering response in Arabidopsis, as the CO protein is crucial for the day-length dependent *FT*-activation. The accumulation of *CO* mRNA abundance is highest in the dark both under LD and SD conditions,

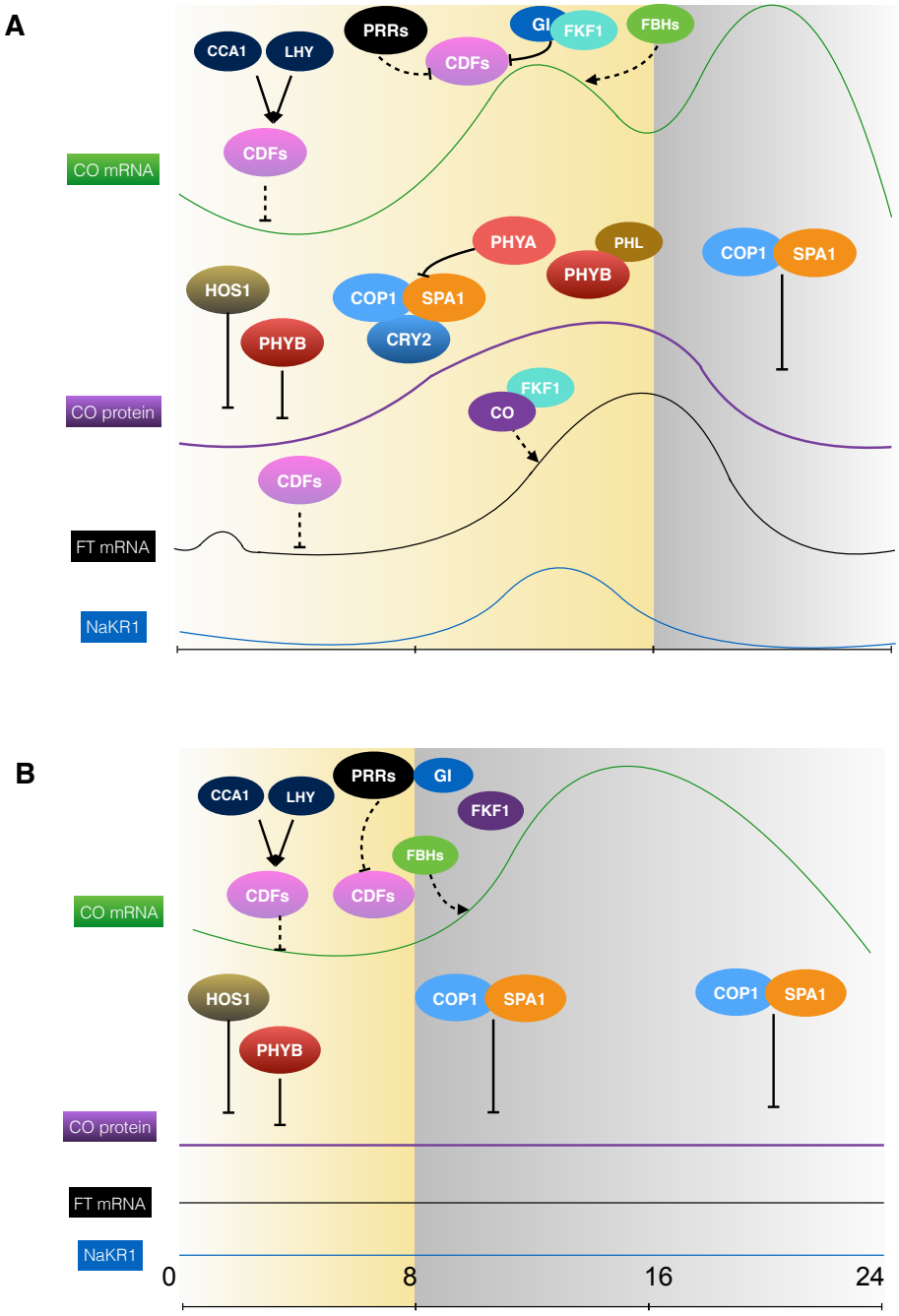


Figure I-5. Transcriptional and post-translational regulation of *CONSTANTS (CO)* controls photoperiodic flowering in *Arabidopsis thaliana*.

(A) *CO* regulation under long days (LD) of spring or early summer. The *CO* mRNA abundance peaks at 12-16 hours after dawn under long day, thus CO protein accumulates at the end of the day to activate *FT*. NaKR1 is induced in late afternoon and essential to assist the movement of FT protein.
(B) *CO* regulation under short days (SD) of winter of autumn. *CO* mRNA only peaks in the dark and CO protein cannot be stabilized, thus *FT* transcription is not activated. Arrows indicate post-transcriptional activation, perpendicular lines indicate post-transcriptional repression. Dotted Arrows indicate transcriptional activation, dotted perpendicular lines indicate transcriptional repression. Figure based on André & Coupland (2012), Shim et al., (2016).

however, CO protein only accumulates in the late afternoon in LD, which accounts for the peak expression of *FT* at dusk in LD (Suárez-López et al., 2001; Jang et al., 2008). The regulation of CO protein requires the COP1/SPA complex (Briggs & Olney, 2001; Liu et al, 2008). In short day conditions, as the *CO* mRNA accumulation only occur in darkness, the CO protein is degraded actively by the COP1-SPA1 complex. This is consistent with the findings that *cop1* and *spa* mutants flower early under SD (Laubinger et al., 2006; Jang et al., 2008), which are a consequence of the stabilized CO protein in darkness. The degradation of CO by COP1-SPA complex is, at least in part, repressed by light in late afternoon, where activate CRY2 directly interacts and reduces the catalytic activity of COP1-SPA1 complex (Liu et al., 2008a; Wang et al., 2001). CO is also stabilized by far-red light through PHYA in the afternoon, which possibly disrupts the COP1-SPA complex function (Sheerin et al., 2015). These are consistent with mutant studies of *cry2* and *phyA* which are late flowering under inductive LD conditions. The post-translational stablization of CO protein in late afternoon is further enhanced by the direct interaction of FKF1 and CO (Song et al., 2012). Additionally, any CO protein present in the beginning of the day is degraded through PHYB, the red-light receptor (Valverde et al., 2004). The regulation is possibly through two distinct mechanisms: HOS1 and PHYTOCHROME-DEPENDENT-LATE-FLOWERING (PHL) (Endo et al., 2013; Lazaro et al., 2015; Golembeski et al., 2014; Shim et al., 2016). The HOS1 protein, a RING-finger-containing E3 ubiquitin ligase, mediates the degradation of CO in the morning. It is possible that HOS1 is genetically in the same pathway as PHYB (Lazaro et al., 2015). PHL interferes with the phyB-dependent destabilization of CO in late afternoon, proved by its interaction with phyB and CO in red light conditions (Endo et al., 2013).

The expression of *FT* is activated in the end of the long day directly by CO. In the morning, *FT* transcription can be repressed by CDFs which associate with the *FT* promoter. Furthermore, FT protein is a floral signal synthesized in the leaves and moves to function in the shoot apical meristem (see I.2.4 for detailed introduction).

The SDP rice (*Oryza sativa*) has conserved circadian regulation mechanism to control photoperiodic flowering. The homologues of *CO* and *FT*, *HD1* and *HD3A,* were identified from initial molecular genetic studies (Yano et al., 2000). In contrast to Arabidopsis, under long days, the transcription of *HD3A* gene is inhibited by *HD1*, whereas under short days *HD1* promotes the *FT*-like genes *HD3A* and its praralogue *RICE FT-LIKE1* (*RFT1*) (Hayama et al., 2004). However, identification of further genes in rice which have key functions in photoperiodic flowering but are absent in *Arabidopsis* make the conservation of mechanism between rice and *Arabidopsis* more complicated. In particular, *EARLY HEADING DATE 1*(*EHD1*), which encodes a B-type response regulators, activates the transcription of *HD3A* and *RFT1*, in a *HD1*-independent pathway (Doi et al., 2004). However, the transcription factors encoding B-type

response regulator in Arabidopsis are mostly involved in cytokinin signal transduction pathway. Furthermore, the GHD7 protein, which encodes a CCT-domain protein, as CO in Arabidopsis, has a robust role in photoperiodic flowering in rice, but no counterparts were found in Arabidopsis (Xue et al., 2008). Current models of short-day flowering in rice suggest that three are conserved as well as diverged mechanisms controlling the transcription response of FT-like genes to photoperiod.

I.2.2 Vernalization pathway

Many plants, including winter annuals, most biennials and perennials, require a prolonged exposure to low winter temperature for an optimal duration to induce flowering, which is called vernalization response. *Arabidopsis thaliana* accessions consist of summer annuals and winter annuals, in the basis of their flowering responses to cold, analysis of the genetic differences revealed active alleles at two loci, FLC and FRI in winter annuals, whereas mutations occur in one or both of these genes in summer annuals (Sheldon et al., 1999; Johanson et al., 2000; Shindo et al, 2005).

FRI protein is required for the up-regulation of *FLC* — a MADS-box transcription factor which directly represses the expression of floral promoting genes (Johanson et al., 2000). The expression of *FLC* is repressed by vernalization, and can be considered in two phases: the repression of *FLC* transcription during winter vernalization, and the stabilization of low *FLC* mRNA after vernalization (André and Coupland 2012). The maintenance of the repression of *FLC* then allows plants to flower in the following spring. Recent studies of the molecular components required for the repression of *FLC* transcription during vernalization revealed a range of mechanisms, including chromatin remodeling and processing of non-coding RNAs *COOLAIR* and *COLDAIR* (Swiezewski et al., 2009), as well as Ploycomb silencing (Angel et al., 2011). The expression of antisense non-coding RNA *COOLAIR* is instrumental in reducing *FLC* expression in the cold, but not for *FLC* silencing (Helliwell et al., 2011). The sense non-coding RNA *COLDAIR* is essential for silencing *FLC* expression after vernalization, and for binding of proteins that are required for chromatin changes (Heo & Sung., 2011). Components of Polycom repressive complex (PRC) are required for stable silencing of *FLC* after vernalization (Angel et al., 2011).

FLC functions in the shoot apical meristem and vascular tissue, and at least directly binds to the flowering genes *SOC1* and *FT* to repress flowering (Searle et al., 2006). As *FT* and *SOC1* are key regulators from photoperiodic pathway, the inhibition of their expression by FLC indicates the convergence of photoperiod and vernalization pathways. Another MADS box protein SVP acts together with FLC to repress flowering, by direct interaction with FLC, as well as by targeting *SOC1* and *FT* to repress their transcription (Fujiwara et al., 2008). The mechanism of

FLC-SVP complex is unclear, but possibly the late flowering in winter-annual accessions of Arabidopsis owes to the direct binding of FLC-SVP to target genes, hence recruiting chromatin modifiers responsible for blocking transcription (André and Coupland 2012).

In vernalization-requiring varieties of temperate cereals crops, wheat and barley, the floral induction by long day is blocked until they are exposed to winter temperatures for a period of time. This photoperiod and vernalization pathways are tightly interlinked in cereals and integrated by VRN2, which has no counterpart in *Arabidopsis*. VRN2 is a floral repressor under long days, by inhibiting the expression of at least one of the FT homologues in cereals. Upon exposure to vernalization, the MADS box transcription factor *VRN1* is expressed and represses the expression of *VRN2*, thereby the FT-like genes can be activated to induce flowering after vernalization (Dubcovsky et al., 2006).

I.2.3 Other flowering pathways

Very recently, the aging flowering pathway was proposed, in which two evolutionarily conserved microRNAs (miRNAs), miR156 and miR172, are key players to promote transition to flowering. miR156 targets mRNAs of 11 genes encoding *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) transcription factors, which were recently identified to promote transition from juvenile to adult and to flowering (Wu and Poethig, 2006). miR156 promotes the juvenile phase, showing highest level at the seedling stage and decreases in the adult phase (Wang et al., 2009). The down-regulation of miRNA156 during adult stage allows the activation of several *SPL* genes to promote transition to flowering (Wu and Poethig, 2006; Wang et al., 2009a). The SPL proteins have also been shown to be able to bind and promote floral integrator genes, such as *SOC1*, as well as floral meristems genes *LFY*, *FUL* and *AP1 (*Wang et al., 2009a*)*.

By contrast, miR172 has an opposite expression pattern as miR156, suggesting a complementary regulatory relationship between miR156 and miR172. miR172 regulates six members of *APETALA2* (*AP2*) transcription factor family which have been shown to be involved in transition to flowering and flower development (Aukerman and Sakai, 2003; Jung et al., 2007, 2011; Mathieu et al., 2009). In contrast to miR156, the level of which is controlled by plant age, the expression of miR172 is likely under photoperiodic control and promote photoperiodic flowering independently of CO (Jung et al., 2007). Whole-genome mapping of AP2 binding sites showed that the miR172-AP2 module regulates *FT* in leaves, as well as other flowering time regulators downstream of FT in the shoot apex (Mathieu et al., 2009). Furthermore, the miR156 targeted *SPL* genes regulate the expression of miR172 and thus the AP2-like floral repressors. Despite all the knowledge we have obtained, there is very little known about factors which regulate the temporal and spatial expression of the miRNA genes themselves. For example, it is not clear how plants measure and determine ‘aging’ and thus regulating gene expression

(Huijser & Schmid 2011). Recently in Arabidopsis, a trehalose-6-phosphate (T6P) pathway was proposed, in which T6P acts as a proxy for carbohydrate status in plants and regulates flowering through expression of key floral regulators in the leaves and meristems (Wahl et al., 2013). In addition, the T6P pathway regulates the expression of *SPL3/4/SPL5* in the shoot apical meristem, both dependently and independently via miR156.

The functions of miR156 and miR172 and their targets are evolutionary conserved, in maize and potato. The short response period since germination to flower induction in Arabidopsis hampers the study of how miR156 and miR172 are involved in age-related competence to flower. This is addressed by studies in *Arabis alpina*, and introduced in detail in later section.

The autonomous pathway was identified via a group of mutants that were characterized as late flowering irrespective of day length and are highly responsive to vernalization (Marquardt et al. 2006). The late flowering phenotype of these mutants can be overcome by vernalization treatment. Indeed, the mutants, identified so far as *LUMINIDE-PENDENS* (*LD*), *FCA*, *FY*, *FPA*, *FLOWERING LOCUS D* (*FLD*), *FVE*, *FLOWERING LOCUS K* (*FLK*), and *REF6*, are all involved in repressing the common target FLC (Simpson et al, 2003; Bäurle and Dean, 2006). Therefore, the autonomous and vernalization pathway have been suggested to function in parallel in flower regulation. The regulation on the expression of *FLC*, however, occurs at different levels.

The growth regulator gibberellin (GA) also regulates flowering in Arabidopsis in a biphasic manner. Elevated GA first promotes the end of vegetative development, subsequent reduced GA then negatively regulate flower formation (Yamaguchi et al., 2014). Mutants analysis revealed genes with key functions in GA signalling, including *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR OF GA1-3* (*RGA*), *RGA-LIKE 1* (*RGL1*). The integration of signaling of GA involves *LFY*, *SOC1* in the meristem (Moon et al., 2003) and *FT* in the leaves (Hisamatsu and King 2008). The transcription factor LFY induces catabolism of GA, the elevated level of GA promotes the termination of the vegetative phase, subsequently the expression of genes encoding transcription factors such as the *SPLs* and *LFY* are increased (Yamaguchi et al., 2014). GA induces *FT* in the leaves, indicating the collaboratively modulation of flowering by GA and photoperiod pathway (Hisamatsu and King 2008).

In addition to the flowering pathway introduced here, there are other environmental factors involved in flowering regulation. These environmental conditions generally act as stress signals to induce flowering to produce seeds for next generation, or delay flowering by slowing metabolism (Cho et al., 2016). The factors consist of high or low light intensity, UV light, high or low temperature, poor nutrition, nitrogen deficiency, drought, low oxygen, crowding, root removal, pathogenic microbes and mechanical stimulation.

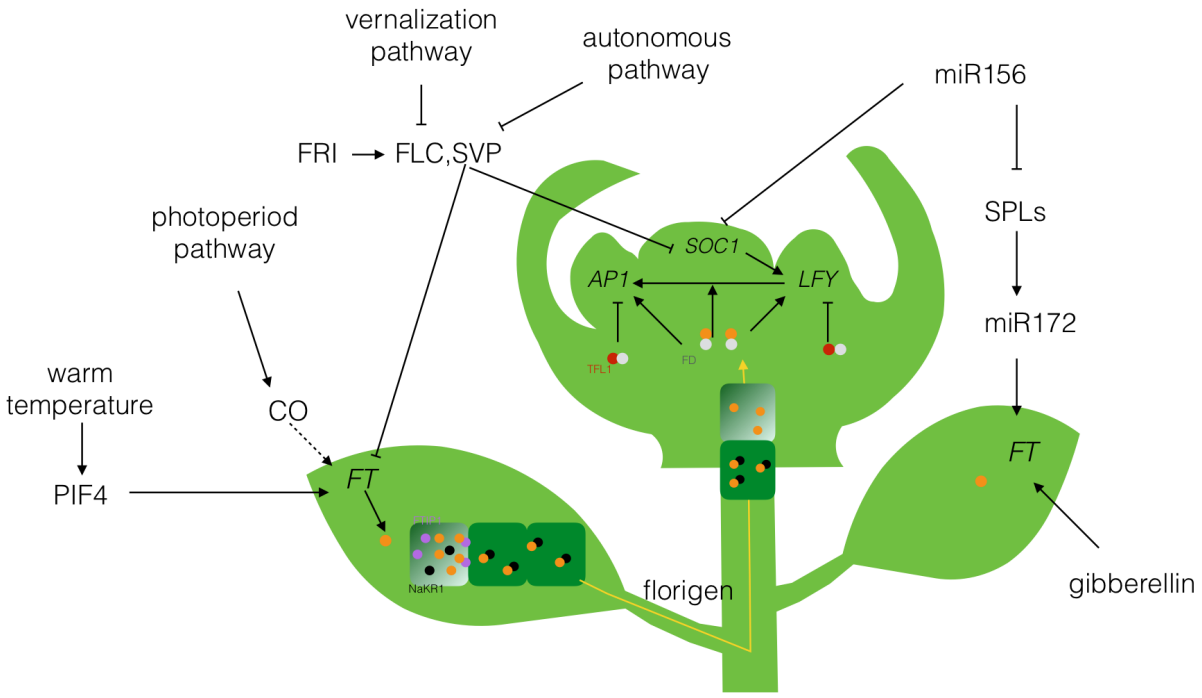


Figure I-6. Outline of flowering pathways in Arabidopsis and the movement of FT protein.

Five independent pathways have been identified up to date: vernalization, photoperiod, autonomous, aging and gibberellin pathway. In addition, warm temperature can also initiate flowering through FT. The photoperiod pathway activates the *FT* transcription through CO. Vernalization and autonomous pathways repress the activity of *FLC*, which is a repressor of flowering by acting on *FT* and *SOC1* transcription. Environmental signals integrated by FT in the leaves and SOC1 in the meristem activate the expression of floral meristem identity genes *AP1* and *FLY* to start flower formation. The movement of FT protein is assisted by FTIP1 to sieve elements, and NaKR1 through the phloem. Once FT reaches the shoot apical meristem, it interacts with FD to switch on the subsequent floral development program. Arrows represent positive regulation, perpendicular lines represent negative regulation, dotted lines represent transcriptional activation.

I.2.4 Floral Integrator FT and initiation of floral meristems

In Arabidopsis, the transcription of *FT* is a convergence point of multiple flowering pathways (Figure I-6). In addition to the direct up-regulation by CO, other photoperiod pathway components GI, FKF1 and CDFs were also reported to bind directly to the *FT* locus (Sawa et al., 2007; Song et al., 2012; Sawa et al., 2011). Furthermore, in response to high temperature and blue light, respectively, the basic helix-loop-helix transcription factor PIF4 and CRYPTOCHROME-INTERACTING BASIC HELIX–LOOP–HELIX (CIB1) bind directly to the proximal region of *FT* promoter and activates *FT* transcription (Kumar et al., 2012; Liu et al.,

2008b). The TPS1 in the T6P pathway is also required for the induction of *FT* expression even under inductive photoperiod (Wahl et al., 2013). The transcription of *FT* is repressed by epigenetic mechanisms, known as repressive chromatin marks formed by LHP1, a Polycomb-repressive complex 1 and histone H3 modified by trimethylation on lysine 27 (H3K27me3), a Polycomb-repressive complex2, which widely covers the *FT* locus (Adrian et al., 2010). Those regions that are free of repressive marks constitute a window of open chromatin that is accessible to regulatory factors (Adrian et al., 2010). Many other proteins that bind to the *FT* locus are also transcription repressors, including SVP and FLC (introduced in I.2.2), AP2-like transcription factors TEM1 and TEM2, a small group of AP2-like genes and mRNAs targeted by miR172 such as *APETALA2* (*AP2*), *TARGET OF EAT 1* (*TOE1*), *TOE2*, *SCHNARCHZAPFEN* (*SNZ*) and *SCHLAFMÜTZE* (*SMZ*) (Mathieu et al., 2009).

The floral integrator *FT* encodes a 20kDa protein that is homologous to the phosphatidylethanolamine-binding (PEBP) or Raf kinase inhibitor proteins (Kardailsky et al., 1999). Once FT protein is synthesized in the companion cells of leaf phloem, it travels with the phloem translocation stream to function in the shoot apex (Corbesier et al., 2007; Putterill & Varkonyi-Gasic, 2016). Two membrane-associated proteins, FT-INTERACTING PROTEIN 1 (FTIP1) and SODIUM POTASSIUM ROOT DEFECTIVE 1 (NaKR1), assist the transport of FT movement (Liu et al., 2012; Zhu et al., 2016). FTIP1 interacts with FT in the companion cells and mediates the movement of FT to sieve elements (Liu et al., 2012), whereas NaKR1 assists the long distance movement of FT through sieve elements. The expression of *NaKR1* is highly induced in late afternoon under long days, by direct induction of CO which physically binds to the *NaKR1* promoter (Zhu et al., 2016). Therefore, the photoperiodic flowering mechanism in companion cells facilitate, at least partially, the movement of FT protein to shoot apex meristem.

Once FT reaches the shoot apex, it unloads from the phloem and moves cell to cell to interact with the meristem-expressed bZIP transcription factor FD (Taoka et al., 2011; Wigge 2011). Subsequently the floral development program is switched on, through the initiation of floral meristem identity genes such as *APETELA1* (*AP1*) and *LEAFY* (*LFY*), as well as floral promoters such as *SOC1*. *AP1* can be activated directly by FT-FD complex (Wigge et al., 2005). *SOC1*, a MADs box transcription factor, is the earliest activated gene in the shoot meristem in response to long day condition (Samach et al., 2000). Together with FUL, they are essential for the promotion of flowering by *FT* (Torti et al., 2012). Furthermore, the transcription of *LFY* can also be activated by the interaction of SOC1 with AGL24, another MADS box transcription factor (Lee et al., 2008).

TSF is a highly related protein with 82% identity and proposed to act redundantly as FT. The spatial expression pattern of *TSF* and *FT* in Arabidopsis seedlings are non-overlapping, with *TSF* expressed mainly in the vascular (phloem) tissue of hypocotyl and petiole, whereas *FT*

mostly in the vascular tissue of cotyledons and leaves (Yamaguchi et al., 2005). The expression of *TSF* is also generally lower than that of *FT* in Arabidopsis seedlings and is exclusively present in shoot apical region (Yamaguchi et al., 2005). It was recently reported that TSF protein is less stable, and has less protein mobility in comparison to FT (Jin et al., 2015). Another protein TERMINAL FLOWER1 (TFL1), with 71% similar amino acid residues to FT, represses the expression of meristem identity genes *AP1* and *LFY* in the meristem (Liljegren et al., 1999; Shannon et al., 1993). The transcriptional repression of TFL1 was reported to depend on its integration with FD (Hanano and Goto 2011), suggesting a pivotal role of FD. A very comprehensive functional mutagenesis on FT protein examined how over-expression of hundreds of FT point mutations affect flowering time (Ho and Weigel 2014), and revealed four new point mutations in FT that could convert FT to a TFL1-like repressor. Studies on TFL1-like repressors in woody perennials highlight their importance not only in flowering time, but also in controlling the juvenile length. Moreover, it was reported that FT is also present in the axillary buds together with *BRANCHED1/TEOSINTE BRANCHED1-LIKE1* (*BRC1*), and likely prevent premature floral transition in axillary meristems by their direct interaction (Khan et al., 2014).

I.2.5 Natural genetic variation in flowering regulation in Arabidopsis

Natural genetic variation in flowering responses within species has been described in Arabidopsis and other crops (Giakountis et al., 2010; Alonso-Blanco et al, 2009). QTL linkage mapping and association analyses of flowering time showed several candidate genes. Among this, *FRI* and *FLC* are involved in the regulation of flowering by vernalization (detailed introduction in section 1.1.2), and three photoreceptor genes *CRY2*, *PHYC* and *PHYD* cause natural variation for the photoperiodic flowering responses (El-Assal et al., 2001; Balasubramanian et al., 2006; Aukerman et al., 1997). Different accessions of Arabidopsis require varied period of low temperature for vernalization response, which is likely due to the allelic variation in *FLC* (Shindo et al., 2006). The loss-of-function alleles of *PHYC* and *PHYD*, gain-of-function of *CRY2* contribute to natural variation in photoperiod response, which is often associated with early flowering under short days, thereby reduced photoperiod response.

I.3 Perenniality and flowering control in woody perennials

Most studies facilitating our understanding in the mechanisms of plant development were carried out in the annual species, such as Arabidopsis, rice, wheat and barley, maize. In the past decades, there have been some efforts expended in studying flowering control in perennials, nonetheless small and slow progress was made. The main hindrances for genetic analysis lie in the extended length of juvenile phase and the usually large plant size in woody

perennials. The lack of availability of crossing varieties as well as difficulties with plant transformation further limit the research progress. Nonetheless, important progress has been made in underlying the flowering mechanisms in woody perennials, notably in strawberry, apple and rose within the Rosaceae family, in the tree species *Populus*, and in close relatives of Arabidopsis within the Brasicaceae family.

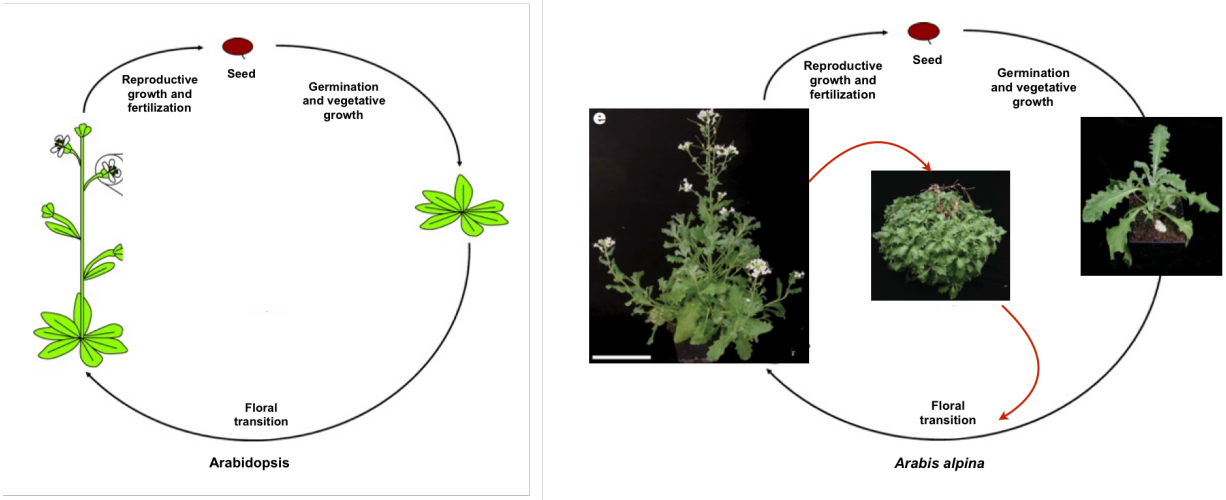


Figure I-7. Different flowering behavior contributes to the different life strategies of the annual Arabidopsis and the perennial *Arabis alpina*.

The life strategy of Arabidopsis, or an annual plant, is characterized by massive flowering followed by seeds production, whole plant senescence and death of the plant. *A. alpina*, or an perennial plant, however, has the ability to resume vegetative growth every year after flowering.

I.3.1 The evolution of annual and perennial life strategies

In annual species, the switch from vegetative to reproductive phase occurs only once in their life time, followed by senescence and death of the whole plant. In perennial species, however, plants cycle between periods of flowering and vegetative growth, thus being reproductive multiple times in their life time (Figure I-7). Although many nature ecosystems are dominated by polycarpic perennials, a large number of group of plants adopted both annual and perennial life strategies (Albani and Coupland 2010; André and Coupland 2012). For example, *Arabidopsis thaliana* and *Arabidopsis lyrata* are close relatives but adopted annual and perennial life strategy respectively. Similar variation in life strategies can also be found in the sister species of rice and maize. Therefore, in these groups mapping the major QTLs linked to perennials traits can be performed using the crosses between annual and perennial species. Indeed, populations generated by the crosses in rice revealed not only the unidirectional evolution from perennial to

annuals, but also the complex genetic basis of the evolution of life strategy (Grillo et al., 2009). The distinction between perennials and annuals have evolved many times in flowering plants. The fact that perenniality is ancestral to annuality is common in most species, which is supported by many phylogenetic studies (e.g. Datson et al., 2008). Nevertheless, shifts from annuals to perennials can also occur in some genera (Tank and Olmstead, 2008). Annual species are mostly distributed in areas which require fast reproduction and easy survival of seed banks. Therefore, the shift from perennials to annuals was likely a adaptive response to climate change during evolution (Datson et al., 2008).

In the annual plant *Arabidopsis*, the shoot apical meristem and all meristems forming axillary shoots flower. In perennials, the cycling between vegetative and reproductive phases is due to the different behavior of the meristems — while some undergo floral transition the others stay vegetative (Albani and Coupland 2010). Therefore, the meristems in a perennial plant hold varied reproductive competence — upon optimal environmental conditions only competent meristems perceive flower inductive signals (Albani and Coupland 2010). In contrast to annuals, the juvenile phase is extended up to multiple years in perennials. The transition from juvenile to adult phase is known to be regulated by microRNAs, similar as in annuals (Bergonzi et al., 2013). Flowering in perennials only occurs in adult phase, and is limited to a subset of axillary meristems so that vegetative growth is maintained (Albani and Coupland 2010).

I.3.2 Flowering control in *A. alpina* and other woody perennials

In comparison to the extensive study of florigen in Arabidopsis and annual crops, perennial flowering is complex and knowledge on the molecular regulation is still limited. Studies so far have focused on several distantly related species, such as poplar, citrus, tomato, apple and so on. Here I summarize and focus on the regulation of flowering in a few representative perennial species, with special emphasis on the new model plant *Arabis alpina*.

Arabis alpina (Alpine rock cress) is widely used as decorative plants in gardens. It belongs to the Brassicacae family, and Arabis comprises of 338 genera and 3,700 species (Koch et al., 1999; Warwick et al., 2006). Several characteristics of *A. alpina* make it a promising perennial model species for studying flowering time control and perenniality. First, being in the same Brassicacae family as *Arabidopsis*, *A. alpina* was diverged from *Arabidopsis* 20 million years ago. Most studies in modern plant biology were carried out in Arabidopsis and can be easily transferred to the studies in *A. alpina*. The knowledge obtained from studying *A. alpina* can be further applied to other Brassica species, which are more closely related to *A. alpina* than to Arabidopsis (Bailey et al., 2006). Second, *A. alpina* is diploid (2n=16) and self-compatible (Koch et al., 1999; 2000), which makes genetic analysis straightforward. The small genome size of *A. alpina* (392Mb) was fully sequenced and partially annotated (Willing et al., 2015) and the

absence of genome triplication suggests less redundant sequences, making it easier to study functions of individual genes (Johnston et al., 2005; Lysak and Lexer, 2006). Furthermore, more than 140 accessions of *A. alpina* have been collected from a wide range of habitats (Koch et al., 2006). Therefore, the genetic divergence among accessions makes map-based cloning strategy feasible. Finally, in contrast to most woody perennials, *A. alpina* has a relatively small plant size (40cm in height) and short life cycle (seed-to-seed in optimal conditions 6 to 8 months), making large scale genetic analysis durable both in the laboratory and in the greenhouse.

Most perennials flower seasonally, in which flowers occur only for a short period of time between vegetative growth phases, thus ensuring successful development seeds and fruit set before winter. Seasonal flowering plants require vernalization for floral initiation. Some perennials, on the other hand, flower as long as they perceive optimal environmental conditions, thus called perpetual flowering. Within the Rosaceae family several strawberry and rose species flower perpetually. In *A. alpina*, both seasonal and perpetual flowering accessions were collected from natural habitats (Albani et al., 2012). Recently, Wang et al. (2009) demonstrated that the floral repressor *PEP1*, a *FLC* orthologue, is the regulator of seasonal flowering. *A. alpina* wild plant *Pajares* requires vernalization to flower, whereas the *pep1* mutant flowers continuously without vernalization and without returning to vegetative growth. Therefore, *PEP1* plays an essential role not only in restricting the flowering to a short period, but also in regulating return to vegetative growth after flowering in *A. alpina*. This is correlated with the temporal expression pattern of *PEP1.* Instead of being stably repressed by cold as *FLC* as in Arabidopsis, *PEP1* mRNA abundance rises again after vernalization, thus suppressing flowering in shoots that were not induced to flower.

A further study by Wang et al. (2011) suggested that the homolog of the floral repressor *TFL1* in *A. alpina* has not only a conserved role as a floral repressor, but also regulates several perennial traits. When old *A. alpina* plants are exposed to vernalization, the reduction of *PEP1* expression results in up-regulation of *AaSOC1* and *AaLFY*, which further inducing flowering. However, in young *A. alpina* plants exposed to vernalization, *AaTFL1* blocks the flowering by inhibiting the expression of *AaLFY* (Wang et al., 2011). In a similar thought, the questions were addressed that whether homologs of *FLC* and *TFL1* have similar roles in regulating perennialism in *populus* trees. However, no functional orthologue of *FLC* were identified in poplar; and *CENTRORADIALISLIKE1* (*CEN1*), the *TFL1* orthologue in poplar, was then proposed to have similar roles in safeguarding SAM indeterminacy during dormancy (Ruonala et al., 2008).

Phylogenetic studies revealed numerous *FT* duplications in different plant species. Grouping of *FT*-likes from all division of plants, which belongs to the PEBP gene family members, showed that *FTs* are exclusively present in angiosperms (Karlgren et al., 2011; Pin and Nilsson 2012).

Therefore, *FT* may have led to the diversification of flowering plants on earth. Indeed, as a key integrator of multiple flowering pathways in Arabidopsis, the homologs of *FT* in a few woody perennials appear to have conserved functions as well as involvement in other responses that are important to perennialism such as bud setting.

The studies on *FT* on *populus* trees are the first ones underlying unexpected functions of *FT* apart from flowering regulation. In *Populus*, the FT-like genes, *PtFT1* and *PtFT2*, are involved in not only flowering regulation in adult trees (Böhlenius et al., 2006; Hsu et al., 2006), but also in growth cessation and bud set induced by SD condition (Böhlenius et al.2006; Hsu et al.,2011). Particularly, the over-expression of *PtFT1* reduced juvenile phase by initiating flower structures within 4 weeks in stems, in comparison to the normal flowering time of 8 to 20 years (Böhlenius et al.2006). Transcript profiling study confirmed that the two paralogues of *FT*, although very similar, exhibits distinct expression pattern. *PtFT1* is expressed in late winter when dormancy is released from chilling, whereas *PtFT2* is the predominantly expressed gene during vegetative growth (Rinne et al., 2011; Hsu et al., 2011; Pin and Nilsson 2012). Thereby *PtFT1* might be recruited by chilling in dormant buds to prepare for vegetative growth in following spring and be responsible for floral induction, while *PtFT2* for the vegetative growth control. Nevertheless, no mechanisms proving these hypotheses are identified so far. The interplay between *FT* paralogues in *Populus* suggests their distinct roles in controlling vegetative and reproductive growth cycles in woody perennials.

In addition to Popular, the *FT* homologs in citrus and in apple share roles in promoting flowering induction, suggesting the conserved function of *FT*-likes in these woody perennials (reviewed by Albani and Coupland 2010). One orthologue of *FT* in *A. alpina* was also found and the over-expression of *AaFT* in Arabidopsis confirmed its role in accelerating flowering (Wang, Dissertation, 2007). As *A. alpina* has a relatively long life cycle, the flowering acceleration was proposed to be induced by *FT* only in older plants, but being antagonized by the foral repressor *TFL1* in young plants in juvenile phase (Wang et al., 2011). Two other *FT*- or *TSF*-like genes were also identified, but their roles remain unclear (Wang, Dissertation, 2007).

All recent discoveries in perennials revealed the functional diversification of *FT* in comparison to annual plants. In addition, in potato, one of the *FT* homolog controls stolon- to-tuber meristem differentiation, whereas another *FT* homolog control flower regulation (Navarro et al. 2011). As the detailed study on *FT* and *TFL*-likes in perennials would strongly facilitate the possible customization of aspects of plant flowering, growth and yield from annuals to woody perennials, knowledge of how *FT*-likes function in *A. alpina*, the close perennial relative of *Arabidopsis*, would be rather important. So far, the function of all three *FT* homologs has not yet been studied in detail in *A. alpina*.

Taken together, the recent studies on flowering regulation in woody perennials showed the partially conserved function of *FT*-, *FLC*- and *TFL1*-like genes, as well as their new discovered roles in regulating the transition between juvenile and adult phase and perennialism.

Furthermore, in comparison to annuals, perennials usually have a longer juvenile phase before being competent to induce flowering. Recently, the aging pathway was also studied in detail in *A. alpina* by Bergonzi et al. (2013), in which they showed the increasing age and exposure to winter cold coordinate to establish competence to flower. In *Arabidopsis*, miR156 and miR172 are coupled to regulate the transition from juvenile to adult vegetative phase, as well as from vegetative growth to flowering (Wu and Poethig, 2006). Bergonzi et al. (2013) showed that miR156 decreases as plant ages, thus increasing expression of *SPL* genes to induce flower in response to cold. The age at which plants become sensitive to vernalization can be altered by manipulating the miR156 levels. Although the relationship of miR156 and miR172 was not observed in *A. alpina*, the low level of miR172 during vegetative growth allows high level of *PEP2* which suppresses flowering before vernalization. These findings indicated that miR156 and PEP2/PEP1 act in parallel repressive pathways to ensure the meristems of *A. alpina* be competent to flower only if plants reach a certain age and have been exposed to cold. *AaTFL1*, as mentioned earlier, also puts off the acquisition of age-related response to cold, likely by setting a threshold for *AaSPL* to activate flowering (Wang et al., 2011; Bergonzi et al., 2013).

I.3.3 Natural genetic variation in *A. alpina*

The studies on flowering and perennial growth in *A. alpina* mentioned previously were characterized only in the accession *A. alpina Pajares*, which was collected in the Cardillera Canta ‘brica mountain region of Spain. Despite the seasonal flowering *A. alpina* accessions, naturally occurring perpetual flowering accessions were also found and described (Albani et al., 2012). The difference in the flowering behavior in *A. alpina* natural accessions was conferred by the different PEP1 activity. Five of the accessions Tot, Dor, Wca, Cza and Mug flower perpetually due to loss of function alleles at *PEP1*. These accessions carry different lesions in the *PEP1* gene, indicating that the evolution of *PEP1* occurred independently. However, the natural variation in photoperiod response was not studied in detail in *A. alpina*. Further studies on the inter- and intra- species variation on flowering trait in *A. alpina* accessions are essential to understand the evolution of perennial traits.

II. Aims of this PhD thesis

i) Flowering regulation and photomorphogenesis in *A. alpina* accessions. There has been intensive research on the flowering pathways in the annual model plant Arabidopsis, but very limited studies were performed in perennial species. I, therefore, investigated the effect of day-length on flowering in different accessions of the new perennial model species *A. alpina,* with both physiological and molecular approaches. Aspects of the photomorphogenesis in *A. alpina* were also investigated, with particular focus on shade avoidance response. I performed, therefore, a detailed analysis of shade responses during the juvenile and adult stages of the perennial model species.

ii) The evolutionary conservation/ divergence of *COP1* and *SPA* genes involved in photomorphogenesis between *A. alpina* and Arabidopsis. The function of *COP1* and *SPA* genes was studied only in limited species, and has never been previously investigated in perennial species. Therefore, I examined the function of *A. alpina* COP1 and SPA1 homologs in Arabidopsis, by expressing the open reading frames of the *A. alpina* homologs in Arabidopsis *cop1* and *spa* mutants, respectively. To completely understand the role of *A. alpina* COP1 and SPA1 homologs, I attempted to generate mutants in *AaCOP1* and *AaSPA1* using amiRNA-mediated silencing as well as the CRISPR/Cas9 system. The photoreceptors homologs in *A. alpina*, phytochrome B in particular, was also sequenced and analyzed in comparison to Arabidopsis.

III. Results

III.1 Effect of day length on flowering in *Arabis alpina* perpetual flowering accessions and the *pep1* mutant

In the summer-annual accessions of *Arabidopsis*, LD condition accelerates flowering through a major component of florigen — the FT protein (Corbesier et al., 2007). Albani et al. (2012) identified one of the perpetual flowering accessions, *Arabis alpina* Dor, as a facultative long day plant, which flowers significantly earlier under LD than SD. In order to study the effect of photoperiod on flowering time in *A. alpina*, I examined the flowering responses in the *pep1* mutant, and perpetual flowering accessions *A. alpina* Wca, Dor and Tot.

III.1.1 *A. alpina* Wca plants are photoperiod-insensitive

The flowering time was expressed in terms of the number of days as well as the number of leaves produced on the primary stems upon flowering. As shown in Figure III-1, LD conditions accelerated flowering both in the *pep1* mutant and the Dor plants, whereas under SD, the *pep1* mutant and Dor plants did not flower. The Tot plants failed to induce flowering both under SD and LD conditions (data not shown). However, previous report (Albani et al., 2012) showed that Tot plants flower under LD condition. The different flowering behavior of Tot in both study is likely due to the different chamber conditions used. The Wca plants, interestingly, flowered with approximately 27 leaves regardless of the photoperiod, hence behaving as photoperiod-insensitive plants. In accordance with the flowering time observation in *Arabidopsis thaliana* (Arabidopsis) (Alonso-Blanco et al., 2009), these results indicate occurring natural variation among the *A. alpina* accessions.

III.1.2 The identification of ortholog(s) of *CO*, *FT*, *LFY* and *AP1* in *A. alpina*

Subsequently, I attempted to examine the expression of flowering time genes in *A. alpina* Wca and Dor, notably the homologs of *CO*, *FT*, *LFY* and *AP1*. Based on previous work (Wang, Dissertation, 2007), most if not all *A. alpina* plants in nature flower as facultative LD species. As Dor is a relatively well-characterized LD plant, it is used here as a reference accession for Wca.
In Arabidopsis, the CO protein accumulates upon exposure to LD conditions, which activates the downstream targets *FT and TSF* (André and Coupland 2012). *FT* integrates signals from multiple flowering pathways to induce the expression of the downstream floral meristem identity

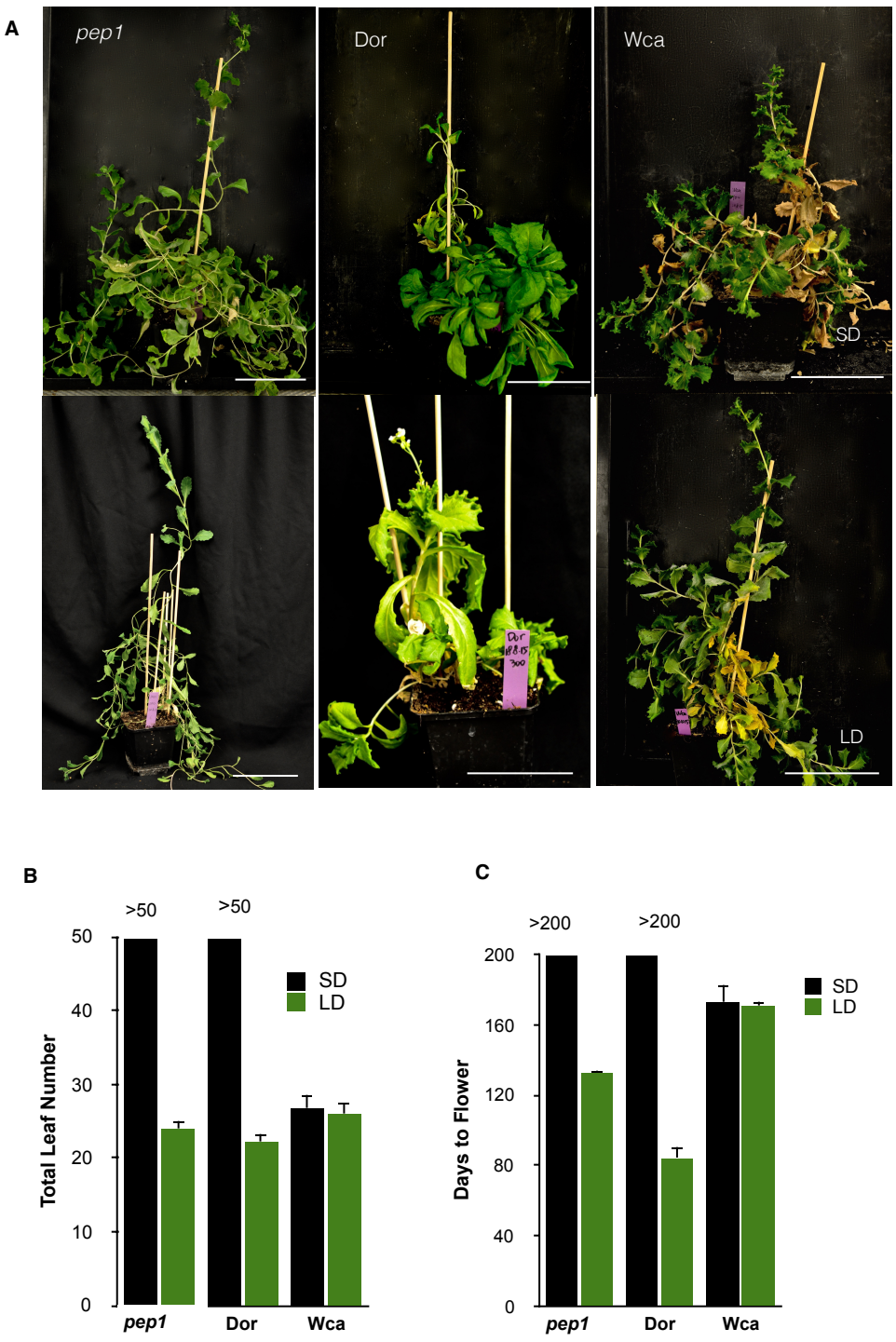


Figure III-1. Flowering time in *pep1*, Dor and Wca in response to day length (SD & LD).

(A) Visual phenotype of *A. alpina* accessions and the *pep1* mutant at flowering under LD condition, and 150 days under SD condition. (B) Flowering time expressed in terms of the total leaf number. (C) Flowering time expressed in terms of days to flower. Plants were grown in SD and LD chamber with 100 µmol/m²/s, 21°C. Data shown as mean of 10-15 plants ±SE. Bar = 9 cm.

genes *LFY* and *AP1* and flowering (André and Coupland 2012). If the orthologs of these genes have conserved functions in *A. alpina*, they would contribute to the phenotypic differences observed in Wca and Dor. To this end, I investigated the expression of the orthologs in Wca and Dor.

Based on the homology with *A. thaliana FT* (*AtFT*), we retrieved three homologs of *AtFT* with 91%, 83%, 81% identity, residing on chromosome 1, 7 and 3, respectively (Figure S1-2). All of them showed similar genomic organization to *AtFT* or *AtTSF*, consisting of 4 exons and 3 introns (Figure S1-2). Moreover, the synteny between *A. alpina AaFT* and Arabidopsis *AtFT* are conserved: a homolog of gene At1g65470, which resides immediately upstream of *AtFT* gene of Arabidopsis genome, was also found immediately upstream of *AaFT*. Wang (Dissertation, 2007) confirmed the function of the true ortholog *AaFT* (91% identity) as a flowering-promoter in Arabidopsis. The deduced amino acid sequences were compared for phylogenetic analysis (Wang, Dissertation, 2007), and two of the FT/TSF like proteins, which carry 176 and 175 amino acids, clustered together with AtTSF, thus were named as AaTSF1 and AaTSF2. So far very little is known about AaTSF1 and AaTSF2. I attempted to detect their mRNA expression level by RT-PCR in various tissues at various developmental stage in *A. alpina* Pajares (Paj), yet I was not successful. Therefore, I analyzed the ortholog *AaFT* for further experiments.

Genes showing high homology to *AtCO*, *AtLFY* and *AtAP1* were also retrieved from Basic Local Alignment Search Tool (BLAST) searches, and all of them showed similar genomic organization and conserved synteny (Figure S3). *AaCO* resides on chromosome 8 and encodes a 389-amino-acid BBOX and CCT domain protein, with 76% identity to AtCO which has 373 amino acids. AtCO acts as a network hub to integrate external as well as internal signals into the photoperiod flowering pathway, and the BBOX and CCT domains are essential for its stabilization, degradation and interaction with other proteins (Shim et al., 2016). Consisting of highly identical BBOX and CCT domains, AaCO might share similar roles in the photoperiod flowering pathway in *A. alpina*. The AaLFY protein consists of 417 amino acids, and is 86% identical to AtLFY with 420 amino acids. The expression of *AaLFY* in shoot apical meristem of *A. alpina* Paj was analyzed in detail by Wang et al. (2011), where they showed *AaLFY* acts also as a marker for the floral transition in *A. alpina*. *AaAP1* encodes a 256 amino acids MADS domain protein, which is highly identical (96%) to AtAP1. Taken together, the high sequence similarity of AaCO, AaLFY and AaAP1 with their counterparts in Arabidopsis suggests their possibly conserved roles in *A. alpina*.

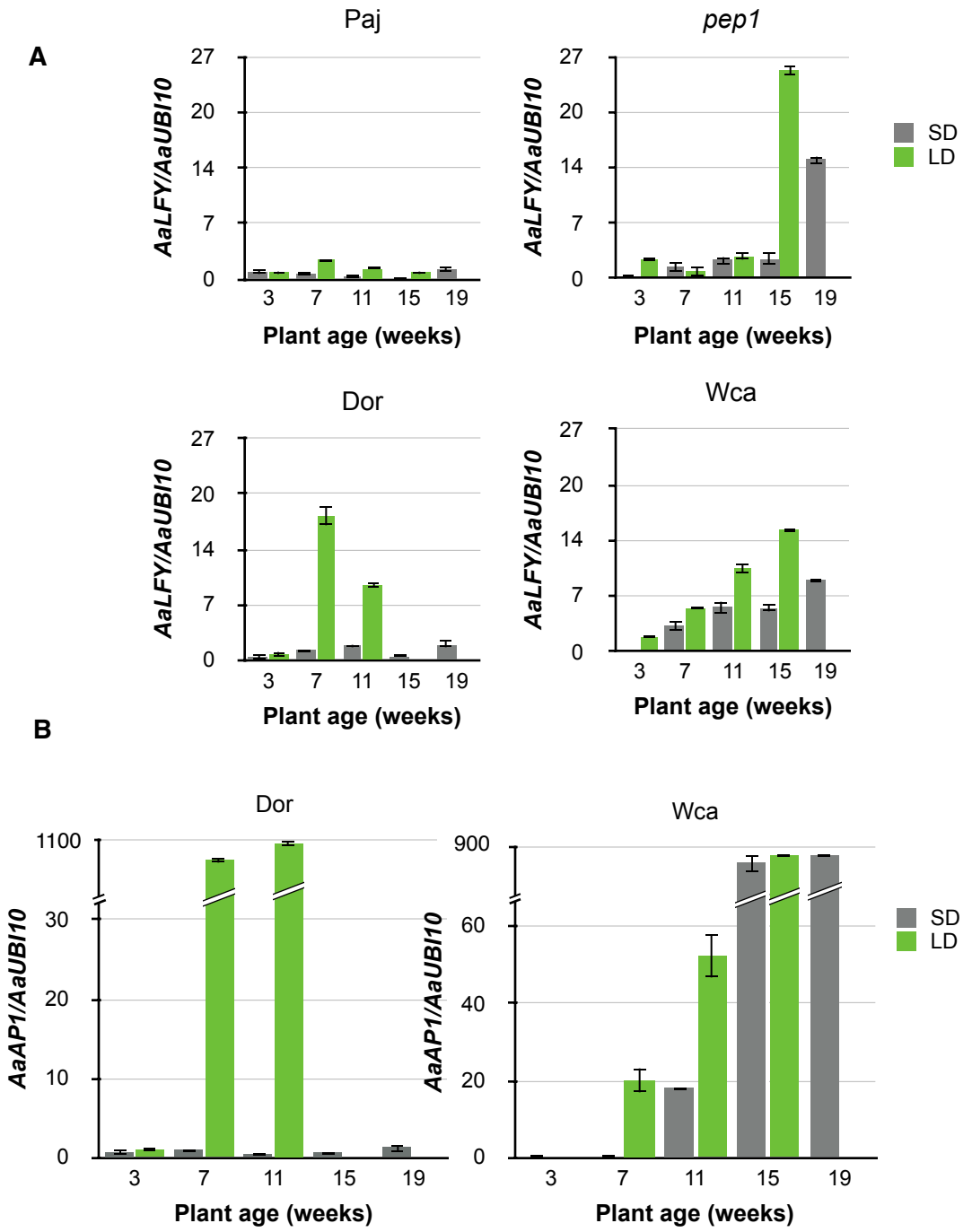


Figure III-2. The expression of *AaLFY* (A) and *AaAP1* (B) in *A. alpina* accessions and the *pep1* mutant.

Relative transcript levels of *AaLFY* (A) and *AaAP1* (B) were determined by qRT-PCR. Plants were grown in SD or LD conditions for 3 to 19 weeks before the shoot apices were excised and harvested. Shoot apices from 10 to 15 plants were pooled. *AaUBI10* was used as the normalization gene. Data were calibrated to the 3-week-old time point under SD condition for both genes. Data represent mean±SE of one biological replicate and two technical replicates each.

III.1.3 The expression of *AaLFY*, *AaAP1* in the shoot apices support the phenotyping results

The wild type *A. alpina* Paj, two accessions Wca and Dor, and the *pep1* mutant were grown for 3 to 19 weeks under SD and LD conditions, before the shoot apices were excised and sampled

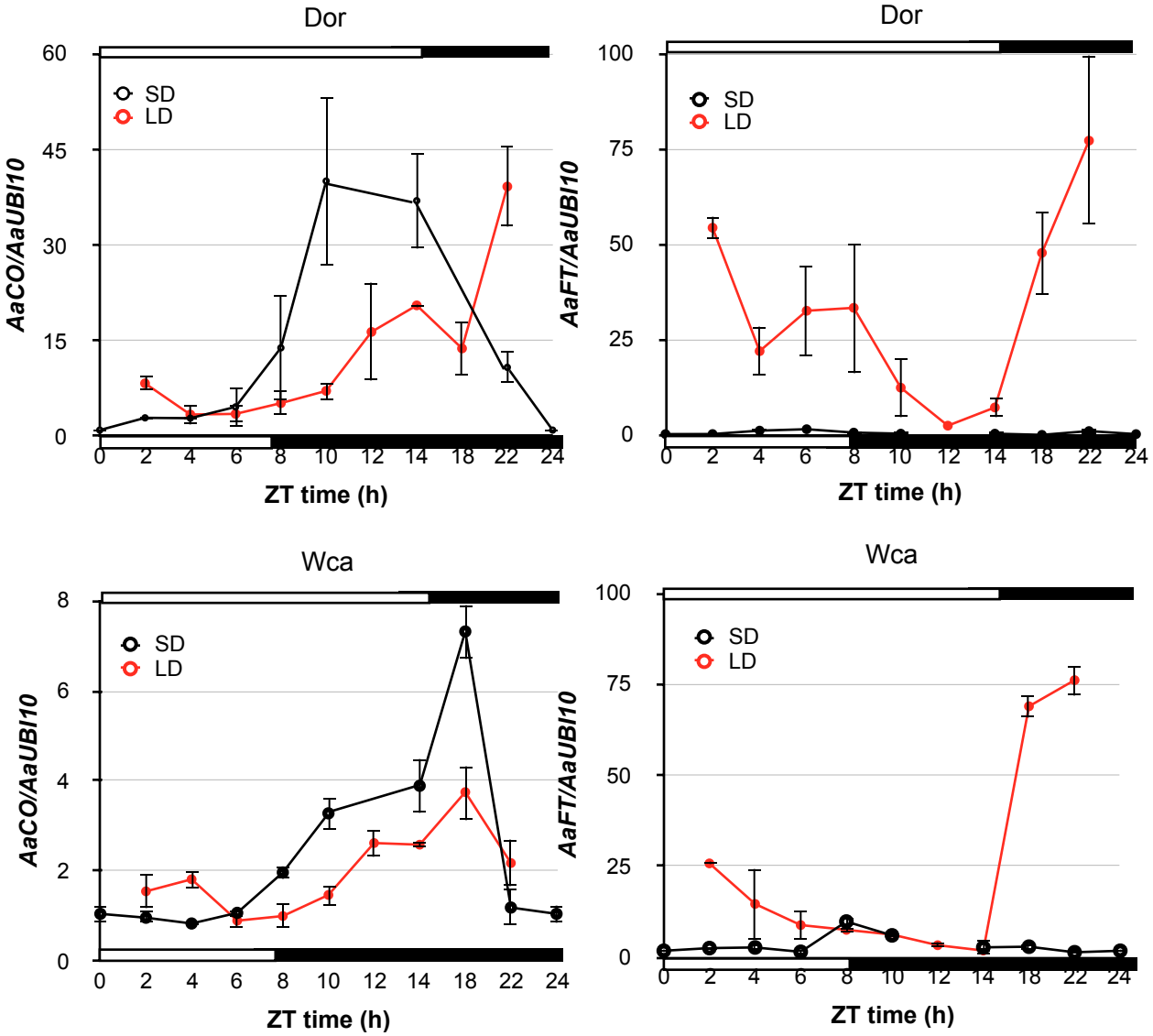


Figure III-3. Diurnal rhythmic expression patterns of *AaCO* and *AaFT* in *A. alpina* Dor and Wca, under SD and LD conditions.

Relative transcript levels of *AaCO* and *AaFT* were determined by qRT-PCR. 4-week-old whole plants were harvested at the indicated ZT time. Plants were grown in SD and LD chambers under 100µmol/m²/s, 21°C. *AaUBI10* was used as the normalization gene. Data were calibrated to ZT0 of SD for each gene. Data shown as mean±SE of three biological replicates and two technical replicates each.

(Figure III-2). Subsequently, the transcript levels of floral meristem identity genes *AaLFY*, and *AaAP1* were determined by qRT-PCR analysis in the shoot apical meristem.

In the wild type Paj, which flowers only when exposed to vernalization, the expression of *AaLFY* was constantly low irrespective of the day-length. Under SD condition, *AaLFY* mRNA abundance was low in Dor, or until very late stage (19 weeks) in the *pep1* mutant. Under LD, the expression level of *AaLFY* peaked at 7th week for Dor and 15th week for the *pep1* mutant. These data are in accordance with the observation of the flowering time. In Wca, interestingly, starting from 7th week, the transcript levels of *AaLFY* in the shoot apices under both SD and LD conditions were already similarly up-regulated. Although differences exists in the *AaLFY* mRNA abundance in the following time points, the up-regulation of *AaLFY* were consistent under both conditions in Wca (Figure III-2).

The results were further confirmed by the expression of a second floral meristem identity gene *AaAP1.* In Paj, as expected, the transcript level of *AaAP1* was beyond detection. In Dor, *AaAP1* mRNA abundance was similar as that of *AaLFY*, only induced by LD condition starting from the 7th week. From 11th week on, in Wca plants, the expression of *AaAP1* was significantly high in both SD and LD conditions. This time course experiment suggested that both the LD-flowering Dor and the photoperiod-insensitive flowering of Wca can be confirmed and supported by the expression of floral meristem identity genes.

III.1.4 The diurnal rhythmic expression patterns of *AaCO* and *AaFT* were similar irrespective of the distinctive flowering phenotype in Dor and Wca

In Arabidopsis and many other species, *CO* and its homologs were found to be key links between the circadian clock and control of flowering, and the transcription of *AtFT* and its homologs correlated with floral induction (Suárez-López et al. 2001, reviewed by André and Coupland 2012). In the photoperiod-insensitive, perennial plant Tomato, four *FT*-like genes were uncovered: *SFT(SINGLE FLOWER TRUSS)*, *SlSP5G*, *SlSP5G2* and *SlSP5G3*. SFT were shown to be a floral inducer (Molinero-Rosales et al. 2004) whereas all the other three FT-like proteins function as floral inhibitors (Cao et al. 2016). The expression pattern of the *FT*-like genes were found to be controlled differently by photoperiod and they might act antagonistically to regulate the floral initiation in Tomato (Cao et al. 2016).

To investigate the relationships of *AaCO* and *AaFT* with the different flowering behavior in Dor and Wca, I examined their diurnal expression patterns under different photoperiod. To this end, qRT-PCR analysis was performed using 4-week-old whole plants grown in a LD diurnal cycle (16 hour light) or a SD diurnal cycle (8 hour light). As shown in Figure III-3, the expression of *AaCO* showed a broad peak between ZT10 and ZT22 both under LD and SD conditions,

although the peak under LD was narrower than under SD. In comparison to the peak of *AaCO* mRNA under SD which only occurred in darkness, the transient peak of *AaCO* at the end of day under LD possibly led to production of AaCO protein, which might further activated *FT* transcription. The diurnal rhythm in *AaCO* mRNA abundance in Wca followed a similar pattern. Therefore, AaCO in both accessions displayed a photoperiod response rhythm (Figure III-3).

Under LD, the expression of *AaFT* in Dor plants peaked from ZT2 to ZT8, and ZT18 to ZT22, confirming its characteristic LD-promoting flowering habit. At dusk and during the night (ZT10-

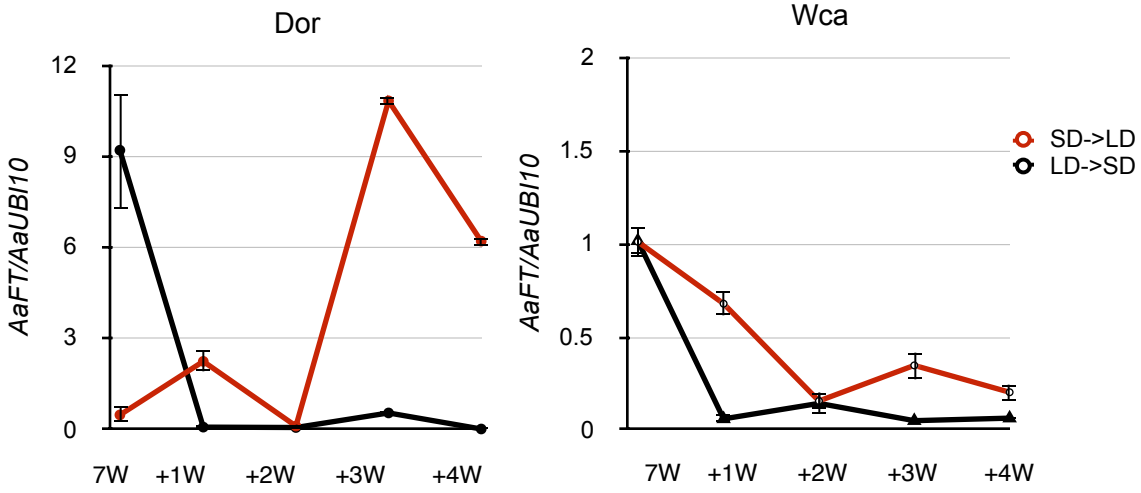


Figure III-4. Regulation of *AaFT* transcript levels in *A. alpina* Dor and Wca plants grown under shifted SD or LD conditions.

Relative transcript levels of *AaFT* were determined by qRT-PCR. Dor or Wca plants were grown in SD or LD conditions for seven weeks (7W), before shifted to LD or SD for another one to four weeks (+1W, +2W, +3W, +4W). Leaves were harvested at ZT8 from both SD and LD conditions. Plants were grown in SD and LD chamber under 100µmol/m²/s, 21°C. *AaUBI10* was used as the normalization gene. Data were calibrated to 7W old leaves under SD condition for both accession. Data shown as mean±SE of three biological replicates and two technical replicates each.

ZT24), Wca *AaFT* mRNA abundance under LD exhibit similar pattern as in Dor. During the day (ZT4-ZT8), however, Dor exhibited higher transcript level of *AaFT* in comparison to Wca. Regardless of the difference, *AaFT* mRNA abundance in Wca plants were still significantly higher in LD than in SD condition.

Under SD, the expression of *AaFT* was low in Dor plants, whereas a peak of *AaFT* expression at ZT8 & ZT10 was observed in Wca plants. The level was about 5-fold higher than ZT0, and as high as that of plants grown under LD at the same time point. Taken together, the diurnal expression pattern of *AaCO* and *AaFT* in Dor indicated its conserved photoperiod response rhythm. Interestingly, in Wca plants, the expression of *AaFT* peaked at dusk under SD as high as LD, and the pattern was similar as what was observed in *phyB* mutant in Arabidopsis under

SD (Cerdán and Chory 2003). Nevertheless, the general expression pattern of *AaFT* in Wca under SD and LD were similar as that in Dor; thus the *AaFT* expression per se may not be, at least solely, the reason for the photoperiod-insensitive flowering phenotype observed.

III.1.5 *AaFT* mRNA abundance in *A. alpina* Wca dropped after day-length shift

From the time course experiment of *AaLFY* and *AaAP1*, we propose that the floral induction begins when Wca plants are around seven weeks old. In Arabidopsis, FT is made in the companion cells of the leaves and is transported to the meristem to promote activation of floral

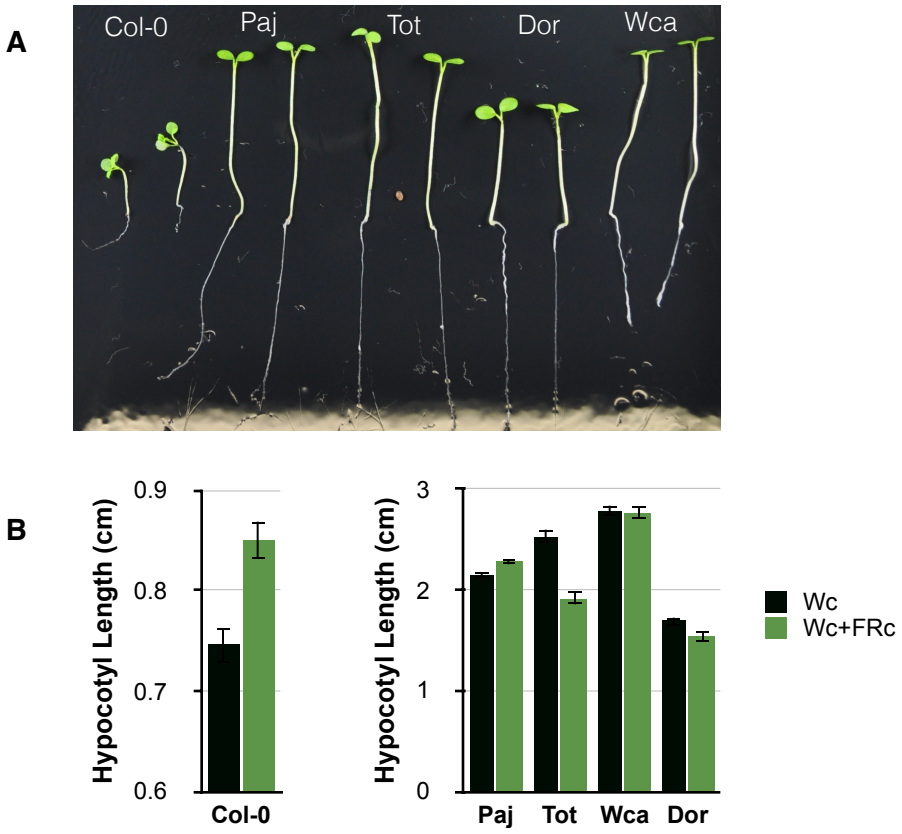


Figure III-5. SAS in two-week-old seedlings of *A. alpina*.

(A) Shade phenotype of two-week-old seedlings grown on black MS media. Within each pair of seedings of one accession, seedlings grown in continuous white light (Wc) are on the left, seedlings grown in continuous white light supplemented with far-red light (Wc+FRc) on the right. *A. alpina* seedlings were grown in Wc for one week, half were moved to Wc+FRc while the other half kept in Wc. Plants were grown in chamber with PAR of 50µmol/m²/s, R:FR= 9.6 (Wc), 21°C, or 50µmol/m²/s, R:FR= 0.25 (Wc +FRc), 21°C. (B) Hypocotyl length of seedlings in Wc and Wc+FR. Data represent mean±SE of > 20 seedlings each.

meristem identity genes *AtLFY* and *AtAP1* (Wigge et al., 2005). Assuming that this mechanism is conserved in *A. alpina*, the expression of *AaFT* should be detectable in the leaves of seven-week-old plants. Therefore, the expression level of *AaFT* was examined by shifting seven-week-old Dor and Wca plants from LD to SD for 4 weeks, and vice versa. Due to chamber space limitation, seven-week-old control plants which were supposed to remain in unchanged SD or LD conditions were not included in this experiment.

As shown in Figure III-4, in Dor plants, down-regulation of *AaFT* was apparent and remained low as early as one week after plants had been transferred from LD to SD. The transfer of Dor plants from SD to LD resulted in a direct increase in *AaFT* expression, even more significantly in the third and fourth week. This result indicate that the expression of *AaFT* in Dor plants was directly regulated by day length.

In Wca plants, the expression level of *AaFT* was very similar in both LD and SD conditions at the ZT8 time point before being shifted. Interestingly, the shift of plants to either SD or LD both down-regulated *AaFT* in Wca. At the second week of day-length shift, *AaFT* mRNA abundance dropped to similar level again under both conditions. Thereafter, in the third and fourth week of day-length shift, the expression of *AaFT* increased slightly under LD but still significantly low in both conditions. This experiment needs repetition with plants constantly grown in SD and LD condition as control. Nonetheless, it indicated that factors other than *AaFT*, such as *AaTSFs*, or unknown factor(s) playing antagonistic role to *AaFT*, might be involved differently under SD or LD conditions between the two accessions, which will be discussed in the discussion section.

III.2. Analysis of shade avoidance syndrome in *A. alpina* accessions

While the shade avoidance syndrome (SAS) has been well studied in Arabidopsis, very limited research has been conducted on perennial species. Here, I used the *A. alpina* accessions mentioned previously to study the SAS in *A. alpina*.

III.2.1 Two-week-old *A. alpina* does not respond to low R:FR, except for the accession Tot

The simulated shade conditions employed in this study consisted of continuous white light (Wc) supplemented with additional continuous FR light (Wc+FRc), resulting in low R:FR ratio in comparison with the Wc light condition alone, but an unchanged PAR. Experiment set up was performed as described in Rolauffs et al. (2012), and always with Arabidopsis wild type Col-0 as a control. I investigated the SAS in two-week-old *A. alpina* seedlings, by growing them in Wc for seven days and subsequently shifting them to low R:FR for additional seven days, while a

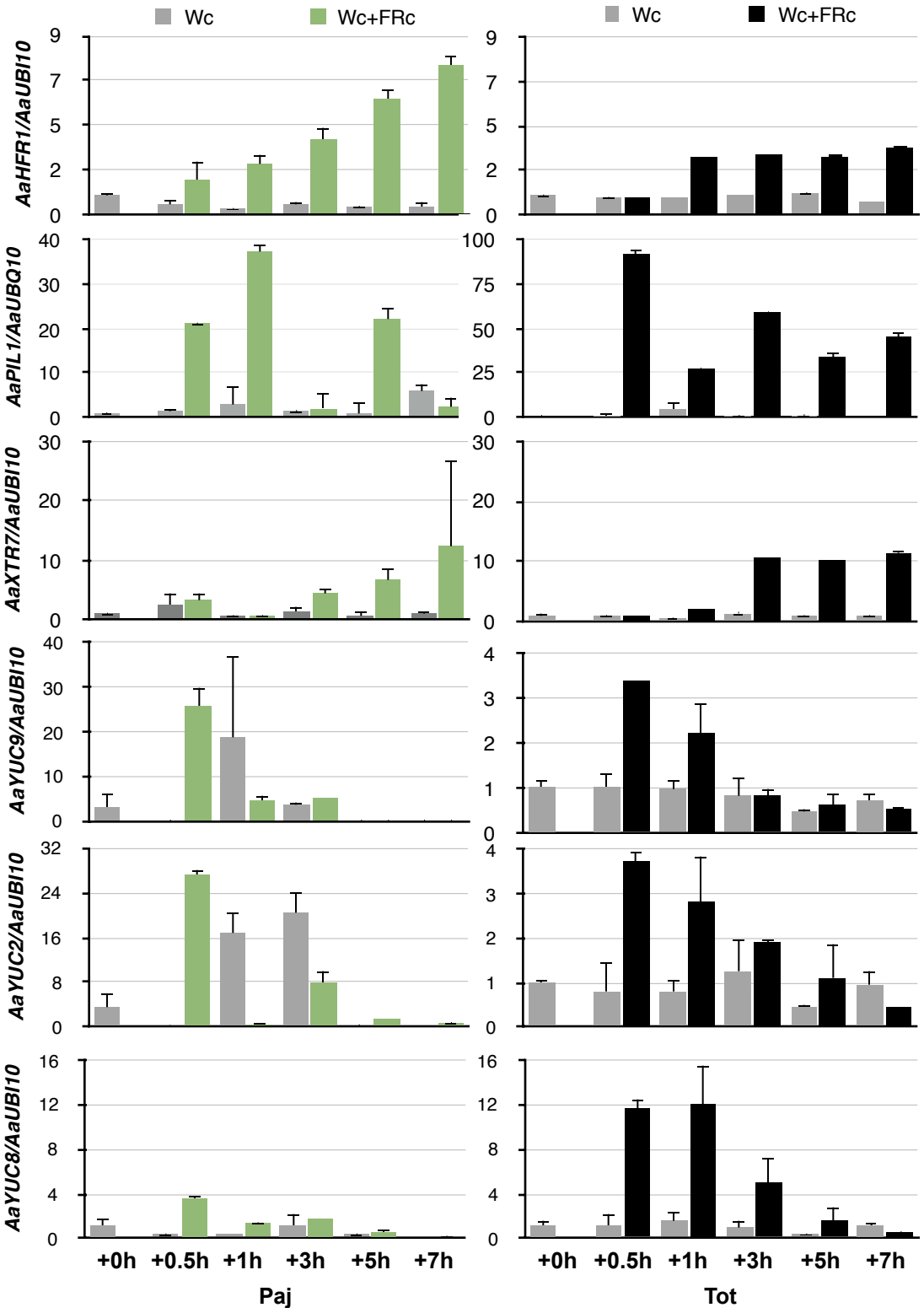


Figure III-6. Expression of early shade marker genes and auxin biosynthesis genes in response to simulated shade in two-week-old *A. alpina* seedlings.
Transcript levels of *AaHFR1*, *AaPIL1*, *AaXTR7*, *AaYUC9*, *AaYUC2* and *AaYUC8* were analyzed by qRT-PCR. Seedlings were grown in Wc for one week, half was moved to Wc+FRc for 30min (0.5h) to 7hours (7h), while the other half was kept in Wc. Plants were grown in chamber with continuous PAR of 50µmol/m²/s, R:FR= 9.6 (Wc) or 50µmol/m²/s, R:FR= 0.25, 21°C. *AaUBI10* was used as the normalization gene. All data were calibrated to the 0hr Wc sample of Paj or Tot. Data represent mean +SE of three biological replicates with two technical replicates each.

second set was kept in Wc. The two-week-old session was determined due to the later germination and larger seedling size of *A. alpina* in comparison to Arabidopsis, which typically is phenotyped in six-day-old seedlings.

A. alpina Paj, together with Wca and Dor, exhibited similar hypocotyl length under Wc and Wc +FRc, suggesting that they were unable to respond to simulated shade condition (Figure III-5). The accession Tot, however, responded to the low R:FR treatment with reduced hypocotyl length, exhibiting about one third shorter hypocotyl length when compared to the seedlings grown under Wc.

The reduced hypocotyl length in response to low R:FR treatment in Tot was similar as what observed in the *phyB* mutant in Arabidopsis (Smith et al., 1997). To confirm whether the SAS response in Tot was due to a loss-of-function mutation in its *AaPHYB* gene, the *AaPHYB* was amplified and sequenced from Tot, as well as from the wild type Paj as a reference. The sequence alignment revealed four nucleotide changes, which resulted in one amino acid change from Valine (Val) in Paj into Isoleucine (Ile) in Tot at position 731 in the signal-sensing PAS domain (Figure S5). This change was found common in other relatives of the Brassicaceae family (Figure S7), and was not reported formerly to be critical for *AtPHYB* functionality. Therefore, it is not likely that the SAS observed in Tot was due to any changes in its *AaPHYB* gene function. Another hypothesis for the low R:FR treatment-induced reduction of hypocotyl length in Tot was that it might be a *PHYA*-hypersensitive plant. This hypothesis had to be rejected after performing the fluence rate response curve under red and far-red condition, which was further explained in Section 3.1.1 (Figure III-9).

Taken together, the different responses of *A. alpina* accessions to simulated shade indicated natural variation of *A. alpina* seedlings in response to shade.

III.2.2 Transcript analysis of the homologs of shade marker genes and auxin biosynthesis genes

In Arabidopsis, the early shade marker genes, which are swiftly up-regulated by shade conditions, include transcription factors *ATHB-2*, *PIL1 (PHYTOCHROME INTERACTING FACTOR LIKE1)* and *HFR1 (LONG HYPOCOTYL IN FAR-RED LIGHT)* (Lorrain et al., 2008), genes encoding enzymes that are involved in cell wall modification, such as *XTR7/XTH15* and a set of auxin responsive genes (Sessa et al., 2005; Roig-Villanova et al., 2006,2007; Hornitschek et al., 2012). As loss of shade-induced hypocotyl responses was observed in *A. alpina* Paj, and *phyB*-mutant-like phenotype was observed in Tot, I therefore investigated the expression of the homologs of early shade marker genes and auxin biosynthesis genes in these two accessions. To this end, the homologs of *AtHFR1*, *AtPIL1*, *AtXTR7*, *AtYUC9, AtYUC2, AtYUC8* were

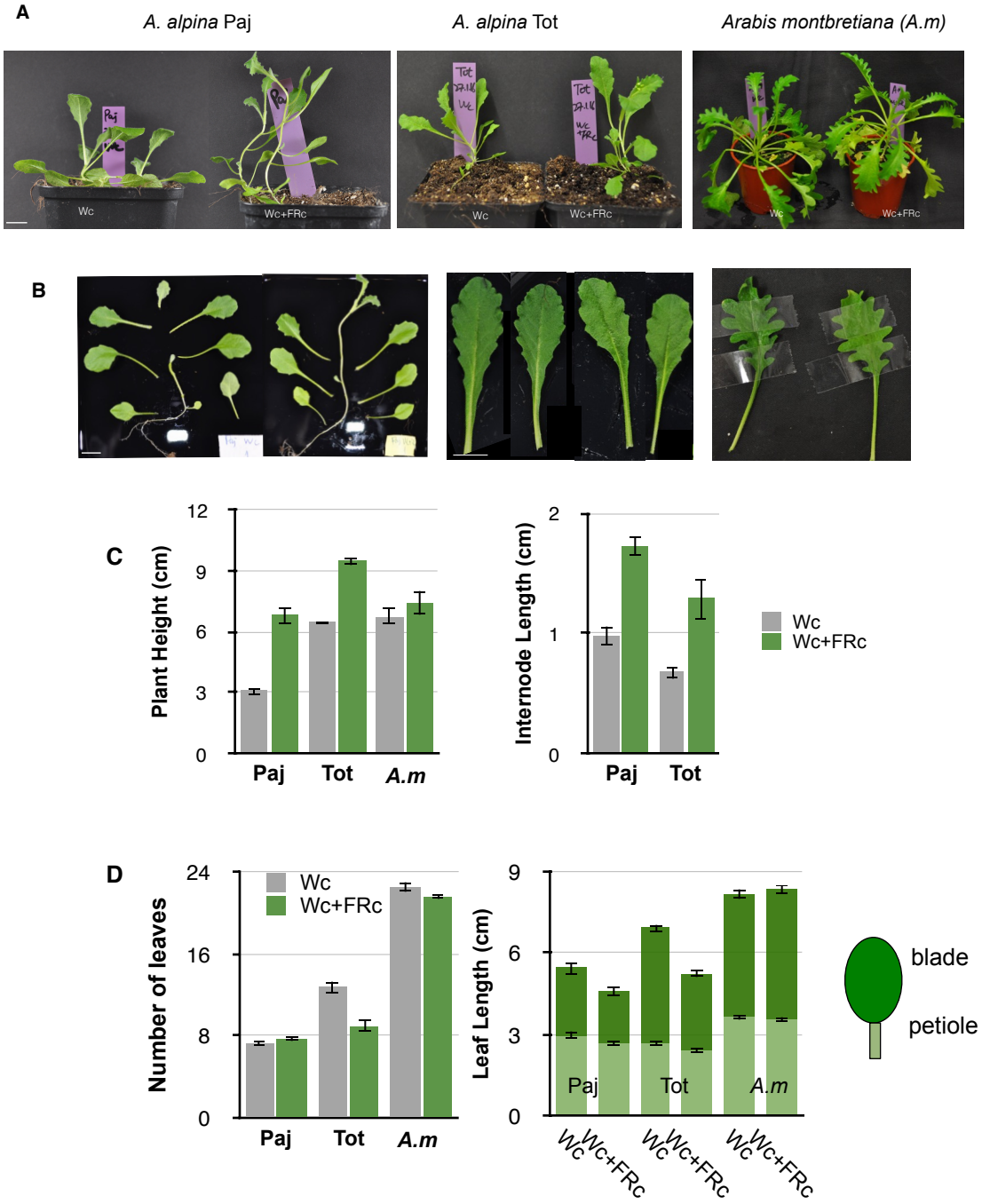


Figure III-7. SAS in eight-week-old *A. alpina* and *Arabis montebretiana* (*A.m*) adult plants.
(A) Shade phenotype of eight-week-old adult plants of *A. alpina* Paj and Tot, *A.montbretiana*. Plants were grown in Wc for one week, half were moved to Wc+FRc while the other half was kept in Wc. Growth condition in chamber with continuous 50μmol/m²/s, R:FR= 9.6 (Wc) or 50μmol/m²/s, R:FR= 0.25, 21°C. Bar = 1cm. (B) Shade phenotype of eight-week-old leaves. Leaves of adult plants grown in continuous white light (Wc) are on the left, leaves of adult plants grown in continuous white light supplemented with far-red light (Wc+FRc) on the right. The fifth and sixth leaves of Tot were shown, the 12th leaf of *A.m* were shown. Bar = 1cm. (C) Quantification of plant height and internode length. Data represent mean±SE. (D) Number of leaves of eight-week-old plants (>10 plants were measured for each accession). The leaf length (petiole and blade) were quantified by averaging the first 6 leaves for Paj, the first 8 leaves for Tot, and the eighth and twelfth leaves for *A. m*. Data represent mean±SE.

identified in *A. alpina* by BLAST searches. All their gene homologs in *A. alpina* showed similar genomic organization and conserved synteny as their counterparts in Arabidopsis (Figure S4). Seedlings were grown for seven days in Wc and were subsequently shifted to Wc+FRc (low R:FR) conditions or kept in Wc for the indicated time; subsequently the transcript levels of shade marker genes and auxin biosynthesis genes were determined (Figure III-6).

A significant up-regulation of *AaHFR1*, *AaPIL1* and *AaYUC9* was observed both in *A. alpina* Paj and Tot as early as 30 minutes after the onset of the shade treatment, which was the earliest time point tested. The cell wall modification gene *AaXTR7* was up-regulated after three hours of shade treatment for Paj, while after one hour for Tot. However, commonly for the shade marker genes *AaHFR1*, *AaPIL1* and *AaXTR7*, the up-regulation was constant until the last time point examined (seven hours).

The transcript level of the auxin biosynthesis gene *AaYUC9* was rapidly up-regulated by low R:FR. Nonetheless, starting from the 3rd hour of low R:FR treatment, the expression *AaYUC9* decreased to the same or even lower level as Wc both for Paj and Tot. Similarly, the low R:FR-induced rapid up-regulation of *AaYUC2* and *AaYUC8* was followed by their reduced levels at subsequent time points (Figure III-6). Among these, the expression *AaYUC2* was decreased as early as 1h after low R:FR treatment in Paj, and 3h in Tot. *AaYUC8* was also rapidly up-regulated upon exposure to low R:FR, and decreased to similar levels as Wc at 3h in Paj, and 7h in Tot. Surprisingly in Wc, the expression of the *AaYUC2* and *AaYUC9* genes first increased (until 3h), then decreased in Paj. The transcript level of *AaYUC8* in Paj was however low. The expression of all the *AaYUC* genes in Tot, in Wc condition, was also constantly low.

Combining the results together, early shade marker genes *AaHFR1*, *AaPIL1* and *AaXTR7* were strongly up-regulated by shade treatment in Paj and Tot, irrespective of the lack of the elongation of hypocotyl observed in shade. The transcript levels of auxin biosynthesis genes, *AaYUC9*, *AaYUC2* and *AaYUC8*, however, were down-regulated in shade at the later time point examined and might be related to the phenotype observed.

III.2.3 Eight-week *A. alpina* responded to low R:FR with internode elongation

In comparison to the annual Arabidopsis, as a perennial species, the developmental stage of *A. alpina* consists of a prolonged juvenile phase and adult phase. Therefore the question was addressed, whether the observation of *A. alpina* lacking a SAS response is only representative of the response in the juvenile phase. To this end, I further investigated the response in eight-week-old *A. alpina* adult plants, including the close annual relative *A. montbretina*. The experiment set up was the same as mentioned in section 2.1, with *A. alpina* plants kept until

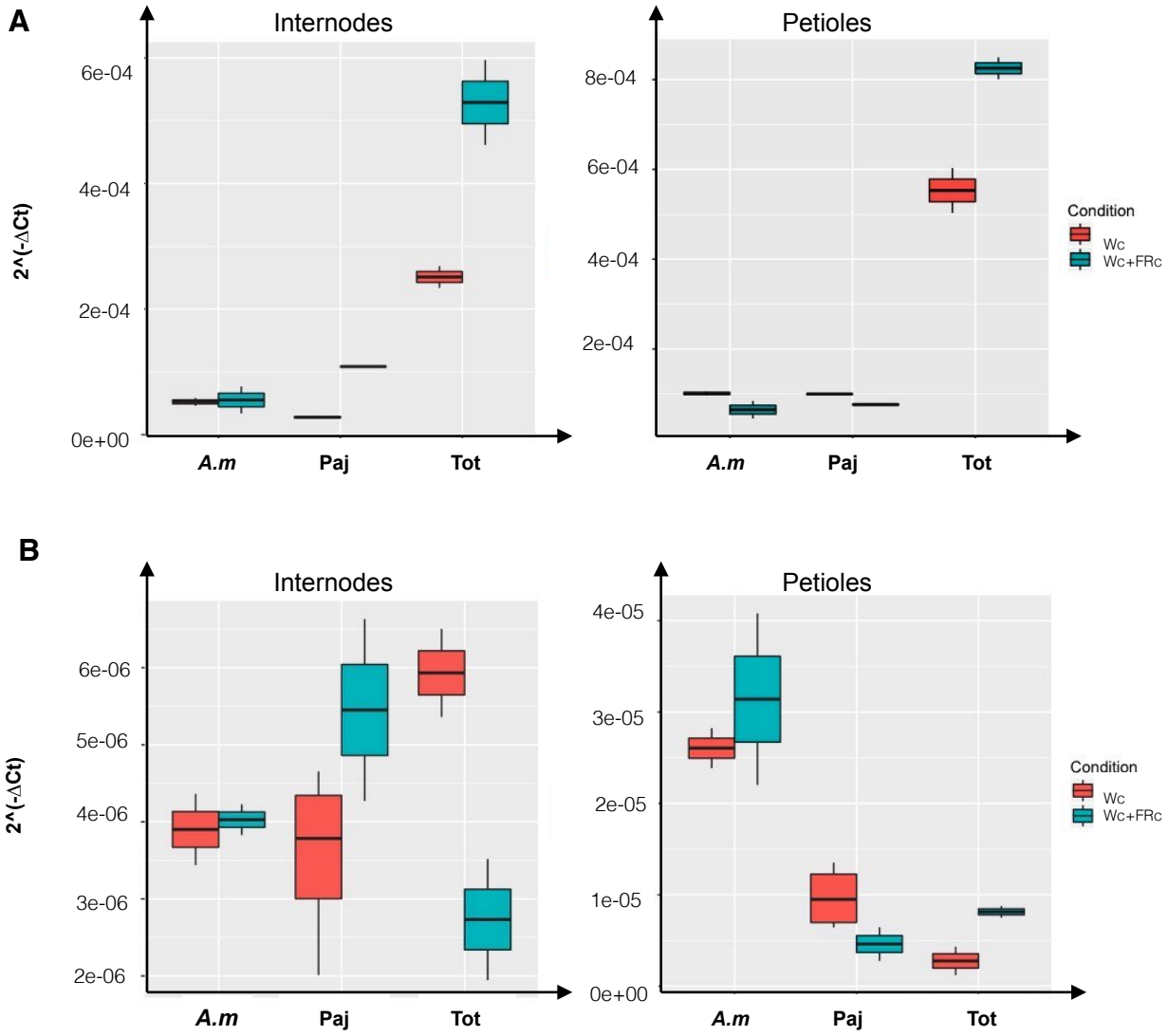


Figure III-8. Relative expression level of auxin biosynthesis gene *AaYUC9* in the internodes and petioles of eight-week-old *A. alpina* and *A. m* adult plants.

Transcript levels of *AaYUC9* were analyzed by qRT-PCR. Plants were grown in Wc for one week, half was moved to Wc+FRc while the other half was kept in Wc for additional seven weeks. The internodes or petioles were harvested at 8th week for analysis. (A) and (B) are two independent experiments. Growth condition in chamber with continuous 50μmol/m²/s, R:FR= 9.6 (Wc) or 50μmol/m²/s, R:FR= 0.25, 21°C. Data represent mean±SE of two biological replicates (A) and three biological replicates with two technical replicates (B). ΔCt value was plotted. Graph generated with R ggplot.

eight weeks old in Wc or Wc+FRc conditions. The SAS in eight-week-old *A. alpina* was expressed in terms of plant height, internode length and petiole length. As shown in Figure III-7A, simulated shade triggered elongation responses in eight-week-old Paj and Tot , but not in their annual relative *Arabis montbretina* (*A. m*), expressed by plant height or internode length

(Figure III-7C). The number of leaves recorded at eight weeks indicated that the plants in Wc or in Wc+FRc were growing at similar speed (Figure III-7D).

Similar as Arabidopsis, the leaf of *A. alpina* and *A.m* consists of a blade (or lamina) and a petiole (Figure III-7D). When Arabidopsis is exposed to simulated shade, the petiole of the rosette leaf is elongated at the expense of blade expansion (de Wit et al., 2015). In *A. alpina* Paj and Tot, however, the simulated shade had no significant effect on the elongation of the petiole (Figure III-7D), but clearly reduced the length of the blade (Figure III-7D). In the annual relative *A.m*, the average length of the eighth and twelfth leaves showed unaltered response to low R:FR.

Furthermore, seven-week-old Tot showed accelerated flowering in response to low R:FR. This was not observed in Paj, which requires vernalization to flower, or in eight-week-old *A.m*. Taken together, this experiment indicates that eight-week-old adult *A. alpina* Paj and Tot did respond to simulated shade, at least by showing elongation response in the internodes.

III.2.4 Transcript analysis of auxin biosynthesis gene *AaYUC9* in *A. alpina* Paj, Tot and *A. montbretiana*

Auxin levels are elevated in Arabidopsis along with the triggered elongation response by low R:FR conditions (Tao et al., 2008; Won et al., 2011). Also the elevation of auxin levels are likely due to up-regulation of *YUC* genes that acts downstream of *TAA1*. As the expression of *AaYUC2*, *AaYUC8* and *AaYUC9* was found likely to correlate with the phenotype of two-week-old *A. alpina* seedlings, we subsequently hypothesized that the elongation response in eight-week-old *A. alpina* was in correlation with the expression of the auxin biosynthesis genes. To this end, I examined the transcript levels of *AaYUC2*, *AaYUC8* and *AaYUC9* in the internodes (stems) and petioles of *A. alpina* eight-week-old plants.

As shown in Figure III-8, the difference in expression of *AaYUC9* was compared among all tested accessions. Since we had two conditions (Wc, Wc+FRc) with three accessions, with the objective to explore the differential expression of *AaYUC9* between the pair-wise combinations of these conditions; therefore, no reference sample was used and the ΔCt value was plotted (instead of $\Delta\Delta$Ct for log fold-change). The expression of *AaYUC9* in the stems and petioles of the annual relative *A.montbretiana* was not altered by low R:FR treatment. By contrast, in the internodes of Paj, an obvious higher expression of *AaYUC9* was triggered by low R:FR. The expression level of *AaYUC9* was similar in Wc and Wc+FRc in the petioles of Paj, with a slight decrease in Wc+FRc. The levels of *AaYUC9* in the internodes of Tot showed two contrasting regulation trends in two independent experiments, making it difficult to draw any conclusions on it. Therefore, a third repetition experiment is necessary. In the petioles of Tot, however, the

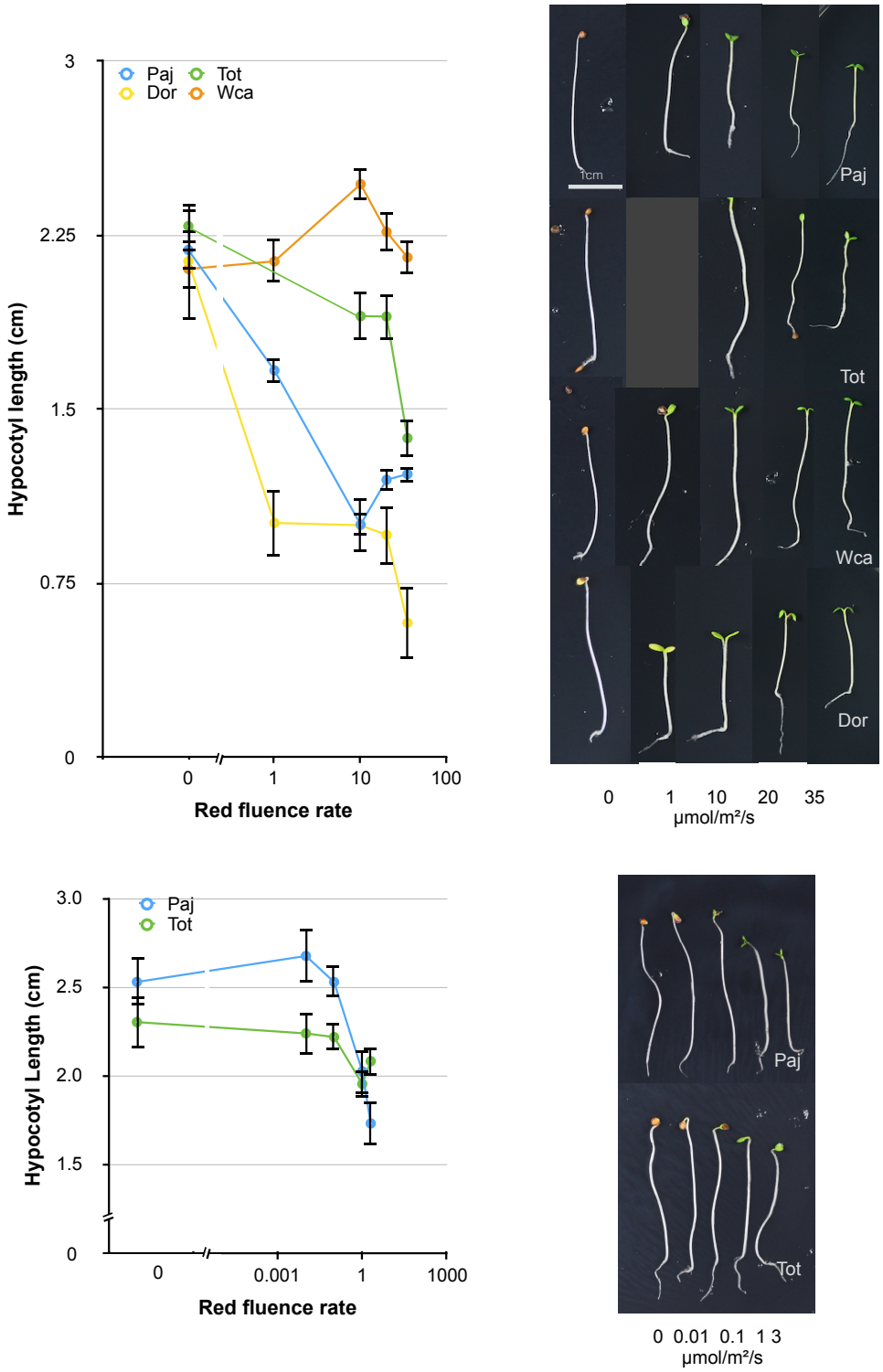


Figure III-9. Fluence rate curve of *A. alpina* accessions under red light.

Hypocotyl length (A) and visual phenotype (B) of Paj, Tot, Dor and Wca seedlings grown in red light. Hypocotyls length (C) and visual phenotype of Paj and Tot seedlings grown in low fluence rate of red light. Seedlings were grown in different fluence rates for 8 days. Data represent mean±SE of >20 seedlings.

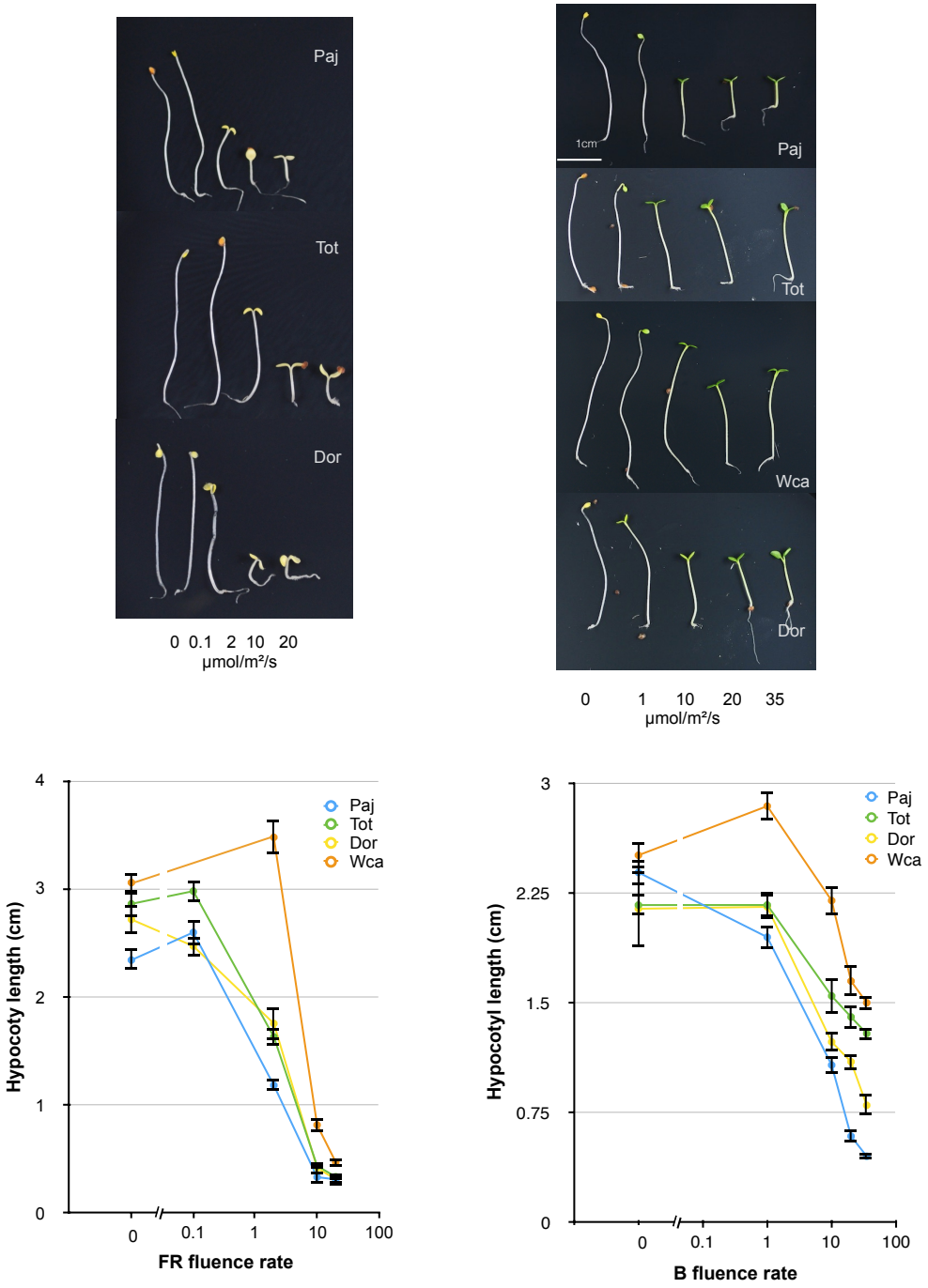


Figure III-10. Fluence rate curve of *A. alpina* accessions under far-red and blue light.

(A, C) Visual phenotype and hypocotyl length of *A. alpina* seedlings grown in far-red light. (B, D) Visual phenotype and hypocotyl length of *A. alpina* seedlings grown in blue light. Seedlings were grown in different fluence rates for 8 days. Data represent mean±SE of >20 seedlings.

expression of *AaYUC9* was relatively high in Wc+FRc, although the petiole length was not promoted by low R:FR (Figure III-7C). The expression level of two other auxin biosynthesis genes *AaYUC2* and *AaYUC8* was also examined in the internodes and petioles. Their expression, however, was hardly detectable in these tissues under both conditions and was not used for further analysis. Taken together, the transcript levels of auxin biosynthesis gene *AaYUC9* can be correlated to the SAS responses observed in *A. alpina* Paj and *A.m*. Nonetheless, more experiments are required to explain the contrasting shade phenotype and *AaYUC9* expression in *A. alpina* Tot.

III.3 Light signal transduction in *Arabis alpina*

Light signal transduction has been well-studied in the model plant Arabidopsis. Downstream of the light-sensing photoreceptors, light signal transduction in Arabidopsis is mediated via highly complex transcriptional regulatory networks. In this study, I attempted to approach part of the networks in *A. alpina,* by examining the roles of phytochrome B, as well as the E3 ubiquitin ligase COP1 and SPA1.

III.3.1 The role of photoreceptors in *A. alpina* accessions

The blast searches against *A. alpina* genome revealed highly conserved photoreceptors (Figure S8). *AaPHYA* showed similar genomic organization and conserved synteny as *AtPHYA,* the deduced protein sequence was also highly identical (96%) (Figure S8). Aacry1 showed 56% identity to cry1 in Arabidopsis (Figure S8). The question was therefore addressed, whether differences exists in the perception of light by photoreceptors in *A. alpina* accessions. To this end, the fluence rate response curve was performed under red, far-red and blue light.

As shown in Figure III-9, in red light, which reflects mainly the function of phytochrome B in *A. alpina*, the degree of responsiveness among the accessions was different. *A. alpina* Tot and Dor responded to increasing red light with arrested hypocotyl growth, comparable to the response in the wild type *A. alpina* Paj. In Wca, however, the effect of red light on the elongation of hypocotyl was likely abolished, and Wca appeared to be insensitive to different doses of red light.

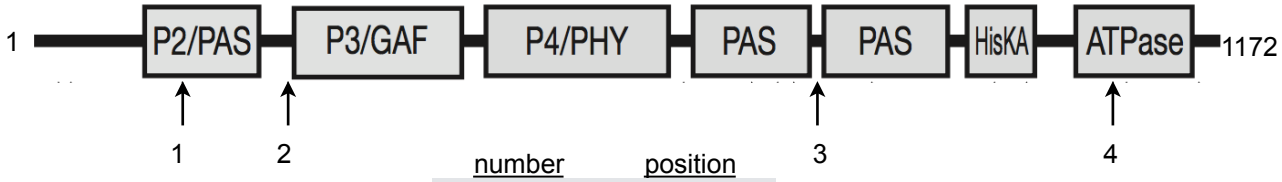
As mentioned in 2.1.1, the shade-induced inhibition of hypocotyl growth in two-week-old Tot seedlings was hypothesized to be a consequence of itself being a phyA-hypersensitive plant. This hypothesis can be easily tested by a very low fluence rate response (VLFR) curve in red light, which was known to be regulated by phyA in Arabidopsis (Casal et al., 2013). The mechanism is that under very low red light, only a small portion of phyA is transformed into the active Pfr form, which was enough to accumulate in the nucleus, and to cause inhibition of

hypocotyl growth. If Tot was a phyA-hypersensitive plant, we would expect a stronger reduction of hypocotyl length in comparison to the wild type Paj under the same fluence rate. As shown in Figure III-9, the length of hypocotyl was not significantly different under low fluence rate in both accessions, thus confirming the hypothesis to be false.

In far-red and blue light (Figure III-10), the responsiveness among all accessions was not significantly different, displaying deceasing hypocotyl length with increasing fluence rate. Taken together, it is likely that among all the *A. alpina* accessions tested, the function of phyA (far-red light) and cryptochromes (blue light) was conserved compared to Arabidopsis as well as among each other. Wca appeared to be insensitive in response to different red fluence rate, at least within the range tested in this study.

III.3.2 Identification of the phytochrome B (phyB) homolog in *A. alpina*

Blast searches against *A. alpina* Paj genome retrieved one homolog of *AtPHYB* with 91% genomic sequence identity, named as *AaPHYB*. The conserved synteny between *AaPHYB* and *AtPHYB* was also found, although at further than 150kb up- and downstream of *AaPHYB* (Figure S6). The ORF of *AaPHYB* was subsequently amplified and sequenced. The alignment of the deduced AaphyB protein sequence with AtphyB showed their high identity (95%), but also revealed four amino acid changes in AaphyB (Figure III-11 & Figure S9). Three of amino acid changes in AaphyB were previously found to be the polymorphisms differentiating the Arabidopsis accession Ler and Cvi, and reported to be linked to phyB function (Filiault et al.,



number	position
1*	I143L
2*	A247S
3	G766A
4*	L1071S

Figure III-11. Amino acid changes in *A. alpina* phyB in comparison to Arabidopsis phyB.
A schematic diagram of AaphyB domains is shown, with arrows indicating each amino acid change. The table below indicates the specific amino acid change. The amino acid on the left of the position number indicates the residue in the reference genome from Arabidopsis Col-0, whereas on the right of the position number indicates the residue in *A. alpina* Paj. * represents the amino acid changes which were also found to be the polymorphisms differentiating the Arabidopsis accession Ler and Cvi (Filiault et al., 2008). Protein structure analysis by SMART, see Figure S5.

2008). In addition, the amino acid change found in the PAS domain (G766A) of AaphyB was reported previously to be essential for its nuclear localization (Bai & Choi 2008).

On the other hand, the accession Wca showed strongly reduced sensitivity to red light - which reflects the function of phyB - in comparison to all the other *A. alpina* accessions examined (Figure III-9). I subsequently investigated whether sequence variation occurs in the *AaPHYB* of Wca, in comparison to Paj. Although the sequence amplification was not finished, the 3'-end of *AaPHYB* in Wca, at least, was very conserved in comparison to *AaPHYB* in Paj (Figure S10).

III.3.3 The interaction of AaphyB with AaSPA1 by Yeast Two-Hybrid analysis

I used yeast-two-hybrid analysis to examine if an interaction between AaphyB with AaSPA1 occurs and if the four amino acid changes in AaphyB would possibly result in altered protein function. Yeast is unable to synthesize the naturally occurring chromophore of plant phytochromes, phytochromobilin. Therefore, I used phycocyanobilin (PCB) extracted from cyanobacteria as a substitute, as described by Sheerin et al. (2015). The identification of the *AaSPA1* gene from *A. alpina* is described in the next section. The premise behind the Yeast-Two-Hybrid system is the activation of downstream reporter gene by the binding of the

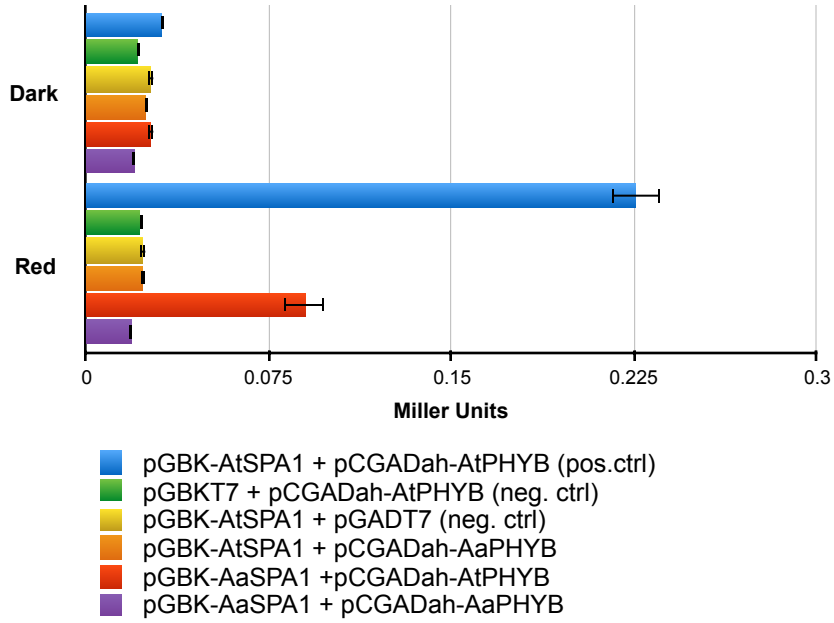


Figure III-12. Interaction of AaphyB with AaSPA1 by yeast-two-hybrid analysis.
AtSPA1 and AaSPA1 are fused to the biding domain (BD) and AtphyB and AaphyB are fused to the activating domain (AD). Empty vectors expressing BD and AD are served as negative controls. ONPG assay for 200 mins. Data represent mean±SE of three biological replicates, four technical replicates.

transcription factor onto an upstream activated sequence. The transcription factor in Yeast-Two-Hybrid system is split into two fragments: the binding domain (BD) and the activating domain (AD). In this study, the BD was fused to AtSPA1 or AaSPA1 (pGBK-AtSPA1, pGBK-AaSPA1), and the AD was fused to AtphyB or AaphyB (pCGADAH-AtphyB, pCGADAH-AaphyB) to investigate the interaction between these proteins.

As Figure III-12 shows, the interaction of AtSPA1 and AtphyB in red light could be easily verified by Yeast-Two-Hybrid using the ONPG assay. The interaction of AaSPA1 with AtphyB was also detected in red light, although it is relatively weak compared to the positive control. As the auto-activation of AtphyB in this system was already analyzed (Sheerin et al. 2015), it is unlikely that the interaction of AaSPA1 and AtPHYB was due to the auto-activation of AtphyB. Surprisingly, AaphyB can interact neither with AtSPA1 nor AaSPA1 in this analysis.

III.3.4 Identification of *SPA* and *COP1* homologs in *Arabis alpina*

Arabidopsis *SPA* genes encode a group of plant specific proteins, which act together with COP1 as a complex to repress light signaling (Hoecker 2005). The functions of *COP1* and *SPA* genes have been described in detail in Arabidopsis, the dicotyledonous plant (Laubinger et al., 2004; Hoecker, 2005; Laubinger et al., 2006). Their homologs in non-flowering moss *Physcomitrella patens* and the monocotyledonous rice were also analyzed by Ranjan et al. (2014). However, very little is known about the *COP1* and *SPA* homologs in perennial species. The next section

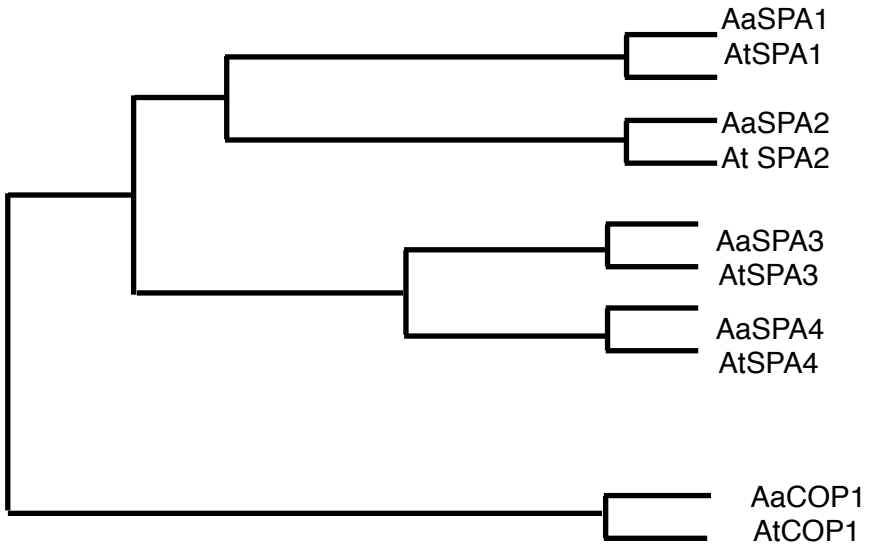


Figure III-13. Phylogenetic tree showing the relationship among the COP1 and SPA proteins of Arabidopsis and *A. alpina.*
The relationship is based on comparison of full-length protein sequences using the CLUSTAL W program.

describes the identification of the *COP1* and *SPA* homologs in *A. alpina*, the investigation of their transcript regulation by light, and the functionality of the homologs in Arabidopsis.

To identify the homologs of *A. alpina SPA1* and *COP1* in *A. alpina*, BLAST searches against *A. alpina* genome databases was performed, using full length Arabidopsis *COP1* (*AtCOP1*) and Arabidopsis *SPA1* (*AtSPA1*) cDNA sequence. The database search retrieved one homolog *AaSPA1* with 87% identity, and one homolog *AaCOP1* with 95% identity, respectively (Figure S11, S12). The cDNA of *AaSPA1* and *AaCOP1* were amplified with specific primers and sequenced before any further analysis. Furthermore, the protein sequences of AaCOP1 and AaSPA1 showed a higher degree of conservation at the C-terminus (protein structure analysis by SMART http://smart.embl-heidelberg.de), but exhibit relatively low sequence similarity at the N-terminus. The homologs for *SPA2*, *SPA3* and *SPA4* were also retrieved with high sequence similarity (Figure S13).

Figure III-13 illustrates the phylogenetic relationship among the SPA and COP1 proteins from Arabidopsis and *A. alpina*. The identical number of SPAs and COP1 between *Arabidopsis* and *A. alpina* suggests their possible conserved functions as light signaling intermediates, between annual and perennial species within the Brassicaceae family.

III.3.5 *A. alpina* SPA1 is functional in Arabidopsis

In Arabidopsis, the *SPA* genes regulate seedling development, leaf size and photoperiodic flowering with redundant and specific functions (Laubinger et al., 2004; 2006). *AtSPA1,* in particular*,* predominantly regulates seedling photomorphogenesis and photoperiodic flowering with a significant contribution to leaf size regulation (Hoecker 2005; Balcerowicz et al., 2011).

In order to address the evolutionary conservation of SPA1 function between annual and perennial species within the Brassicaceae family, the cDNA of *AaSPA1* was placed under the control of the Arabidopsis *AtSPA1* endogenous promoter and subsequently introduced into the segregating *spa1 spa2 spa3 spa4/SPA4* mutants. For further protein detection in the transgenic plants,the 3xHA tag was added to the sequence, both for *AtSPA1* and *AaSPA1*. The resulting transgenic plants in the T1 generation were selected for either homozygous *spa1spa2spa3* or *spa* quadruple mutants. The transgenic plants in T2 generation were further analyzed for the complementation of *spa* mutant phenotypes.

The *spa1 spa2 spa3* mutant has a similar but weaker defect in suppressing photomorphogenesis in the dark and in the light as the *spa* quadruple mutant (Laubinger et al., 2004; Fittinghof et al., 2006); therefore, the phenotype analysis of AaSPA1 complementation in T2 was performed both in the *spa1 spa2 spa3* and in the *spa* quadruple background. As shown in Figure III-14A, transgenic *spa1spa2spa3* seedlings expressing *AtSPA1::cAaSPA1* exhibited, like wild-type seedlings, long hypocotyls and closed cotyledons, indicating that AaSPA1 was

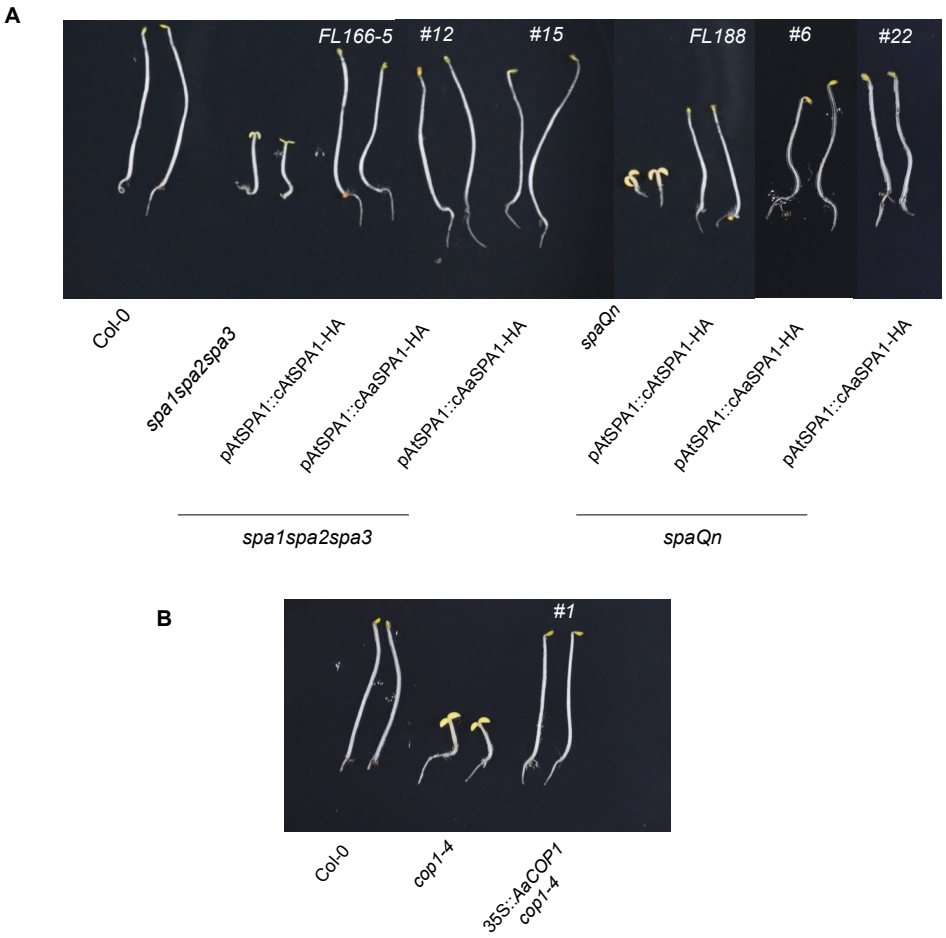


Figure III-14. *A. alpina* SPA1 and COP1 can complement the seedling phenotype of Arabidopsis *spa1spa2spa3, spaQn* mutant, and *cop1-4* mutant, respectively.

(A) Visual phenotype of transgenic T2 *spa1spa2spa3* and *spaQn* seedlings carrying *pAtSPA1::cAaSPA1* construct. Four-day-old dark-grown seedlings of two representative transgenic line for each transgene, along with wild- type Col-0, *spa1 spa2 spa3* and *spaQn* seedlings are shown. (B) Visual phenotype of four-day-old dark-grown transgenic T2 *cop1-4* seedlings carrying *35S:AaCOP1*.

fully functional under *AtSPA1* promoter in Arabidopsis. Two *spaQn* mutants carrying *AtSPA1::cAaSPA1* were selected from the T1 generation, the T2 seedlings grown in darkness also fully complemented the dwarf phenotype of *spaQn* (Figure III-14A).

In adult plants, the leaf shape in three of the T2 transgenic *spa1spa2spa3* lines expressing *AtSPA1::cAaSPA1* mimic that of *A. alpina* Paj, by showing curly leaf edges observed only in Paj vegetative plants, but not in *Arabidopsis* wild type Col-0 (Figure III-15A). Furthermore, The *spaQn* transgenic line expressing *AtSPA1::cAaSPA1* showed full complementation of the plant size of the phenotype of the *spaQn* mutant (Figure III-15B).

III.3.6 AaCOP1 is functional in Arabidopsis

In order to address the evolutionary conservation of COP1 function between annual and perennial species within the Brassicaceae family, the cDNA of *AaCOP1* and - as a control - *AtCOP1,* was placed under the control of the 35S constitutive promoter and introduced into the hypomorphic *cop1-4* mutant. While the *cop1* null mutant is lethal, the *cop1-4* mutant remains fertile and viable, and produces a truncated COP1 protein retaining the N-terminal part with coiled-coil domain. The seedling phenotype of transgenic *cop1-4* plants expressing *AtCOP1* or *AaCOP1* was analyzed in the T2 generation.

The *cop1-4* mutant shows constitutive photomorphogenesis in darkness by displaying reduced hypocotyl length and open cotyledons (McNellis et al., 1994).Transgenic seedlings expressing *35S:AaCOP1* fully etiolated in darkness and resembled the wild type phenotype (Figure III-14B).

Figure III-15. *A. alpina* SPA1 complement the adult phenotype of Arabidopsis *spa1spa2spa3* and spaQn mutant.

(A) Visual phenotype of three adult transgenic T2 *spa1spa2spa3* expressing *pAtSPA1:cAaSPA1* constructs. In two of the transgenic line, the leaf shape mimic that of A. alpina vegetative leaf shape. (B) Visual phenotype of one transgenic T2 *spaQn* expressing *pAtSPA1:cAaSPA1,* AaSPA1 was able to fully complement the dwarfed plant size of *spaQn*.

Therefore, *AaCOP1* was able to fully complement the seedling phenotype of the *cop1-4* mutant in darkness. This experiment indicates that AaCOP1 is fully functional in Arabidopsis seedlings. In addition, the *cop1-4* mutant adult plants are small and dwarfed, with accelerated flowering in comparison to the wild type (McNellis et al., 1994). Transgenic *AaCOP1* and *AtCOP1 cop1-4* mutant lines in T2 generation were similar as the wild type in leaf size (Figure in preparation). Hence, *AaCOP1*, like *AtCOP1*, can fully complement the dwarfed *cop1-4* mutant phenotypes.

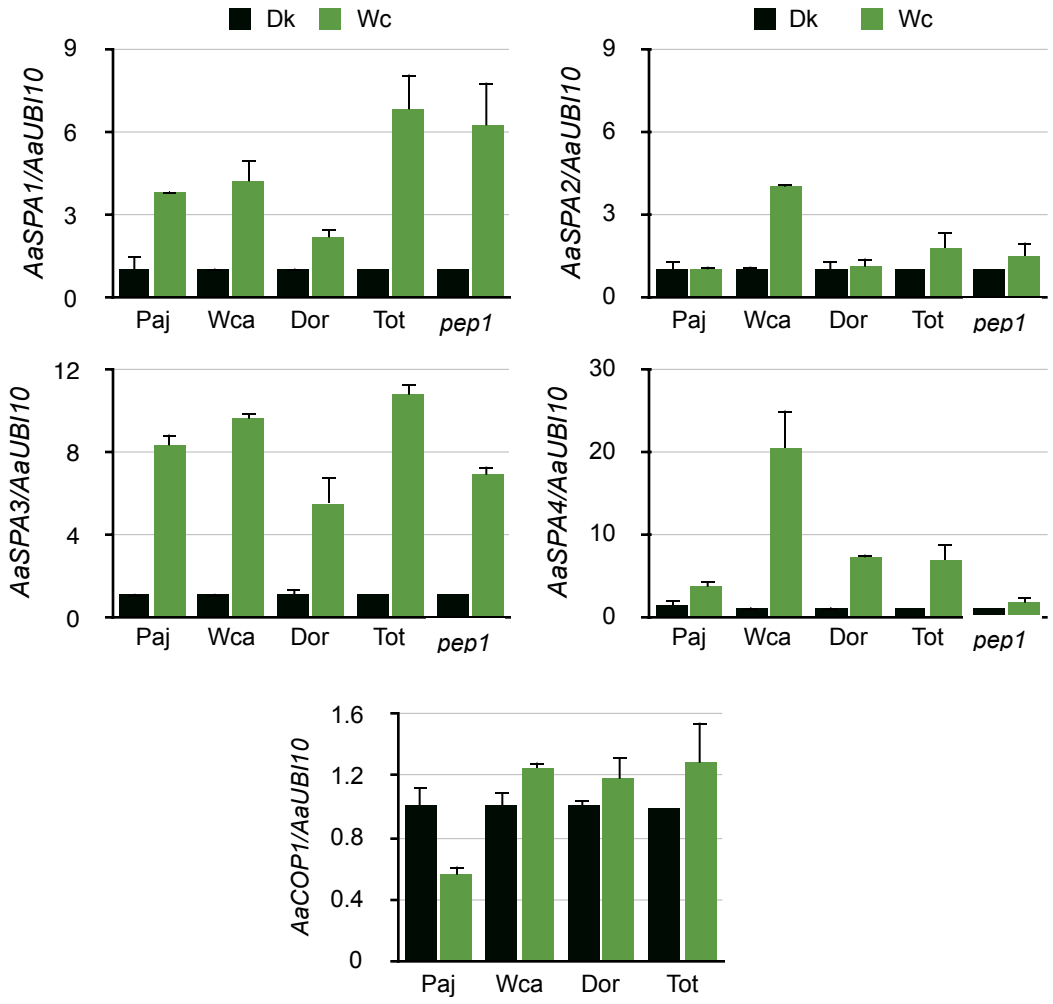


Figure III-16. Transcript analysis of *AaSPA1*, *AaSPA2*, *AaSPA3*, *AaSPA4, AaCOP1* in the wild type Paj, accessions Wca, Dor, Tot and the mutant *pep1*.

Seedlings were grown under darkness (Dk) or white light (Wc) (50µmol/m²/s) for eight days before harvested. *AaUBI10* was used as the normalization gene. Data represent mean±SE of three biological replicates with two technical replicates.

III.3.7 Light-regulation of transcript level of *AaSPAs* and *AaCOP1* in *A. alpina* accessions

In Arabidopsis, the four *SPA*s have overlapping yet distinct functions in light- and dark-grown seedlings. The transcript levels of *AtSPA1*, *AtSPA3* and *AtSPA4* are increased by light, correlating with their function in regulating seedling development (Fittinghof et al. 2006). The function of *AtSPA2*, on the other hand, is mainly restricted to seedling development in darkness (Fittinghof et al. 2006). Therefore, we asked how the transcript levels of the four *AaSPA* homologs and *AaCOP1 in A. alpina* are regulated by light. To this end, total RNA were extracted from dark-grown seedlings as well as light-grown seedings from the wild type *A. alpina* Paj, the *pep1* mutant, and two other accessions Wca and Dor. The transcript levels were subsequently determined by quantitative real-time PCR.

In accordance with the regulation of *SPA* transcripts in Arabidopsis, in the wild type *A. alpina* Paj, the *AaSPA1*, *AaSPA3* and *AaSPA4* mRNA abundance was increased by white light, whereas the *AaSPA2* level was not altered by light (Figure III-15). A similar transcript regulation by light was observed in the other two accessions Tot and Dor, as well as in the *pep1* mutant. Surprisingly, in *A. alpina* Wca, the *AaSPA2* mRNA abundance was also increased by light, suggesting a different light regulation mechanism on Wca *AaSPA2* in comparison to the wild Paj.

AaCOP1 is highly conserved to *AtCOP1*, and was proved to be functional in *Arabidopsis* in this study. I examined the transcripts of *AaCOP1* regulated by light in *A. alpina*, expecting it to be similarly regulated as in Arabidopsis (Deng et al., 1991). Indeed, as it is shown in Figure III-15, the transcript levels of *AaCOP1* were constantly low in darkness or light in all of the *A. alpina* accessions examined, and was not significantly altered by light.

III.3.8 Generation of mutants in *AaSPA1* and *AaCOP1*

A complete understanding of the role of *AaSPA1* and *AaCOP1* in *A. alpina* requires respective mutants. Therefore, I attempted to generate mutants in *AaSPA1* and *AaCOP1* using amiRNAs and CRISPR/Cas9 system.

Artificial miroRNAs (amiRNAs) targeting gene-silencing method was used to silence *AaSPA1* and *AaCOP1* (described in Materials and Methods). Since the *A. alpina* genome database was not successfully uploaded to the web-based tool (http://wmd3.weigelworld.org), the design of amiRNAs for targeting *AaSPA1* and *AaCOP1* was instead performed manually by fulfilling the principles described by Schwab et al. (2006).

The 21 nucleotides amiRNA 5´- UAACUUACAAGGUACGUCAUC-3´ was designed to target *AaSPA1*. The screening of the T1 generation which carried basta resistance identified thirteen transformants. The transformants were subsequently grown under SD or flowering observation

and leaf tissues were collected to examine the expression level of *AaSPA1*. Under SD, the thirteen transformants flowered as wild type, and did not show the *spa1* mutant early-flowering phenotype as in Arabidopsis. Real-time PCR analysis quantified similar amount of expression levels of *AaSPA1* in the transformants in comparison to the wild type, indicating that *AaSPA1* transcript levels was not altered. Therefore, although positive transformants carrying basta resistance were selectable, the amiRNA did not target and silence *AaSPA1* successfully in *A. alpina* Paj.

The partial loss of function *AtCOP1* allele in Arabidopsis, *cop1-4*, is a weak mutant which is defective only in repressing photomorphogenesis but remains fertile and viable, whereas severe *cop1* alleles are seedling lethal (Stacey et al., 2000). To generate a viable and fertile *cop1* mutant in *A. alpina*, the amiRNA targeting *AaCOP1* was selected covering the WD-repeat domain. The 21 nucleotides were selected manually, 5'-UGACUUUUCACGUACAG**C**ACC-3', covering *COP1* gene position 1401-1420 (456 in protein, WD repeat domain). Similar as the amiRNA designed for *AaSPA1*, transformants were successfully selected but no *cop1*-like dwarf phenotype was observed. The gene expression of *AaCOP1* in the transformants was also not different as the wild type. Therefore, the first attempt to generate mutants in *AaSPA1* and *AaCOP1* was not successful. However, this might be due to low number of amiRNAs designed. Using several amiRNAs may increase the successful rate.

In the second attempt of generating mutants in *AaSPA1* and *AaCOP1*, I adapted the CRISPR/ Cas9 system as described by Hyun et al. (2015) in Arabidopsis. Hyun et al. (2015) reported the RNA-guided endonuclease (RGEN) derived from bacterial clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 protein system, which can be used to efficiently generate heritable mutations by site-directed mutagenesis. To induce mutagenesis in the gene of interest in proliferating tissues throughout the plant life cycle, the *single guide RNA* (*sgRNA*) and Cas9 DNA endonuclease were expressed under *U6 snRNA* and *INCURVATA2* promoters. The sgRNAs were designed manually by selecting a 20-nucleotide-long sequence followed by the PAM sequence NGG. Four sgRNAs each were selected for *AaSPA1* and *AaCOP1*. The off-targets were checked by Cas-OFFinder (http://www.rgenome.net/cas-offinder/), assuming that similar ones would be found in *A. alpina*, due to its high sequence similarity with Arabidopsis. From the screening of the T1 seeds, only one transformant was selected for *AaSPA1*, and none for *AaCOP1*. As the T1 plant is chimeric, the homozygous line will be obtained in T3 plants. The subsequent screening in T2 and T3 generation will be taken over by other members of the group.

IV. Discussion

IV.1 Natural variation in photoperiod flowering in *A. alpina* accessions

The phenotypic differences caused by intra-specific natural variation (hereafter natural variation) could be due to single-gene (monogenic) allelic variants, or most commonly determined by molecular polymorphisms at multiple loci and genes which is referred to as quantitative trait loci (QTL) and qualitative trait genes (QTGs). Genetic analysis of natural variation has been performed extensively in annual species like *A. thaliana*, rice, wheat, barley and maize; genes and their nucleotide polymorphisms with major effects in photoperiod flowering were identified (Alonso-Blanco et al., 2009). Genetic analysis of flowering time in short-lived perennials *Fragaria vesca* (Albani et al., 2004) and long-lived woody perennials *Populus* (Frewen et al, 2000) also revealed multiple casual genes and polymorphisms. However, similar studies have never been performed in the perennial *A. alpina,* which is a close relative of Arabidopsis. In this study, phenotypic and genetic analysis of natural variation in flowering time in *A. alpina* accessions was performed.

IV.1.1 A dysfunctional phyB may contribute to the photoperiod-insensitivity in *A. alpina* Wca

The *A. alpina* accession Wca behaved as a photoperiod-insensitive plant (Figure III-1), with similar number of leaves at flowering under both SD and LD conditions. In Arabidopsis, the expression of *AP1* in developing flower primordia is an indication of the end of the floral induction process and start of the floral development (Hempel et al., 1997). *LFY* is also responsible for the acquisition of floral meristem identity (Shannon & Meeks-Wagner 1993). *LFY* and *AP1* enhance the expression of each other (Hempel et al., 1997). Due to the high homology (86% for *AaLFY* and 96% for *AaAP1*) and similar genomic organization with conserved synteny in comparison to their counterparts in Arabidopsis, we assumed that *AaLFY* and *AaAP1* play similar roles in *A. alpina*. Indeed, we could confirm the photoperiod insensitivity in Wca by the expression of floral meristem identity genes *AaLFY* and *AaAP1* (Figure III-2).

It is possible that the phenotype observed in Wca is a consequence of mutations in the photoreceptor genes, particularly *phyB* and/or *phyA* (Hanumappa et al., 1999; Johnson et al., 1994), which are mainly involved in the photoperiod response; or in *phyC* (Balasubramanian et al., 2005), *phyD* (Aukerman et al., 1997) and *cry2* (El-Assal et al., 2001). The loss- or gain-of-

function of these genes could lead to reduced photoperiod response in natural accessions of Arabidopsis.

Both phyA and phyB have been reported previously to be involved in day-length perception in Arabidopsis. The *phyA* mutant is deficient in sensing inductive day-length (Johnson et al., 1994); when *phyA* is over-expressed, the resulting transgenic plants *phyA* flower early in all day lengths, approaching day-neutrality (Bagnall et al., 1995). Moreover, the photoperiod-insensitive cultivars in Soybean [*Glycine max (L.) Merrill*] are caused by dysfunctional alleles of *phyA* (Watanabe et al., 2009). Since phyA primarily perceives far-red light (Casal et al., 2014), subsequently the function of phyA in the photoperiod-insensitive Wca was analyzed under different fluence rate of far-red light (Figure III-10). The inhibition of hypocotyl elongation in response to increasing dose of far-red light in Wca was similar compared to the wild type Paj and two other accessions Tot and Dor (Figure III-10). Therefore, the phyA in Wca should be functional, and the photoperiod insensitivity in Wca is not likely a consequence of a dysfunctional phyA.

Apart from *phyA*, it has been shown that Arabidopsis plants, when lacking a functional *phyB,* flower early regardless of photoperiod (Halliday et al., 1994). phyB is a typical light-stable form of phytochrome; however, in one cultivar of barley, phyB became light-liable and caused photoperiod-insensitivity with early flowering under SD (Hanumappa et al., 1999). To examine the role of the phyB homolog in the photoperiod-insensitive *A. alpina* Wca, the function of phyB was also analyzed under different fluence rates of red light (Figure III-9). Paj, Dor and Tot exhibited inhibition of hypocotyl elongation in response to increasing red fluence rate. By contrast, Wca appeared to be strongly insensitive to the different fluence rate of red light, displaying almost unaltered hypocotyl length (Figure III-9). Therefore, the photoperiod insensitivity in Wca could be a consequence of either the mutated *phyB* sequence, which would lead to a naturally occurred *phyB* mutant; or the destabilization of the phyB protein, possibly resulting from a reduced rate of transcription and/or a decrease in its messanger stability in the light. A third possibility is that in Wca the interacting partner(s) of phyB are different when compared to Paj. The first hypothesis can be verified by amplifying the *AaPHYB* sequence and comparing to its counterpart in Arabidopsis. In this study, only part of the *AaPHYB* from Wca was successfully amplified (Figure S10), and further analyses are necessary. The later hypotheses require examination on the stability of the Wca phyB protein in darkness as well as in light.

IV.1.2 The diurnal oscillation of *AaCO* and *AaFT* expression in Dor and Wca could not fully be correlated to their distinct flowering phenotype

In Arabidopsis, many photoperiod pathway-related genes exhibit diurnal oscillation of gene expression, such as *FT*, *CO* and *SOC1* under LD conditions (Michael et al., 2008). Among these, *CO* expression in the leaves is a crucial mechanism to precisely measure the difference in day length (Golembeski et al., 2014). CO protein is stabilized only at a narrow time window of the day, involving multiple photoreceptors and E3 ubiquitin ligases (Andrés and Coupland 2012; Shim and Imaizumi 2015). *FT* transcription could be activated directly by stabilized CO protein as it is a direct target of CO (Suárez-López et al., 2001). Furthermore, *FT* could also be induced independently of CO by CIB (reviewed by Greenham & McClung 2015). The *CO-FT* module is also found to be conserved in the photoperiodic flowering in rice and *populus* in addition to Arabidopsis (Kojima et al., 2002; Böhlenius et al., 2006).

We first asked if the photoperiod-insensitive in Wca could be due to any changes in the circadian clock-regulation of *AaCO* expression, which can lead to day-length-independent early flowering, such as the *toc1* mutant in Arabidopsis. *TIMING OF CHLOROPHYLL A/B BINDING PROTEIN1* (*TOC1*) is part of the central mechanism that generates circadian rhythms in plants (Searle and Coupland 2004). If the expression of *CO* starts earlier in SD, which could overlap with the light exposure, then Wca would flower early in SD. Consistent with this, the diurnal expression of AaCO was investigated. The diurnal expression pattern of *AaCO* in Dor and Wca were similar, and both followed the pattern of a facultative LD plant, with only a transient peak of *AaCO* mRNA abundance at the end of the day under LD to ensure AaCO protein accumulation (Figure III-3). Therefore, it is unlikely that the photoperiod-insensitivity in Wca is due to any changes in the circadian clock-regulation of *AaCO* expression. The lack of significant correlation between *AaCO* mRNA level with Wca flowering time suggests that the AaCO protein activity in Wca might be differentially regulated when compared to Arabidopsis. This possibility requires further analyses on the function of AaCO protein.

In an Arabidopsis mutant with dysfunctional *phyB*, the downstream CO protein was stabilized post-transcriptionally in early morning (Valverde et al., 2004), which would further result in an increased expression level of *FT*. Presuming this pathway is conserved in *A. alpina*, the transcript level of *AaFT* in Wca during the day is expected to be similarly induced, both under SD and LD conditions. This hypothesis was tested by analyzing the diurnal expression pattern of *AaFT* in Wca with its reference accession Dor, which is an established LD plant (Figure III-3, 4). Interestingly, under SD conditions, *AaFT* mRNA abundance in Dor exhibited induced level at early morning ZT4 & ZT6, which is commonly observed in the early morning of LD grown plants. However, the very low level was further repressed at later time point, thus was not likely able to

induce flowering. Whereas in Wca, the increase in *AaFT* mRNA levels in the late afternoon at ZT10 & ZT12 (Figure III-3) is similar as what is observed in *phyB* mutant in Arabidopsis (Cerdán and Chory 2003), and one may speculate that if this *AaFT* expression could be the direct reason to induce flowering under SD.

As was reported in Arabidopsis, phyB could directly regulate *FT* mRNA levels without involvement of changes in the mRNA levels of *CO* or *SOC1* (Cerdán and Chory 2003). Similarly, it is possible that the expression of *AaFT* mRNA levels in Wca was not activated by *AaCO*. Therefore, it is possible that, at least in Wca, AaphyB might regulate *AaFT* mRNA level in a CO-independent mechanism. In Arabidopsis, *PFT1* was identified to act in-between phyB and *FT* transcription (Cerdán and Chory 2003). Therefore, a *PFT1* counterpart or other unknown factor(s) in *A. alpina* might play a similar role to activate *AaFT* transcription and to induce flowering, which might also explain the differential regulation of *AaFT* under SD and LD conditions during night and in the early morning. Further studies with *AaphyB* mutant, for example, will be helpful to support this hypothesis.

Taken together, the differences of photoperiod flowering between Dor and Wca may be a consequence of a dysfunctional *phyB* in Wca, which may result into a direct increase of *AaFT* mRNA levels under SD condition, indicating natural variation in the measuring of day length by photoreceptors in *A. alpina*. Other factor(s) independent of AaCO may act in the late afternoon under SD differently, to regulate the transcription of *AaFT* in Dor and Wca. However, there is not enough evidence so far showing that the increase of *AaFT* mRNA levels under SD condition in Wca is sufficient to induce flowering, as the *AaFT* mRNA levels in SD are much lower than in LD. Therefore, we came up with three hypotheses. First, Wca flowers in SD as early as in LD might be due to that the low *AaFT* abundance could already surpass the threshold of *AaFT* required for flowering, and the additional *AaFT* in LD may not further increase flowering induction. Second, some factor(s) may increase the sensitivity of Wca plants to FT levels specifically under SD condition. Finally, an *AaFT*-independent mechanism might exist in Wca to assure the flowering induction both in SD and LD. On the other hand, Wang (Dissertation 2007) showed that in 4-week-old Paj adult plants, *AaFT* is expressed mainly in the leaves, but also at relatively low levels in the apices. Since the experiment sampled 4-week-old whole plant with all different tissues (Figure III-3), the *AaFT* mRNA levels investigated could represent the additive abundance both in the leaves and the shoot apices.

IV.1.3 The expression of *AaFT* may not account solely for the floral transition in *A. alpina* Wca

The expression of floral meristem identity genes *AaLFY* and *AaAP1* in the time scale experiment suggested that the floral initiation occurred in around 7-week-old plants (Figure III-1). Therefore, the expression of *AaFT* was examined in 7-week-old plants (as control), and in plants shifted from LD to SD, or vice versa, for additional one to four weeks (Figure III-4). In the leaves of 7-week-old adult plants, the level of *AaFT* in Wca was similar (sampled at ZT8) and decreased when shifted from LD to SD, or vice versa (Figure III-4). Although plants growing continuously under SD or LD as controls are essential for further speculations, the decreased levels of *AaFT* in aging Wca could be explained by two possibilities. First, it is likely that the exposure to SD condition at any time during/before the floral induction deactivates the transcription of *AaFT* in Wca. However, as all plants flowered at the end of the SD/LD shift experiment (data not shown), the decreased *AaFT* mRNA levels cannot be directly correlated with the flowering response. The second possibility is that *AaFT* might not account for, at least solely, the floral induction in Wca.

TSF is closely related to *FT* in Arabidopsis and is also targeted by CO, with 82% identity in the deduced amino acid sequence (Yamaguchi et al., 2005). In *A. alpina,* two homologs of *TSF*s were also identified with 82% and 81% identity to *AaFT*, respectively (Figure S1, S2; Wang 2007). In the pioneer work by Koornneef et al. (1991), *ft* mutant flowers late under LD conditions, and is only slightly affected under SD. By contrast, *tsf* mutation does not affect flowering under LD but only exhibits late flowering phenotype under SD (Yamaguchi et al., 2005). This suggests that *TSF* not only has a redundant role in promoting flowering with *FT* in LD conditions, but also has distinct contribution to flowering in SD conditions. Recent work has shown that *TSF* is involved in flowering promotion by the phytohormone cytokinin under non-inductive SD conditions (D'Aloia et al., 2011), suggesting that different transcriptional control of *FT* and *TSF* could be the basis of different flowering response to various photoperiod conditions.

In *A. alpina*, although the roles of the two *TSF* homologs *AaTSF1* and *AaTSF2* (Figure S1, S2; Wang, Dissertation, 2007) remain unclear; considering the distinctive role of *TSF* in accelerating flowering under SD in Arabidopsis, we speculate that the highly conserved *AaTSF*s may play any particular roles in the flowering response under SD conditions in *A. alpina,* especially in the photoperiod insensitive Wca accession. In this study, multiple attempts to detect the mRNA abundance of *AaTSF*s were performed, yet was not successful until the completion of this thesis. Further experiments are necessary to detect the *AaTSF* mRNA abundance.

Finally, it is common to find several *FT*-like genes that promote flowering with overlapping and/ or distinct roles in plants. For example, *FT* and *TSF* in Arabidopsis, *Hd3a* and *RFT1* in rice, two FT-like genes in sugar beet, four FT-like ones in tomato (*SFT*, *SlSP5G*, *SlSP5G2* and *SlSP5G3*), and two each in strawberry and *populus* (*PtFT1*, *PtFT2)*. Among these, some *FT*-likes were found to act as anti-florigens, such as *FT1* of sugar beet and three *FT*-likes in tomato (Pin et al., 2010; Molinero-Rosales et al., 2004; Cao et al., 2016). However, *FT*-likes were found to be involved in more than flowering regulation. Some *FT*-like genes in potato and onion have functions in storage organ differentiation (Navarro et al., 2011; Lee et al., 2013); and as mentioned previously, *populus FT* genes have diverse roles in seasonal phenology (Böhlenius et al.2006; Ding and Nilsson 2016). Although detailed research is still essential, the *populus FT1* (*PtFT1*) was proposed to be recruited by chilling in dormant buds to prepare for vegetative growth in following spring, and be responsible for floral induction, while *PtFT2* for the vegetative growth control (Rinne et al., 2011; Hsu et al., 2011; Pin and Nilsson 2012). The interplay between *FT* paralogues in *Populus* suggests their distinct roles in controlling vegetative and reproductive growth cycles in woody perennials. In *A. alpina*, *AaFT* was proposed to be involved in flower induction only in adult plants, whereas in young plants the *AaTFL1* antagonizes the flower promotion (Wang et al., 2011). Therefore, the possibility cannot be excluded that the function of *AaFT* and two *TSF*-likes is linked to the juvenile or adult stages, thus acting more than floral inducer(s) of *A. alpina* plants.

IV.2 The SAS of *A. alpina* during juvenile and adult stages

In contrast to short-lived annual plants, perennial species usually have a prolonged vegetative juvenile phase during which they are unable to respond to floral inductive signals (Baurle & Dean 2006). Molecular genetic analysis of the juvenile and adult phase changes in maize and Arabidopsis, and also very recently in the perennial species *A. alpina*, revealed the important role of abundant miR156 in maintaining the juvenile phase (Wu and Poethig, 2006; Bergonzi et al., 2013). Despite of the recent progress in understanding the vegetative phase change, many questions remain to be answered: How and at what growth stage, miR156 gets repressed in order to transit to an adult stage? What is the role of environmental factors such as light in vegetative phase change? So far, only a few studies have been performed in woody perennial species, where juvenile and adult phase change was first discovered and where this is of practical significance.

For example, there is little information about, how light perception and transduction occur differently between the juvenile and adult phase in perennial plants. To our knowledge, no previous study was performed in perennial species on the shade avoidance syndrome (SAS) responses in adult stages and reproductive tissues (Reviewed by Roig-Villanova & Martínez-

García 2016), and there is still no data available on the systemic analysis of SAS in perennial plants including both the juvenile and adult phases. Therefore, in this study, the response of perennial *A. alpina* under simulated shade condition was investigated, with detailed analysis of the elongation response, and the expression of auxin biosynthesis genes both during the juvenile and adult stages.

IV.2.1 The age-dependent SAS responses in *A. alpina*

Arabidopsis responds to low R:FR ratio with elongated hypocotyl and petiole at seedlings stage (Roig-Villanova et al., 2007). In the adult tissues, Arabidopsis responds to shade with the elongation of petioles in the rosette leaves, inhibition of leaf blade expansion and accelerated flowering in adult plants, which are important for its survival (reviewed by Roig-Villanova & Martínez-García 2016). Interestingly, in two-week-old *A. alpina* seedlings, simulated shade (low R:FR) was not able to induce any elongation responses in Paj, Wca and Dor (Figure III-5), suggesting that these accessions are more tolerant to simulated shade, at least in the seedlings stages. However, Tot displayed significantly reduced length of hypocotyl in response to low R:FR (Figure III-5). In Arabidopsis, phyA and phyB play important roles in shade avoidance response (Martínez-García et al., 2014). Possibility of Tot being a *phyB* mutant analyzed by sequencing the *PHYB* gene, as well as by the fluence rate response curve under red light. The *PHYB* gene and the deduced protein sequence in Tot does not have subtle mutations in comparison to those of Paj (Figure S5, S6; Bae and Choi, 2008; Filiault et al., 2008). The sequence alignment revealed four nucleotide changes, which resulted in one amino acid change from Valine (Val) in Paj into Isoleucine (Ile) in Tot at position 731 in the signal-sensing PAS domain (Figure S5). This change was found common in other relatives of the Brassicaceae family (Figure S7), and was not reported formerly to be critical for *AtPHYB* functionality. Possibility of a light destabilized phyB protein in Tot was also excluded, by the arrested hypocotyl elongation in response to increasing red light (Figure III-9). In addition, Tot is also not a phyA-hypersensitive plant as it responded to very low red fluence rate, which is regulated by phyA, similarly as the wild type Paj (Figure III-9).

A. alpina has a prolonged juvenility in comparison to its close relative Arabidopsis (Wang, Dissertation, 2007). During the juvenile phase, *A. alpina* Paj does not respond to environmental signals such as vernalization (Wang et al., 2009), which is controlled by miR156 (Bergonzi et al., 2013). The length of juvenile phase in *A. alpina* is around 5 weeks, as determined by the occurrence of floral induction in response to vernalization treatment (Wang, Dissertation, 2007; Wang et al., 2009). This evidence led us to ask whether the shade tolerant responses in two-week-old *A. alpina* accessions were only representing the juvenile phase.

Indeed, in eight-week-old *A. alpina* plants, Paj and Tot responded to simulated shade with elongated internode (Figure III-7). In addition, exposure to simulated shade suppressed the expansion of leaf blade both in Paj and Tot, but did not promote the elongation of petioleS. In Arabidopsis, spotlight far-red light irradiation of leaf blades, but not that of leaf petioles, promoted the elongation of petioles (Kozuka et al., 2010), suggesting that the low R:FR signal is perceived by the (upper) leaves and subsequently communicated to the petiole and the rest of the plant body. Therefore, the elongation of petiole in Arabidopsis could place the leaf blade in a position that is best suited for light perception to maximize the photosynthesis (Roig-Villanova & Martínez-García 2016). It appears that, the *A. alpina* adult plants invest only in internode elongation at the expense of blade expansion, but not in the petiole elongation (Figure III-7; de Wit et al., 2015). Therefore, it is possible that the hormone-mediated inter-organ communication may also exist in *A. alpina* Paj and Tot. Finally, at the end of 7th week, Tot exhibited accelerated flowering in response to low R:FR, suggesting similar SAS response during its reproductive stage as Arabidopsis. As there is a lack of specific knowledge about the perception of shade in reproductive tissues in Arabidopsis, the SAS in reproductive stage in *A. alpina* will also not be discussed in this study.

Taken together, the different SAS responses in the two-week-old, and the eight-week-old *A. alpina* plants suggest that the SAS might be age-dependent in *A. alpina*. On the other hand, the natural habitat of *A. alpina* is at high altitude on mountains, and *A. alpina* plants are exposed to strong light intensity consistently. Therefore, this hypothesis requires further analyses from shade experiments with stronger light intensity (PAR) set-up. Moreover, the simulated shade in this study mimics vegetation shade in nature — lower R:FR but unaltered PAR. In Arabidopsis, it is known that the regulatory mechanisms differ between proximity shade and dense canopy shade (Hersch et al., 2014); therefore, further studies of the SAS response in *A. alpina* under direct plant canopy shade, in which both the PAR and R:FR is strongly reduced, will further facilitate our understanding of SAS in perennial species.

IV.2.2 The low R:FR signaling might at least be partially conserved in *A. alpina*

In Arabidopsis, the up-regulation of *YUC2/5/8/9* is controlled by PIF7 and *YCU5/8/9* also by PIF4 and PIF5 (Hornitschek et al., 2012; Li et al., 2012). The PIFs are positive regulators in response to low R:FR (Fraser et al., 2016). The expression of *PIF* genes, however, is not affected by simulated shade (Roig-Villanova & Martínez-García 2016). Similarly, in *A. alpina* Paj and Tot seedlings, the expression of *AaPIF4* was not altered by low R:FR (Figure S15). The bHLH transcription factors *HFR1* and *PIL1* in Arabidopsis act as negative regulators in the SAS

responses, and are swiftly up-regulated by simulated shade (Sessa et al., 2005). In this study, the transcript levels of the homologs of both *HFR1* and *PIL1* were up-regulated by simulated shade in young *A. alpina* Paj and Tot seedlings, irrespective of the lack of SAS phenotype observed. Therefore, we propose that the low R:FR signaling on *AaHFR1* and *AaPIL1* is conserved in *A. alpina*. Whether *AaHFR1* acts to repress auxin production in *A. alpina* as in Arabidopsis (Hersch et al., 2014) requires further experiment.

The cell wall modification gene, *XTR7/XTH15,* which with other *XTH*s are required for cellular expansion that fuels elongation responses, is up-regulated by low R:FR in Arabidopsis (Sasidharan et al., 2010), and also in this study in *A. alpina* Paj and Tot (Figure III-6). However, the up-regulation of cell modification gene *AaXTR7* cannot explain the low R:FR phenotype observed in Paj and Tot. Nevertheless, Arabidopsis has 33 *XTH* genes (Sasidharan et al., 2010); similarly in *A. alpina*, it would be inaccurate to label any single cell wall modifying gene as the sole regulator in the cellular expansion process. Therefore, further analysis with other *XTH*s family genes in *A. alpina* are required.

Taken together, we propose that the low R:FR signaling on *PIF*s and *HFR1* may be similar between *A. alpina* and Arabidopsis, although various mutants and more expression data are still necessary to further confirm this hypothesis.

IV.2.3 The correlation of auxin biosynthesis genes *AaYUC*s with the age-dependent SAS phenotype in *A. alpina*

To further support the phenotypic analysis, the expression of auxin biosynthesis genes were analyzed in the juvenile (seedlings) and adult (internodes and petioles) stages of *A. alpina* Paj, Tot and the annual relative *Arabis montbretiana* (Figure III-6, III-8).

In Arabidopsis, several features of the SAS, such as stem growth and leaf petiole elongation, are characteristic of high auxin levels (Hornitschek et al., 2012; Li et al., 2012). The auxin biosynthesis genes, notably *YUC2*, *YUC5*, *YUC8* and *YUC9*, are induced by low R:FR to directly increase the auxin levels and subsequently induce SAS (Hornitschek et al., 2012; Li et al., 2012; Tao et al., 2008). As all the homologs of *YUC2*, *YUC8* and *YUC9* were identified in *A. alpina* Paj with similar genomic organization and conserved synteny (Figure S4), we hypothesize that these auxin biosynthesis gene homologs play similar roles in *A. alpina*.

In the seedlings of *A. alpina* Paj, the simulated shade immediately up-regulated the transcript abundance of *AaYUC2*, *AaYUC8* and *AaYUC9,* but followed by rapid reduction of their expression at the later time points (Figure III-6). This can be correlated to the unaltered seedling hypocotyl length in response to simulated shade (Figure III-5). Taken together, low R:FR is able to elevate the expression of auxin biosynthesis genes in *A. alpina* Paj upon exposure to the

simulated shade, but not for more than three hours. In Arabidopsis, the *YUC2/5/8/9* genes are directly activated by the SAS positive regulator PIFs (Casal 2013), and control the rate-limiting step in auxin biosynthesis (Hornitschek et al., 2012). If this mechanism is conserved in *A. alpina*, we could then speculate that the repression on the expression of *AaYUC2/8/9* at later time points might be due to other important mechanism(s) that prevent the over-synthesis of auxin through *AaYUC*s in juvenile *A. alpina* seedlings, regardless of the surrounding environmental signals. On the other hand, the seedlings that were kept in Wc exhibited constantly low expression of *AaYUC8* and mostly that of *AaYUC9* except at 1hr Wc. The expression of the *AaYUC2* gene first increased (until 3h), then decreased in Wc (Figure III-6).

For *A. alpina* Tot, on the other hand, a lower expression of *AaYUC* genes in simulated shade than in Wc is expected to explain the shorter hypocotyl length observed under simulated shade (Figure III-5). However, the transcript levels of *AaYUC2*, *AaYUC8* and *AaYUC9* were all rapidly up-regulated under low R:FR and then decreased to lower levels similar as what was observed in Wc (Figure III-6). The expression of all *AaYUC* genes in Wc condition was constantly low. Therefore, although juvenile seedlings of Tot exhibited arrested hypocotyl elongation in response to low R:FR, the auxin biosynthesis through *AaYUC*s appears to be similarly regulated as in Paj — that other mechanism may play an important role in regulating the expression of *AaYUC* genes to maintain auxin biosynthesis at a certain stable level. Nevertheless, the different sensitivity of *A. alpina* Tot seedlings to auxin under Wc and Wc+FRc is also another possible way to explain the different hypocotyl length observed.

The expression of *YUC2/5/8/9* in Arabidopsis is increased by shade within 15min in the cotyledons (Kohnen et al., 2016), similarly, the expression of *AaYUC2/8/9* was induced by shade within 30min (Figure III-6). However, the expression is transitory and low R:FR is not able to effectively increase the expression of *AaYUC2/8/9* for more than 3 hours in the juvenile seedlings of *A. alpina* (Figure III-6). This has not been previously observed in Arabidopsis or in the related natural populations (Botto et al., 2002; Filiault and Maloof 2012).

On the other hand, the limitation of this observation is that the seven-hour treatment is probably too short to represent the auxin biosynthesis of the whole juvenile phase of *A. alpina,* which is around 5 weeks long (Wang, Dissertation 2007; Wang et al., 2009). Consequently, to further support this hypothesis, the time course experiment examining the expression of *AaYUC genes* needs to be extended to longer time, at least until two weeks when the seedling phenotype of Paj and Tot was observed under simulated shade.

To examine the relation between the expression of auxin biosynthesis genes and the elongation response observed in the internodes/stems of *A. alpina* adult plants, the transcript levels of *AaYUC2/8/9* were analyzed in the internodes and petioles in *A. alpina* Paj, Tot and in the annual relative *A. montebretiana* (Figure III-8). Surprisingly, the transcript abundance of *AaYUC2* and

AaYUC8 were beyond detection in the internode and petiole tissues in *A. alpina* Paj and Tot. At this stage, we cannot yet explain on the lack of detection of *AaYUC2* and *AaYUC8* in the internodes and petioles of *A. alpina*. However, since their expression could be detected in two-week-old whole seedlings, the transcript levels should also be tested in other tissues (e.g. leaves) to examine the possibility of spatially-limited expression of *AaYUC2* and *AaYUC8* in adult *A. alpina*. The expression of *AaYUC9* can be correlated with the phenotype observed in Paj and *A. montebretiana* under simulated shade, but not in Tot (Figure III-7). Seven weeks of low R:FR treatment increased the expression of *AaYUC9* in the internodes in the adult plants of *A. alpina* Paj (Figure III-8), in contrast to the expression of *AaYUC9* in two-week-old *A. alpina* seedlings (Figure III-6).

Taken together, the expression analyses of *AaYUC* genes in Paj and Tot together suggest that, in seven-day-old *A. alpina* seedlings, the expression of *AaYUC2/8/9* is regulated to be kept in a ubiquitous level, even after exposure to low R:FR. Whereas in adult plants of *A. alpina* Paj, at least the expression levels of *AaYUC9* can be induced by low R:FR. Therefore, we propose that other mechanism play an important role in maintaining the expression of *AaYUC* genes during the juvenile phase; whereas in adult phase, at least for the expression of *AaYUC9* in *A. alpina* Paj, the low R:FR treatment could bypass this mechanism to induce elongation responses. Furthermore, A recent genome-wide associate study has identified that the variants of *YUC5* and *YUC9* genes may potentially contribute to the variation in shade avoidance in Arabidopsis (Filiault and Maloof 2012). Therefore, *AaYUC9* might as well be a potential candidate in analyzing the mechanism underlying the differences of SAS in juvenile and adult phase of *A. alpina*.

Other genes involved in the auxin signaling pathway should also be examined in young *A. alpina* seedlings; since the *AaYUC* genes, similar as in Arabidopsis, should not be the sole factor determining the auxin levels. In addition to auxin, it was shown that a full SAS in Arabidopsis requires also GA (Djakovic-Petrovic et al., 2007; Nozue et al., 2015) and brassinosteroid (BR) (Kozuka et al., 2010). Therefore, future studies on the involvement of GA and BR in the SAS in *A. alpina* is also necessary to fully understand the SAS observed in this study.

IV.3 Comparative study on the evolution of light signaling in *Arabidopsis thaliana* and *A. alpina*

Plants have evolved a remarkably complex system which synchronizes the action of several families of photoreceptors and signal transduction pathways in order to adapt to the ever-changing environments. A plant's ability to maximize its productivity and fitness depends on its

ability to sense, evaluate and respond to light. The crucial life-stages in plants, like transition to flowering or entering into dormancy depends on the responses towards seasonal cues such as day-length. Photoreceptors have been well-characterized in Arabidopsis (reviewed by Chen et al., 2004; Bae and Choi 2008; Franklin and Quail 2010). Among these, the red/far-red sensing phytochromes are most well studied and found in many photosynthetic eukaryotes. However, the evolution and diversity of phytochromes across the photosynthetic eukaryotes remain poorly understood, until a few recent phylogenetic analyses showed that the canonical plant phytochromes originated in a common ancestor of streptophytes (Li et al., 2015). Phytochrome genes have been reported in diverse lineages of plants, including several angiosperms (Mancinelli, 1994) and lower land plants such as Marchantia (Inoue et al., 2016). In this study, I analyzed some of the photoreceptor genes and the downstream components such as *COP1* and *SPA*s in *A. alpina*.

IV.3.1 The photoreceptors are conserved between *A. alpina* and Arabidopsis

BLAST searches identified identical number of the phytochromes and cryptochromes in *A. alpina* as in Arabidopsis (Figure S8), suggesting the conservation of photoreceptors during evolution between perennial and annual species in the Brassicaceae family. This was expected, since in several other angiosperms such as rice and maize, at least the phytochromes were also reported to be highly conserved as in Arabidopsis (Reddy and Sharma, 1998; Xie et al., 2014). Among the five phytochromes in *A. alpina*, *AaPHYA* showed similar genomic organization and conserved synteny as *AtPHYA* (Figure S8), *AaPHYB* showed high identity to *AtPHYB* both in the genomic sequence and the deduced protein sequence (Figure S6, S9), and with conserved synteny upstream of *AaPHYB* (Figure S6). However, AaphyB protein contains four amino acid changes that was previously reported to be linked to its function (Figure III-11; Bae and Choi, 2008; Filiault et al., 2008). The protein sequences of Aa1 and AaCRY2 have 96% and 93% identity to AtCRY1 and AtCRY2, respectively (Figure S8).

The hypocotyl growth in response to various fluence rates of continuous red, far-red or blue light should reflect the function of phyB, phyA and cryptochromes, respectively (Laubinger and Hoecker 2003; Fittinghoff et al., 2006). Therefore, we compared the hypocotyl length in response to far-red and blue light among all the *A. alpina* accessions (Figure III-10), the similar arrested hypocotyl growth indicates that phyA and cryptochromes in these accessions are fully functional. The responsiveness of *A. alpina* Paj, Tot and Dor to different fluence rates of red light suggests the presence of a fully functional phyB in these accessions (Figure III-9). The lack of responsiveness to red light in the accession Wca might be due to an intra-specific variation in

phyB, similar to what was found in one cultivar of barley (Hanumappa et al., 1999). The detailed analysis of phyB in Wca was discussed previously in this study.

It appears that early in the evolution, most liverworts have only one single copy of phytochrome (Li et al., 2015; Inoue et al., 2016), whereas later in evolution in mosses (e.g. *P. patens*), ferns and even later in seed plants, phytochromes have been independently diversified (Possart et al., 2014; Li et al., 2015). In several angiosperms, the phytochrome genes have been reported to have highly conserved functions (Reddy and Sharma, 1998; Xie et al., 2014). This study also showed that the phytochromes (at least phyA and phyB) in the perennial *A. alpina* are highly conserved as in Arabidopsis. In the more distant woody perennial *Populus*, one *PHYA* and two *PHYB* homologs were identified with diverged function, at least in shade avoidance responses (Karve et al., 2012); and the *PHYD* and *PHYE* members are not present (Karve et al., 2012). Therefore, despite the suggested conservation of PHYs in seed plants, it would be of particular interest to infer their diverged function among each other, especially between annuals and perennials in which the comparative analyses on photoreceptors were scarcely performed.

IV.3.2 The conserved basic mechanisms of *AaSPA1* and *AaCOP1* between *A. alpina* and Arabidopsis

In the dicotyledonous plant Arabidopsis, the functions of *COP1* and *SPA* genes have been described in detail. *COP1* and *SPAs* are involved in the light-regulation of growth and development (Laubinger et al., 2004; Hoecker, 2005; Laubinger et al., 2006). Although SPAs appear to be plant-specific, COP1 can be found also in mammals, and previous studies have identified some conserved basic mechanism of COP1 in plant and animal kingdoms (Wang et al., 1999). The function of the homologs of SPAs and COP1 in rice and *Physcomitrella patens* was also studied (Ranjan et al., 2014). Nevertheless, knowledge about their conserved or diverged function in many other plant species, in particular in perennials, is rather limited. In this study, I identified the homologs of *SPA*s and *COP1* in the perennial species *A. alpina* and conducted pilot studies to examine their functionality.

Database searches identified the identical numbers of *SPAs* and *COP1* homologs in *A. alpina* as in Arabidopsis, suggesting the retention of *SPA*s and *COP1* homologs during the evolution from perennials to annuals in the Brassicaceae family. *A. alpina* COP1 (AaCOP1) is highly identical to Arabidopsis COP1 (AtCOP1), sharing 95% identity (Figure S12, III-13). In fact, COP1 from other plant species such as rice and Physcomitrella show more than 60% identity to AtCOP1, and was reported to be functionally conserved during evolution (Richardt et al., 2007; Ranjan et al., 2014; Tsuge et al., 2001). In this study, consistent with the high identity of *AaCOP1* with *AtCOP1*, *AaCOP1* was able to complement all of the *cop1-4* mutant phenotypes

observed for seedling and adult plant, hence AaCOP1 is functional in Arabidopsis (Figure III-14B). Therefore, it is very likely that AaCOP1 also functions as a E3-ubiquitin ligase in *A. alpina*. In addition, rice and Physcomitrella COP1 was able to mostly complement the Arabidopsis *cop1-5* null mutant phenotype (Ranjan et al., 2014), supporting the idea that their rescue of the *cop1-4* mutant phenotype is not due to the presence of the truncated COP1-4 protein. Accordingly, we speculate that the rescue of Arabidopsis *cop1-4* mutant phenotype by the highly identical *AaCOP1* is also a consequence of the expression of *AaCOP1* per se.

By contrast, the SPA gene family appears to have undergone more functional divergence than COP1 in seed plants (Ranjan et al., 2014). The four *SPA* genes in Arabidopsis are divided into two subgroups depending on whether the long N-terminus and NLS are present (Laubinger and Hoecker, 2003; Laubinger et al., 2004). In this study, four homologs of the *SPA* gene family were also retrieved in *A. alpina* with high identity to the respective Arabidopsis *SPA* genes (Figure S11, 13), thus they can also be divided into two similar subgroups. In the distantly related Physcomitrella, and rice which are intermediate in evolution, only two SPA homologs were found, and they were not able to function fully in Arabidopsis (Ranjan et al., 2014). This indicates the functional divergence of SPA gene family in the course of land plant evolution. Arabidopsis and *A. alpina* diverged about 20 million years ago (Koch et al., 2006), the highly similar *SPA* gene family between these two species suggests the possible functional conservation of *SPA*s during the evolution between perennial and annual species.

Distinct from COP1, all the SPA protein sequences contain a kinase-like domain in the N-terminus (Fittinghoff et al., 2006). Very recently, Chen et al. (2016) reported that the N-terminal kinase-like domain is functionally important for the distinctive light responsiveness of SPA1 and SPA2. The kinase-like domain at the N-terminus of SPA1 is important for its activity in darkness, but not in light (Holtkotte et al., 2016). In Physcomitrella and rice, the diverged sequences in the kinase-like domain suggests its early evolution in land plant (Ranjan et al., 2014). Similarly, AaSPA1 exhibits 87% identity to AtSPA1, but the sequences in N-terminal are more diverged in the Serine/Threonine protein kinase motif (Figure S11). Nevertheless, the *AaSPA1* from *A. alpina* Paj could fully complement the Arabidopsis *spa* triple (*spa1spa2spa3)* as well as quadruple mutant (*spaQn)* seedling phenotype in darkness, and the *spaQn* dwarfed adult phenotype (Figure III-14A, 15). This indicates that AaSPA1 is fully functional under *AtSPA1* promoter in Arabidopsis.

Evolutionary transitions between perenniality and annuality are one the most common transitions that occurred in angiosperms. Although transitions in both directions were found in nature (Albani and Coupland 2010), phylogenetic studies generally support that annuals are derived from perennial ancestors, favored by environmental selections (Cruz-Mazo et al., 2009). The most distinct traits between an annual and perennial plant is the different decisions on the

allocation to vegetative growth versus flowering, and the timing of these decisions (Friedman and Rubin 2015). So far very little is known about the selective mechanisms and genetic changes that are responsible for this perennial-annual transition. Recently intensive investigations on the life history, detailed molecular mechanisms and genetic architecture of flowering have been performed in a few perennial model species, notably in *A. alpina* (e.g. Wang et al., 2009; Albani and Coupland, 2010 ; Anderson et al., 2011). In this study, the conserved basic mechanisms of AaCOP1 and AaSPA1 indicates that the core COP1/SPA complex, which is a key negative regulator of light signaling in Arabidopsis, may have underwent little evolutionary divergence during the evolution between perennial and annual life history within the Brassicaceae family.

IV.3.3 Light regulation of *SPA*s and *COP1* in *A. alpina* natural accessions

In Arabidopsis, the difference in *SPA* gene expression patterns partially contribute to the divergent function of *SPA1/2/3/4* (Fittinghoff et al., 2006). Light up-regulates the transcript levels of *SPA1*, *SPA3* and *SPA4*, but not *SPA2* (Fittinghoff et al., 2006). The homologs of *SPA2/3/4* in *A. alpina* Paj showed 85%, 91% and 86% identity to their counterparts in Arabidopsis (Figure III-13). The high homology among the SPA proteins between *A. alpina* and Arabidopsis further demonstrate the conservation of the SPA proteins. In agreement with this, in *A. alpina* Paj, the mRNA abundance of *AaSPA1*, *AaSPA3* and *AaSPA4* was all increased upon light treatment (Figure III-16), suggesting their important roles in the phenotypes of light-grown seedlings as in Arabidopsis. *AaSPA2*, as *AtSPA2,* was not induced by light in *A. alpina* Paj. Taken together, it is very likely that the *AaSPA1/2/3/4* have conserved functions in light- or dark- grown *A. alpina* Paj seedlings. To further confirm this idea, complementation analysis of *AaSPA2/3/4* in Arabidopsis *SPA* quadruple mutant will be necessary.

Moreover, *A. alpina* encompasses natural intra-specific variation in life strategies, notably the naturally occurring perpetual flowering accessions which are a consequence of loss of function alleles at *PEP1* (Albani et al., 2012). This is of great importance, as studies of genotypes by environment associations could reveal the differentiation pattern and the molecular mechanisms underlying the divergence (Friedman and Rubin 2015, Figure III-1). We therefore asked whether the light regulation of *AaSPA*s have intra-specific variation in *A. alpina*. Subsequently, in the natural accessions of *A. alpina*: Wca, Dor, Tot and the *pep1* mutant, the transcript levels of *AaSPA1/2/3/4* were also examined. The light regulation on *AaSPA1/2/3/4* in Dor, Tot and the *pep1* mutant follows a similar pattern as the wild type *A. alpina* Paj (Figure III-16), suggesting their similar roles in regulating light responses in these accessions and the *pep1* mutant.

Interestingly, in *A. alpina* Wca, the *AaSPA2* mRNA abundance was also increased by light, pointing to a different light regulation mechanism in Wca *AaSPA2* in comparison to the wild type Paj. Interestingly, Wca is also photoperiod-insensitive with respect to flowering (Figure III-1) and in addition shows insensitivity to different red fluence rate (Figure III-9). Moreover, Wca is the only accession that was able to over-produce anthocyanin under high light or stressed conditions, which is absent in other accessions tested (not shown). All these observations together suggest the intra-specific variation of *A. alpina* Wca in comparison to Paj, which might be a very interesting accession to follow up.

V. Materials and Methods

V.1 Materials

V.1.1 Chemicals

All used chemicals in analytical quality were purchased from Ambion (Austin, USA), Applichem (Dermstadt, Germany), Colgate-Palmolive (Hamburg, Germany), Duchefa (Haarlem, Netherlands), Difco (Detriot, USA), Fluka AG (Buchs, Switezerland), Gibco BRL (Eggenstein, Germany), Invitrogen (Karlsruhe, Germany), MBI Fermentas (St. Leon-Rot, Germany), Merck (Dermstadt, Germany), Roth (Karlsruhe, Germany), Serva (Heidelberg, Germany), Sigma-Aldrich (Deisenhofen, Germany) and Macherey-Nagel (Düren, Germany).

V.1.2 Enzymes, kits, molecular biology materials

Restriction enzymes, dNTPs, PCR enzymes and DNase were obtained from MBI- fermentas (St. Leon-Rot, Germany). Gateway cloning enzymes were acquired from Invitrogen (Karlsruhe, Germany).

The kits used in this study followed the manufacturers' protocols: Plasmid Mini and Midi Prep, QIA Gel extraction kit, PCR purification kit and RNeasy Plant Mini Kit (all obtained from Qiagen GmbH, Hilden, Germany).

V.1.3 Growth media

Media used for bacterial and plant growth Media were listed in Table IV-1. All media were sterilized prior to use by autoclaving at 121°C for 20 min. For the addition of antibiotics and heat label compounds the solution was cooled to 60°C. Antibiotics were sterilized using filter sterilization units prior to addition.

Table V-1. Growth media used in this study.

Medium	Components
1.5% Luria-Bertani (LB)	10g/L Tryptone 5g/L Yeast extract 10g/L NaCl
1% Murashige and Skoog (MS) medium	4.44g/L MS salts pH adjusted to 5.8
1% Murashige and Skoog (MS) medium with Charcoal	4.44g/L MS salts pH adjusted to 5.8 0.5% Charcoal

V.1.4 Oligonucleotides

All oligonucleotides were synthesized by Metabion (Martinsried, Germany) or Invitrogen Life Technologies (Karlsruhe, Germany). Sequences of all oligonucleotides that were used in this thesis and their purpose:

Table V-2. Oligonucleotides used in this study.

Oligonucleotides	Sequences (5'-3')	Application
AaPhyB-ORF-0715-F1	CCCATAGACTGAATTCCACC	amplify *AaphyB*, 300bp upstream, fragment 1,length 993
AaPhyB-ORF-0715-R1	AGCCTATCATCTTGAACCAC	amplify *AaphyB*, fragment 1, length 993
AaPhyB-1031-Fw2	GAAAGTGTGAGAGACTTAAC	amplify *AaphyB*, fragment 2, length1108
AaPhyB-ORF-0715-R2	TCTGCAGTTTTAGAGTTCGT	amplify *AaphyB*, fragment 2, length 1108
AaPhyB-ORF-0715-F3	CAGAAATGGATGCGATTCAC	amplify *AaphyB*, fragment 3, length 950
AaPhyB-ORF-0715-R3	TTGGACTGCTAAAGCTTGTT	amplify *AaphyB*, fragment 3, length 950
AaPhyB-2772-Fw4	GCAGGCTAAAGGGTCCTGATG	amplify *AaphyB*, fragment 4, length 1136
AaPhyB-ORF-0715-R4	ACCAAACACAAACAATCTCC	amplify *AaphyB*, fragment 4, length 1136
AaPhyB-attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTTCCGGAGTCGG TGG	*AaphyB* ORF entry clone
AaPhyB-attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTATGGCATCATCAACATC ATG	*AaphyB* ORF entry clone
AaPhyB-XbaI-F	CCCAAGCTTCTAGAAAAATGGTTTCCGGAGTCGGG	Clone *AaphyB* into pCGAHah Y2H vector
AaPhyB-XbaI-R	GGGGTACCTTATCTAGAATATGGCATCATCAGCATCA	Clone *AaphyB* into pCGAHah Y2H vector
PhyA-ATG-43	GCACTTACTTCTTGTTGTCTTG	amplify *AaphyA*, upstream 43kb, fragment 1, length 1164
PhyA-Rv-1121	GTGTTATGACAAACCACCAAACC	amplify *AaphyA*, fragment 1, length 1164
PhyA-1048-Fw	GGGGATGCTCCTGATTCTAC	amplify *AaphyA*, fragment 2, length 1172
PhyA-2220-Rv	GGGCCAGTTACACGCACTGAAG	amplify *AaphyA*, fragment 2, length 1172
PhyA-2136-Fw	CCACTGTAGATGCAAAGCTC	amplify *AaphyA*, fragment 3, length 1224
PhyA-3360-Rv	GACACAATACCTAGGGGAGC	amplify *AaphyA*, fragment 3, length 1224
PhyA-3291-Fw	AGAACATTTTGCGCACACAGAC	amplify *AaphyA*, fragment 4, length 1317
PhyA-Rv	CTTGTTCGCTGCAGCGAG	amplify *AaphyA*, fragment 4, length 1317, no stop codon

AaCOP1-attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAAGAGATTTCAACGGT	*AaCOP1* ORF entry clone
AaCOP1-attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTATCACGCAGCGAGTACAAG AACTT	*AaCOP1* ORF entry clone
COP1-RGEN-A-Fw	TATTGAAGAAAACTTCGGCTGTTTTAGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
COP1-RGEN-A-Rv	AGCCGAAGTTTTCTTCAATAACAATCACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
COP1-RGEN-B-Fw	TGTCCGCTAAAGACATGAAGGTTTTAGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
COP1-RGEN-B-Rv	CTTCATGTCTTTAGCGGACAACAATCACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
COP1-RGEN-C-Fw	TATGAAGGAATAGTAACTGTGGTTTTAGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
COP1-RGEN-C-Rv	CACAGTTACTATTCCTTCATAACAATCACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
COP1-RGEN-**D**-Fw	GAATGAAATAGATCCCCATGCGTTTTAGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
COP1-RGEN-**D**-Rv	GCATGGGGATCTATTTCATTCACAATCACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
COP1-ATG-Fw	ATGGAAGAGATTTCAACGGT	amplify *AaCOP1* ORF, fragment 1
COP1-ORF-R2-0815	TTGTGCACCAAACTTTAACC	amplify *AaCOP1* ORF, fragment 1
COP1-ORF-F2-0815	GAGGAAGCAAAAAGTCGATG	amplify *AaCOP1* ORF, fragment 2
COP1-ORF-R3-0715	TTCCTTGACTATTCGCAGTC	amplify *AaCOP1* ORF, fragment 2
ORF-SPA1c-F1	CACATAATCTCCGTTTGAGA	amplify *AaSPA1* ORF, fragment 1, 875
ORF-SPA1c-R1	CTTCCTGGTTTTTCCCTACT	amplify *AaSPA1* ORF, fragment 1, 875
ORF-SPA1c-F2	CGTTGAGTTCTTCGAGTTTT	amplify *AaSPA1* ORF, fragment 2, 885
ORF-SPA1c-R2	GACGTAAATCTGCCATCATT	amplify *AaSPA1* ORF, fragment 2, 885
ORF-SPA1c-F3	TGTCCAGAGGAGATAAATGG	amplify *AaSPA1* ORF, fragment 3, 874
ORF-SPA1c-R3	CTGCTATGTGTTCCTCATCA	amplify *AaSPA1* ORF, fragment 3, 874
ORF-SPA1c-F4	CAAGTGTGGTCTGTTCATTG	amplify *AaSPA1* ORF, fragment 4, 853
ORF-SPA1c-R4	TGTCCGTTGTCATCAAAGTA	amplify *AaSPA1* ORF, fragment 4, 853
ORF-SPA1c-F5	GCGTGCAATTCTCTTCTTAT	amplify *AaSPA1* ORF, fragment 5, 786
ORF-SPA1c-R5	GTGAAAGATCCAAATCACAG	amplify *AaSPA1* ORF, fragment 5, 786

XmaI-AaSPA1-Fw	tcccCCCGGGACTAGCGGCatgGAA AGAGTTGGTGAAGAAAC	Clone *AaSPA1* into pGBK-T7 Y2H vector
PstI SPA1 Rev	AAAActgcagTCAAACAAGTTTTAGT AGCTTCA	Clone *AaSPA1* into pGBK-T7 Y2H vector, with stop condon
NotI-3`UTR-AaSPA1 -ohneStop-F1	CCTAGCGGCCGCCACAGATTCACT TTCCTGAAGAATCTGAG	Clone *AaSPA1* into pBS vector with *pAtSPA1*
cAaSPA1-NotI-R1	AGCTGCGGCCGCAACAAGTTTTA GTAGCTTCATGTTTC	Clone *AaSPA1* into pBS vector with *pAtSPA1*
SPA1-RGEN-A-Fw	TCGAAGAAACTTAGATATAGGTTTT AGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
SPA1-RGEN-A-Rv	CTATATCTAAGTTTCTTCGAACAAT CACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
SPA1-RGEN-B-Fw	CTTTGAAGAATATCTTCGCTGTTTT AGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
SPA1-RGEN-B-Rv	AGCGAAGATATTCTTCAAAGACAAT CACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
SPA1-RGEN-C-Fw	TATAGCAAGTTTGAAACATG GTTTTAGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
SPA1-RGEN-C-Rv	CATGTTTCAAACTTGCTATA ACAATCACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
SPA1-RGEN-D-Fw	CCGATAACTCTCTCAAGCTT GTTTTAGAGCTAGAAATAGCAA	Forward primer for sgRNA fragment with guide sequence
SPA1-RGEN-D-Rv	AAGCTTGAGAGAGTTATCGG ACAATCACTACTTCGACTCTAGCT	Reverse primer for U6p fragment with guide sequence
sg-1	ATATACTAGTCTAGAGAATGATTAG GCATCGAACCT	Forward primer for *U6p::sgRNA* cassette (Hyun et al., 2015)
sg-2	ATGCAGGAAGACAACTAGTCAA	Reverse primer for *U6p::sgRNA* cassette (Hyun et al., 2015)
RT-AaSPA1-F	TGACTCAGCCCACTCACAAG	AaSPA1 gene expression
RT-AaSPA1-R	CACGGGCCTCTTCTTATTCA	AaSPA1 gene expression
RT-AaSPA2-F	ACGCTGAAAGGAAAGGGAGT	AaSPA2 gene expression
RT-AaSPA2-R	GCCGCCAAAGGGATACTACT	AaSPA2 gene expression
RT-AaSPA3-F	CCTTGAGGGTTTGTGCAGAT	AaSPA3 gene expression
RT-AaSPA3-R	CTTGTTGACACCAGCAGTGG	AaSPA3 gene expression
RT-AaSPA4-F	TGGATTTGACCGTGATGGGG	AaSPA4 gene expression
RT-AaSPA4-R	ACCACTTAGCTTAGAGCGGC	AaSPA4 gene expression
RT-AaCOP1-F-1015	TTCGGACATTCAGAGGGCAC	AaCOP1 gene expression

RT-AaCOP1-R-1015	CGGGTCTCGTGATCTCCTTG	AaCOP1 gene expression
RT-AaUBI10-F	AAGGCCCCAAAACACAAACG	control for RT-PCR, length180
RT-AaUBI19-R	CGTCTCCGTGGTGGTTTCTA	control for RT-PCR, length180
AaSPA1-RTami-0416-F	ATGGAAAGAGTTGGTGAAGA	AaSPA1 gene expression in amiRNA silencing transformants
AaSPA1-RTami-0416-R	CTTCTTCCGGTGACTTATTC	AaSPA1 gene expression in amiRNA silencing transformants
AaCOP1-RTami-0416-F	CTACTAGACAGAGTCTTATG	AaCOP1 gene expression in amiRNA silencing transformants
AaCOP1-RTami-0416-R	TAAGCACACTTGCTTCTTGC	AaCOP1 gene expression in amiRNA silencing transformants
AaCO-RT-Fw	GCTCCGTGGTTGTTCACTAA	*AaCO* gene expression
AaCO-RT-Rv	CACAGGTGACCCCTTGATTC	*AaCO* gene expression
AaFT-F-11.16	CTACACTTTGGTTATGGTGGATC	*AaFT* gene expression
AaFT-R-11.16	TTCTTCTTCCTCCGCAGCCA	*AaFT* gene expression
AaLFY_RT_Fw_26.11	AGGAGGAACTCACGGCATTG	*AaLFY* gene expression
AaLFY_RT_Rv_26.11	GGTGTGTTGGGGATGGAGAG	*AaLFY* gene expression
AaAP1_RT_Fw_26.11	CTGATCCCACTGCTCGTGTT	*AaAP1* gene expression
AaAP1_RT_Rv_26.11	GCAGCAACTTGACACTGCTC	*AaAP1* gene expression
Aa_PIL1-F	TATGGGCGCATCAGTACCAC	*AaPIL1* gene expression
Aa_PIL1-R	ACTGCGGAGAGCAACTTTCA	*AaPIL1* gene expression
Aa_HFR1-F	GCCAAAATCCGGCGAATCAA	*AaHFR1* gene expression
Aa_HFR1-R	GGGAAACAAGGAACCAAACCG	*AaHFR1* gene expression
Aa_PIF4-F	GGGGGCTCCAATGATGTTCA	*AaPIF4* gene expression
Aa_PIF4-R	AACCCGCCGATGTATCTAGC	*AaPIF4* gene expression
Aa_XTR7-F	ACTTGTCGTCAGAAGGAGCG	*AaXTR7* gene expression
Aa_XTR7-R	TGTTGCCTTTCCCTTGAGCA	*AaXTR7* gene expression
Aa_YUC2-F	TTCAATGCTCGTCCTTCGCT	*AaYUC2* gene expression
Aa_YUC2-R	GGTCGAACTAGCCCTAACCG	*AaYUC2* gene expression
Aa_YUC8-F	TCCATGGTCGTTCGAAGCTC	*AaYUC8* gene expression
Aa_YUC8-R	TGGACCCATCTCTGGTCGTT	*AaYUC8* gene expression
Aa_YUC9-1-F	AGATATCGACGCGGTGGTTC	*AaYUC9_1* gene expression

Aa_YUC9-1-R	GATGTTAACGGCGTCTGCTG	*AaYUC9_1* gene expression
Aa_YUC9-6-F	TCCGGGGACGTAGAGATTGT	*AaYUC9_6* gene expression
Aa_YUC9-6-R	TACGGTAACCGGTAGCGAGA	*AaYUC9_6* gene expression
FISH geno1	CTGGGAATGGCGAAATCAAG	Genotyping SPA4 loci
SPA4 geno F1	GGTCAAGAAGCTTCCTCGTG	Genotyping SPA4 loci
SPA4 geno R1	TCATCATCAAGTCCTCCCAAG	Genotyping SPA4 loci

V.1.5 Bacterial strains

For standard cloning, *Escherichia coli* strain *DH5*! was used. For Gateway cloning of destination vectors, the *ccdB* gene resistant *Escherichia coli* strain *DB3.1* (Invitrogen) was used. *Agrobacterium tumefaciens* strain *GV3101 (pMP90RK)* was used for all plant transformations.

V.1.6 Cloning vectors

Plasmid vectors used in this study are listed as following:

Table V-3. Plasmid vectors used in this study.

Vector	Description	Purpose	Reference
pDONR221	Gateway Entry vector. For cloning of products using recombination (Introvigen). Kanamycin (50µg/ml) resistance.	Entry cloning of cDNAs	Introvigen
pGJ2169 GW	Gateway Destination vector. Contain 35S promoter. Spectinomycin (bacteria) and Basta (plant) resistance	Expressing *AaCOP1* gene	George Coupland
pBluescript KF	Binary vector, with *AtSPA1* promoter and 3' end	Expressing *AaSPA1* gene	Fittinghoff 2009 #705 in database
pPZP211	Agrobacterium transformation vector. Spectinomycin (bacteria) and Kanamycin (plant) resistance	Expressing *AaSPA1* gene	this study

pJET	Blunt cloning vector from MBI-Fermentas, St. Leon-Rot, Germany. Ampicillin resistance.	Blunt end cloning of PCR fragments	MBI-Fermentas
pRS300	Contains miR319a precursor in pBSK. Ampicillin resistance.	Template to generate amiRNA containing precursors	Schwab 2005
pRG_ext_CCR5	Contains SgRNA backbone.	Template to generate SgRNA backbone fragment	Cho et al., 2013
pYB196	CRISPR/Cas9 binary vector. Contains *ICU2p::GUS* construction, and Cas9 protein coding sequence. Kanamycin (bacteria) and Basta (plant) resistance.	Insert the different *U6p::sgRNA* constructs of *AaSPA1, AaCOP1*	Hyun et al., 2015
pCGAHah	Yeast two-Hybrid vector Contains GAL4-activation domain (ClontechTM). Resistance in *E.coli:* Ampicillin (100 µg/ml).	Contains AtPHYB, AaPHYB	Sheerin et al., 2015
pGBK-T7	Yeast two-Hybrid vector Vector for conventional cloning, used for Yeast Two-Hybrid analysis. Contains GAL4-binding domain (ClontechTM). Resistance in *E.coli:* Kanamycin (50 µg/ml)	Contains AtSPA1, AaSPA1	Sheerin et al., 2015

V.1.7 Plant lines

The accessions of *A. alpina,* and Arabidopsis mutant lines and transgenic lines used in this study are listed in as following:

Table V-4. Plant accessions and trangenic lines used in this study.

Plant Material	Source Reference
A. alpina Paj	Pajares, collected on Cardillera Cantábrica mountain region of Spain, Wang et al., 2009
A. alpina Tot	Totes Gebirge, collected by Frank Eikelmann, in the Totes Gebirge mountain range of Austria, Albani et al. 2012
A. alpina Wca	West Carpathians, collection from the Marburg botanic garden (SK-0-DR- 016546), Albani et al., 2012
A. alpina Dor	Dorfertal, Dorfertal valley in National Park Hohe Tauern, East Tyrol of Austria, latitude of 1650m, Albani et al., 2012
pep1 mutant	Homolog of *FLC*. Controls seasonal and perpetual flowering. Described in Wang et al., 2009.
cop1-4	McNellis et al., 1994
spa1spa2spa3spa4+/-	Fittinghoff et al., 2006; Balcerowicz et al., 2011
35S::AaCOP1 in *cop1-4*	This study
pAtSPA1::cAaSPA1-HA in *spa1spa2spa3*	This study
pAtSPA1::cAaSPA1-HA in *spaQn*	This study

V.2 Methods

V.2.1 Plant growth and transformation

V.2.1.1 Seed sterilization

For sterile growth of Arabidopsis and *A. alpina* seedlings on MS plates, seeds were surface-sterilized. For liquid sterilization seeds were surface-sterilized first with 70-80% Ethonal for 5 min, then with 2% Sodium hypochlorite (Colgate-Palmolive, Hamburg, Germany) for 3 min (*At*) or 8 min (*Aa*), and washed three times with sterile water, and plated on 1xMS medium. For dry seed sterilization, seeds were incubated with chlorine gas atmosphere (produced by adding 2.5ml 37% HCl to 80ml NaClO) for 4 h prior to plating.

V.2.1.2 Plant growth

For analysis of shade avoidance, sterilized Arabidopsis and *A. alpina* seeds were plated on MS medium supplemented with charcoal without sucrose. Plated seeds were stratified at 4°C for 7 d and subsequently moved to Wc for shade experiment. For dark-grown seedlings, plated seeds were incubated with approximately 5 h of Wc to induce germination, before moved to darkness. White light was produced by Fluora L58W/77 fuorescent tubes (Osram, Munich, Germany),

monochromatic FR was produced by LED light sources (Quantum Devices, Barneveld, WI, USA).

Prior of being sown on soil, *A. alpina* seeds were incubated at 4°C in darkness for 6 d in water. Seeds were sown in a substrate mixed by three parts soil and one part vermiculite. Plants were either grown in the greenhouse for reproduction (16 h light, 8 h darkness, 18°C), in walk-in light chambers for phenotype analysis (Johnson Controls, Milwaukee, WI, USA) under SD conditions (8 h light, 16 h darkness, 21° C) or LD conditions (16 h light, 8 h darkness, 21°C). The light in walk-in chambers were generated by LUmilux L36W/840 white fluorescent tubes. Alternatively, plant phenotypic analyses were perfromed in light chamber supplied by Percival Scientific.

V.2.1.3 *A.tumefaciens*-mediated stable transformation of *A. alpina*

Agrobacterium-mediated transformation of *A. alpina* plants was performed by adapting the transformation method from Arabidopsis previously described by Clough and Bent (1998). Agrobacterium strains were grown overnight in one Liter LB medium with antibiotics. Subsequently cells were harvested by 30 min centrifugation at 4,000prm, pellets were suspended in one liter transformation buffer (50g sucrose, 500µl silvet-L77, pH5.7), the OD (600) was measured to 0.8-1.0. For foral dip, *A. alpina* plants (*pep1* mutant) were grown in the greenhouse until as many unopened floral buds were developed as possible. Each plant was dipped into the transformation mixture for 2 min, and subsequently lay down in a tray with cover, and put into cool place overnight. After incubation, the cover was removed and plants were grown in the greenhouse to develop seeds. To increase transformation efficiency, more than 100 plants were used for each construct.

V.2.2 Bioinformatics and sequence alignment

The CLUSTAL W program was used for alignment and comparison of Arabidopsis and *A. alpina* COP1 and SPA homologs. All the other homolog sequences in this study were retrieved using NCBI BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All the sequence alignments were carried out as well as phylogenetic tree was generated using online resources at http://www.ebi.ac.uk/Tools/msa/clustalo/.

V.2.3 Screening of the transgenic plants

The cDNA of *AaSPA1* was introduced into the segregating *spa1 spa2 spa3 spa4/SPA4* mutants. According the Mendelian inheritance, the resulting T1 generation contains spa quadruple mutants, heterozygous *spa1 spa2 spa3 spa4/SPA4* mutants and the homozygous mutants, in a 1:2:1 fashion. However, approximately 1/25 of the resulting T2 plants were quadruple mutant (4 out of 108). These lines were named as #3, #6, #10, #20, with #3 and #10 failed to express

AaSPA1 thus excluded from the phenotype analyses. The analysis of the respective mutants were performed with the SPA4 geno F1, SPA4 geno R1, and Fish geno R1 primers to verify the alleles of *SPA4* locus.

V.2.4 Shade avoidance setup

For seedling experiments and all transcript determinations under shade, seeds were sterilized and plated on 1% black charcoal medium. Seeds were stratified at 4°C in darkness for 7 days and subsequently moved to Wc (50µmol/m^2/s) which is provided by white light LED sources. The R:FR ration in Wc is around 9.6 to 10.3 (Percival light chamber E-30B equipped with flora LEDs CLF, Plant Climatics GmbH, Germany). The simulated shade conditions with additional far-red light were generated by LED light sources in a chamber of identical construction (Model: E-30B with floral LEDs, CLF, Plant Climatics GmbH, Germany). The PAR was adjusted to be 50µmol/m^2/s but with a R:FR of 0.2 to 0.25.

For adult plant growth analysis, seeds were stratified in water and kept at 4°C for 7 d, before they were sowed on soil and put into chamber in a randomized fashion. Plants were grown in constant Wc at 21°C, 60% humidity for 7 d and were subsequently incubated in continuous low R:FR condition in the upper shelf or kept in the same Wc condition in the lower shelf. The low R:FR was generated by LED light sources (Quantum Devices, Barneveld, WI, USA). The white light PAR was kept at 50µmol/m2/s at both shelves, the R:FR ratio in Wc is around 9.6, while the R:FR in Wc+FRc is around 0.18-0.23.

All photon fluence rates and ratios were quantified using the *SpectroSense2+* (Skye Instruments, Powys, United Kingdom). The sensor was connected to a 1- channel white light sensor or a 4- channel sensor (red and far-red light specific sensors).

The spectral composition of the Wc and Wc+FRc conditions are shown in Figure V-1 (analyzed with spectrometer F600, Stellar Net).

V.2.5 Measurement of hypocotyl and petiole length

The hypocotyl length of seedling was determined by pressing them in 1% MS medium with black charcoal containing 1% agar, and documented with a NIKON D5000 digital camera (Nikon, www.nikon.com). Measurements of hypocotyl length, petiole length and leaf length were carried out with *ImageJ* 1.43u software (Wayne Rasband National Institutes of Health, USA). The measured values were statistically processed with Excel. For the determination of the length of leaf blade, the total leaf length was measured to subtract the petiole length. For each measurement, at least 15 seedlings were included.

V.2.6 Determination of flowering time

The flowering time of all *A. alpina* accessions was determined at the day the white petals emerge from the inflorescence buds which are visible to the unaided eye. The flowering time was expressed in the number of days to flower from the day of sowing, or in the number of true leaves. At least ten plants were analyzed for each accession in each condition. The flowering experiment under SD and LD conditions was performed individually for two times with similar results.

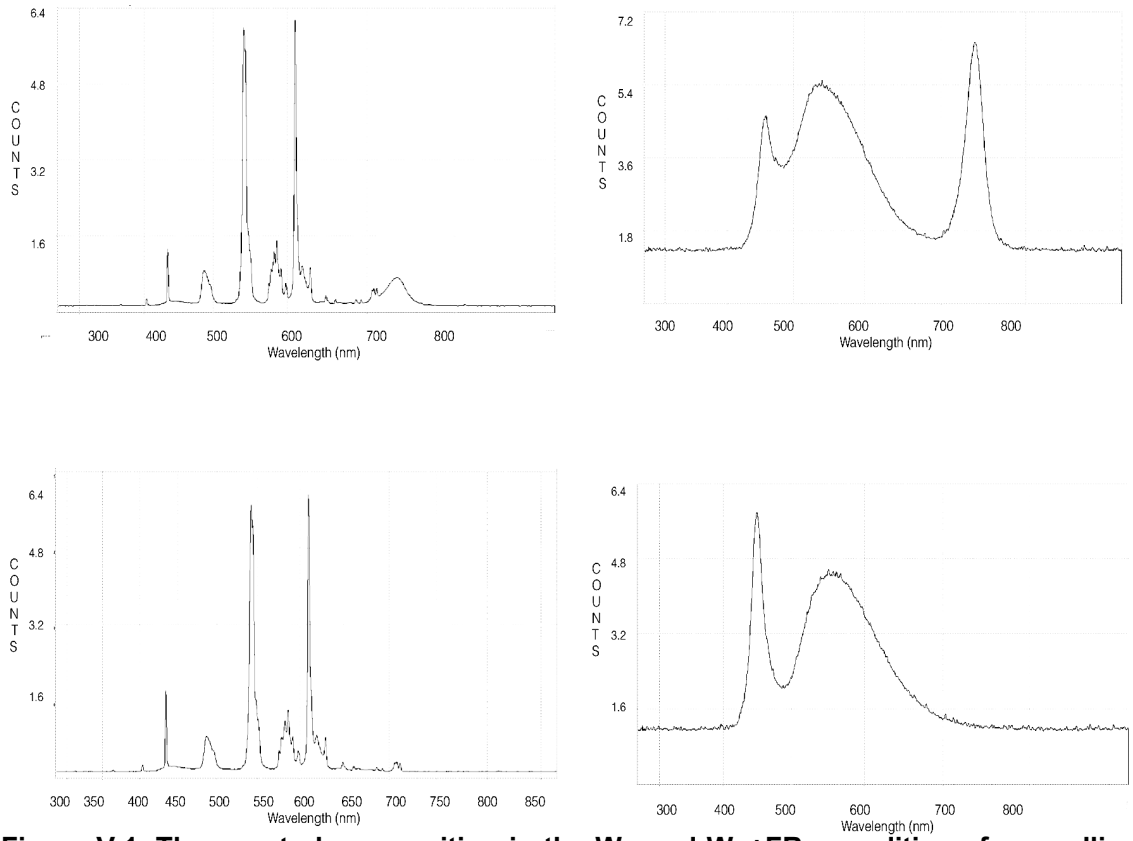


Figure V-1. The spectral composition in the Wc and Wc+FRc conditions for seedling and adult plant growth. The fluences between 300nm and 800nm wavelengths were plotted. (A, C) Spectral composition of the Wc and Wc+FRc conditions used for adult plant growth. (B, D) Spectral compositon of the Wc and Wc+FRc conditions used for seedling experiments.

V.3 Molecular biology methods

V.3.1 Standard molecular biology methods

Standard molecular biology methods, such as DNA gel-electrophoresis, nucleic acid precipitation and staining of DNA fragments, were all performed according to protocol of Sambrook & Russel (2001).

V.3.2 Polymerase chain reaction (PCR)

PCRs were performed with 100ng of genomic DNA from plants, 1µl cDNA or 100ng of plasmid DNA as templates. The reaction tube was in a volume of 20µl when using home-made Taq polymerase (1:20), and 25µl for Q5 polymerase (1:100) protocol.

Standard PCR process consisted of a first step of denaturation by 95°C for 5 min, followed by 40 cycles of denaturing at 95°C for 30 sec, annealing at 56°C for 30 sec and elongation at 72°C for 1 min / 1 kb (72°C for 30 sec / 1 kb for Q5 polymerase).

V.3.3 DNA sequencing and Management

DNA sequences was verified by sequencing at GATC (Konstanz, Germany). The quality of the sequencing results was controlled by examining with the *4peaks* software (Mekentosj B.V., Amsterdam, The Netherlands).

The sequence data were analyzed, edited and stored by *Lasergene*® (DNASTAR, Madison, USA) software packages.

V.3.4 Extraction of total plant RNA

Total RNA from *A. alpina* seedlings or adult plants were obtained by using the *RNeasy Plant Mini Kit* (Qiagen, Hilden, Germany), according to the manufacturer's manual for plant tissue. The concentration of the total RNA was determined in 1 µl of the extract using a Nanodrop® spectrophotometer (Thermo Scientific). The integrity of the total RNA was analyzed on a 2% agarose gel, checking for the visibility of the characteristic rRNA bands.

V.3.5 Reverse transcription of plant mRNA

1µg of total RNA was DNase treated in a 20 µl reaction, with 1 µl DNase (TURBO DNase™, 2 U/µl), 2µl DNase buffer (10x TURBO DNase™ buffer), RNase-free ddH_2O for 1 h at 37°C.

2 µl of EDTA (50mM) was added and to deactivate the enzyme at 75°C for 10 min. 2 µl of the digested RNA was then used for PCR reaction to check the RNA was complete free of contaminated DNA. oligo$(dT)_{18}$ primers were added to the 20 µl of RNA and denatured at 70°C for 10 min. The reaction tube was then put on ice and reverse transcriptase mix (4 µl of 10 mM dNTPs; 8 µl of 5x reverse transcriptase buffer; 1 µl of RevertAID™ H Minus M-MuLV reverse transcriptase (Fermentas)) was added. The sample was put in PCR machine with the process of 5 min at 37°C, 1 h at 42°C and 10 min at 70°C. The synthesized cDNA was stored at -20°C.

V.3.6 Quantitative RT-PCR (qRT-PCR)

1 µl of cDNA from *A. alpina* was used as the template in a 10 µl qRT-PCR reaction including 5 µl POWER SYBR Green PCR mix (KAPA^{TM} SYBR Fast (2X), ABI Prism, Germany), 0.25 µl of each gene specific primer (10 µM) and 3.5 µl of autoclaved ddH_2O. The qRT-PCR was performed and analyzed by the 7300 Real-Time PCR System (Applied Biosystems) or the QuantStudio5 system (Applied Biosystems, Thermo Fisher Scientific). Two to three biological replicates and two technical replicates were used for each analysis. Ct values obtained from the detection were statistically evaluated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). *AaUBI10* was used as the endogenous control.

V.3.7 Cloning

Conventional DNA cloning was performed following standard protocols as described in Sambrook & Russell (2001). Conventional cloning was employed to clone *AaSPA1* in the binary vector pBluescript KS to express *AaSPA1* under *AtSPA1* promoters. *AaSPA1* and *AaphyB* was also cloned into the Yeast-two-Hybrid vectors pCGAHah and pGBK-T7, respectively, through conventional cloning.

All other cloning were performed employing Gateway cloning. BP and LR reaction were performed according to the manufacture's protocol (Invitrogen).

V.3.8 Cloning strategies

V.3.8.1 Generation of *pAtSPA1*::*cAaSPA1*-HA construct

First the cDNA of *AaSPA1* was amplified with the ORF-SPA1 primers from synthesized light-grown *A. alpina* seedling cDNA. The generated cDNA was cloned into pJET and sent for sequencing for the sequence correctness. The *AaSPA1* cDNA was then amplified without the stop codon with the primer pairs cAaSPA1-NotI-R1 and ApaI-cAaSPA1-F1 introducing the 3' ApaI and the 5' NotI restriction site. Subsequently, the AaSPA1 was digested by restriction enzymes ApaI and NotI and ligated into the pBS-KF vector (#705 in plasmid database). The pBS-KF vector contains a 2260bp *AtSPA1* promoter, and 672bp 3' UTR downstream of *AtSPA1*. The construct was then digested with NotI, and the triplicate HA, which was amplified with specific primers carrying both NotI recognition sites and with an artificial stop codon at the end of its sequence, was ligated into the NotI restriction sites. HA insertion was verified by restriction analysis and sequencing.

The *pAtSPA1*::*cAaSPA1*-HA construct was then digested with KpnI restriction enzyme, and ligated into the binary vector pPZP211 for plant transformation.

V.3.8.2 Generation of *35S:AtCOP1/AaCOP1* constructs

The *AaCOP1* cDNA was amplified first with ORF-AaCOP1 primers, from synthesized cDNA of light-grown *A. alpina* Paj seedlings. The *AaCOP1* cDNA was subsequently cloned into pJET for restriction digest and sequencing to confirm the sequence correctness. Then *AaCOP1* cDNA was amplified using gene-specific primers with the attached attB sites and cloned into pDONR221 entry vector. For *AtCOP1*, an existing entry clone was used. The entry clones were then recombined with pGJ2169GW destination vector, which contains *35S* promoter before the gateway cassette.

V.3.8.3 Generation of constructs containing amiRNA

Four oligonucelotide sequences were designed to engineer the artificial microRNA into the endogenous miR319a precursor by site-directed mutagenesis, as described in Schwab et al., 2006. To target *AaSPA1* and *AaCOP1*, the amiRNA was designed manually by fullfiling the requirements and listed as following:

The amiRNA-containing precursor was generated by overlapping PCR as described in Schwab et al., 2006.

The generated miR319a precursor containing amiRNA targeting *AaSPA1* and *AaCOP1* were subsequently cloned into entry vector pDONR221, and then into the destination vector pGJ2169GW for plant transformation.

V.3.8.4 Generation of constructs with the RNA-guided endonuclease (RGEN) derived from the CRISPR/Cas9 protein system

To construct a binary vector for RGEN, pYB196 was used (kindly provided by Dr. Youbung Hyun). pYB196 contains a *ICU2* promoter and the downstream Cas9 protein coding sequence. To generate U6p::sgRNA, overlapping PCR was performed using two amplified products of U6p and sgRNA PCR fragemtns as templates after purification (described in detail in Hyun et al., 2015). The amplified products were directly cloned into pYB196 by conventional cloning. The sequences designed were listed in Table V-2. The generated constructs were listed in Figure V-2.

V.3.9 Bacterial transformation and plasmid isolation

Escherichia coli competent cells were transformed by heat shock method and then cells were plated on selective media and kept at 370C overnight. Agrobacteria cells were transformed using electroporation and then cells were plated on LB media with appropriate antibiotics and incubated at 28°C for two days.

Plasmid DNA from *E. coli* in miniprep or midiprep scale was isolated using Plasmid minikit or midikit (Qiagen) following manufacturer's instructions.

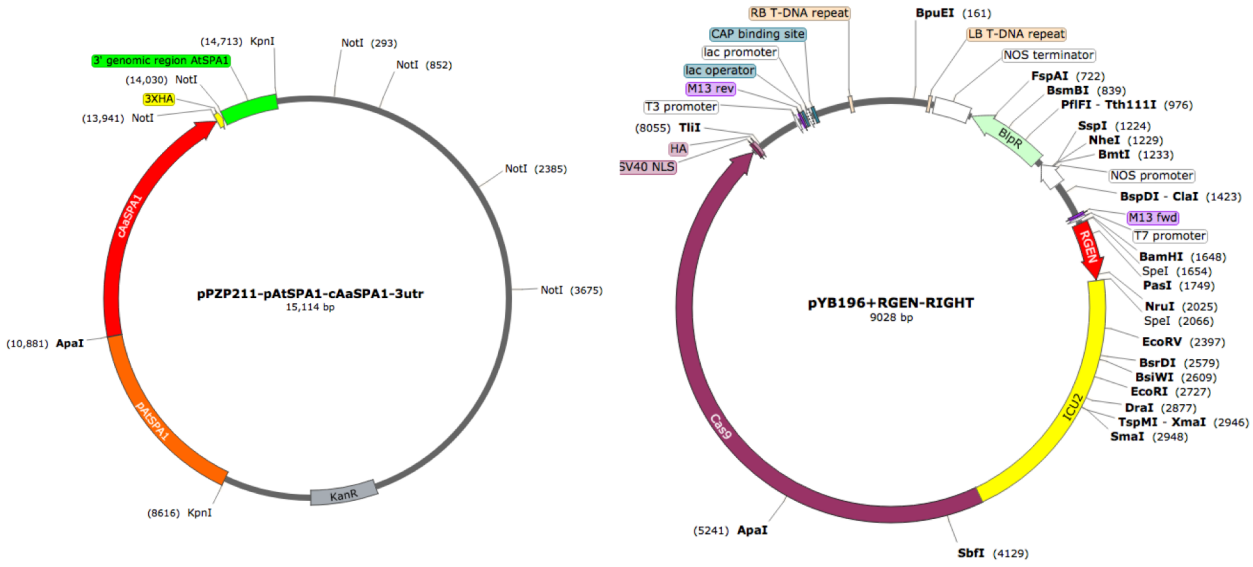


Figure V-2. *pAtSPA1::cAaSPA1* construct and the pYB196+RGEN construct. The insertion site of the sequences indicated in red.

V.3.10 Plasmid DNA manipulations

The correctness of PCR-generated cloned DNA fragments was determined by appropriate restriction enzyme digestion followed by sequencing (GATC, Konstanz). The sequence alignment analysis was performed using Vector NTIsuite software (Invitrogen). Constructs were also designed by using Vector NTIsuite software (Invitrogen).

VI. Supplement

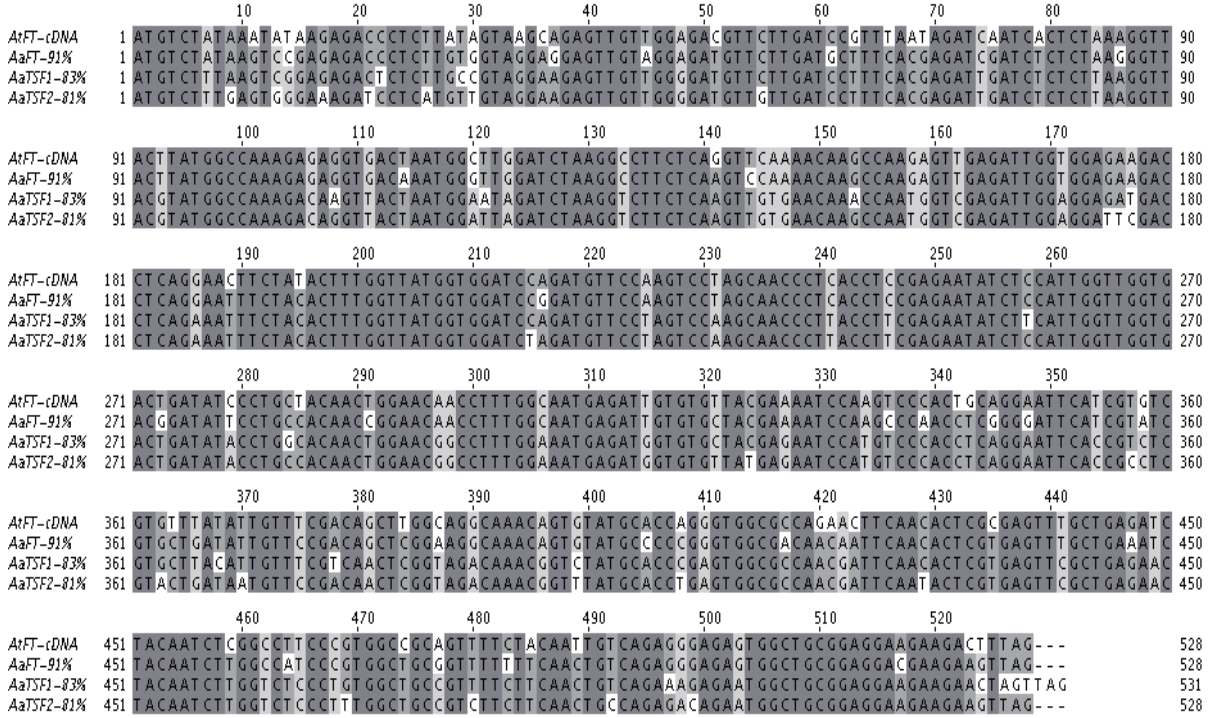


Figure S1. CDS alignment of *AaFT*, *AaTSF1*, *AaTSF2* with *AtFT*. *AaFT* is used in the present study.

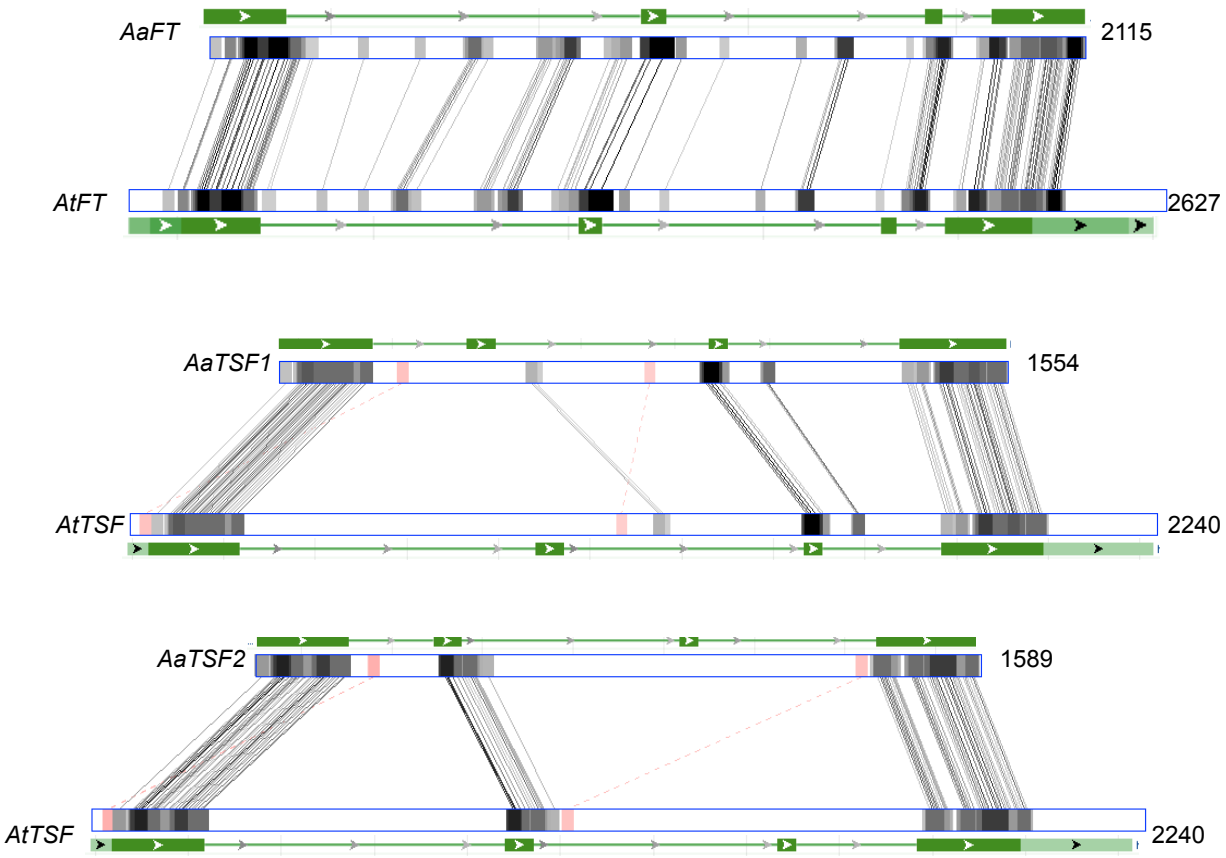


Figure S2. Comparison of genomic regions of *AaFT*, *AaTSF1*, *AaTSF2* with their counterparts in Arabidopsis, residing on chromosome 1, 7, 3, of *A. alpina* Paj respectively. Black to grey lines indicate homologous regions in +/+ direction, light to dark red lines represent inversions.The intensity of the lines relates to the alignment score achieved for the alignment of sequence paris connected by the lines. The green rectangles represents the genomic organization known (At) or predicted (Aa) from NCBI database. Plots generated by GATA plotter.

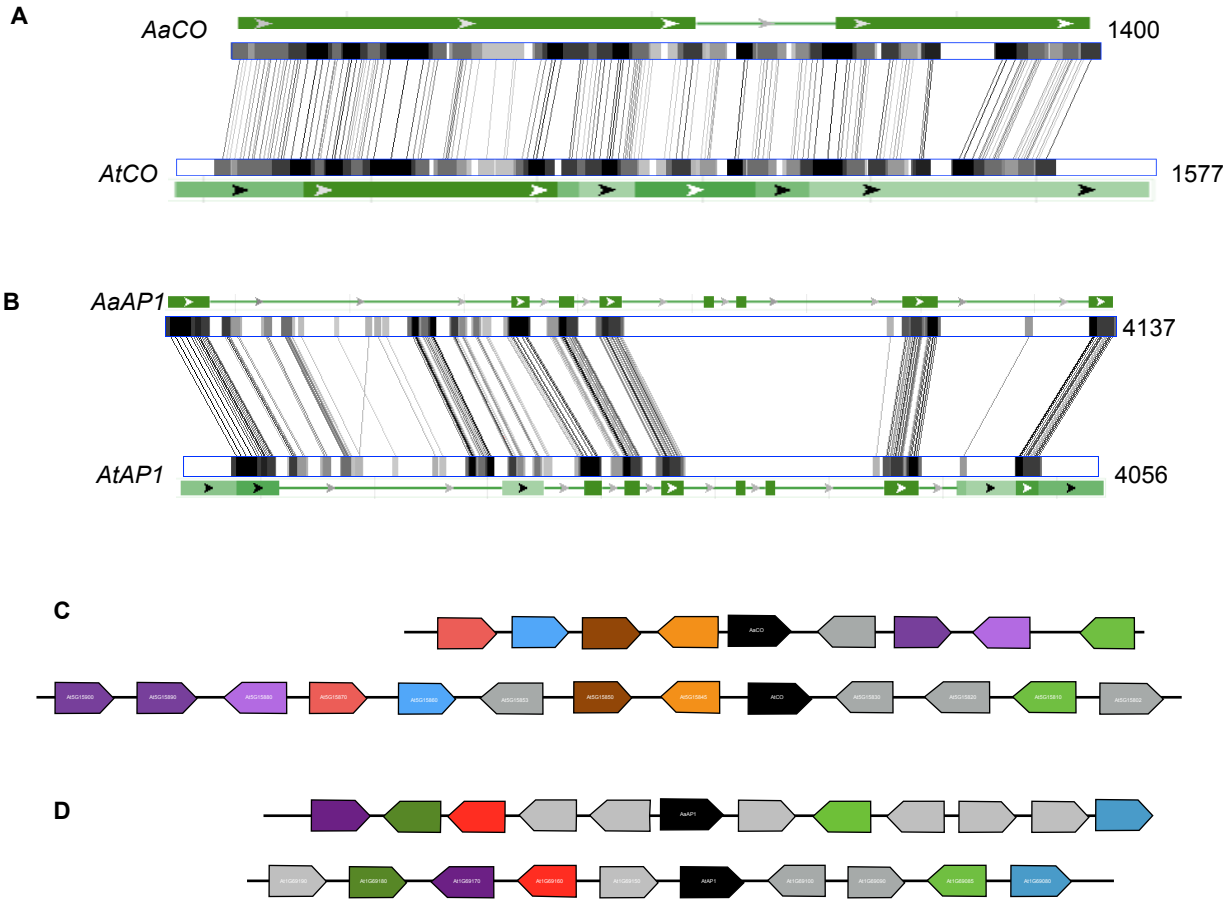


Figure S3. Comparison of genomic regions of *AaCO* and *AaAP1* with their counterparts in Arabidopsis (A, B) and the conserved synteny around *AaCO* (C) and *AaAP1* (D). (A, B) Plots generated by GATA plotter. (C, D) Schematic view of the conserved synteny around AaCO and AaAP (upper lane) in comparison to their counterparts in Arabidopsis (low lane). Grey boxes indicates the genes have no corresponding homologs, color boxes represent different genes that are conserved. The direction of the boxes indicates the direction of the genes. The distance between genes does not represent the actual distance in the respective genome.

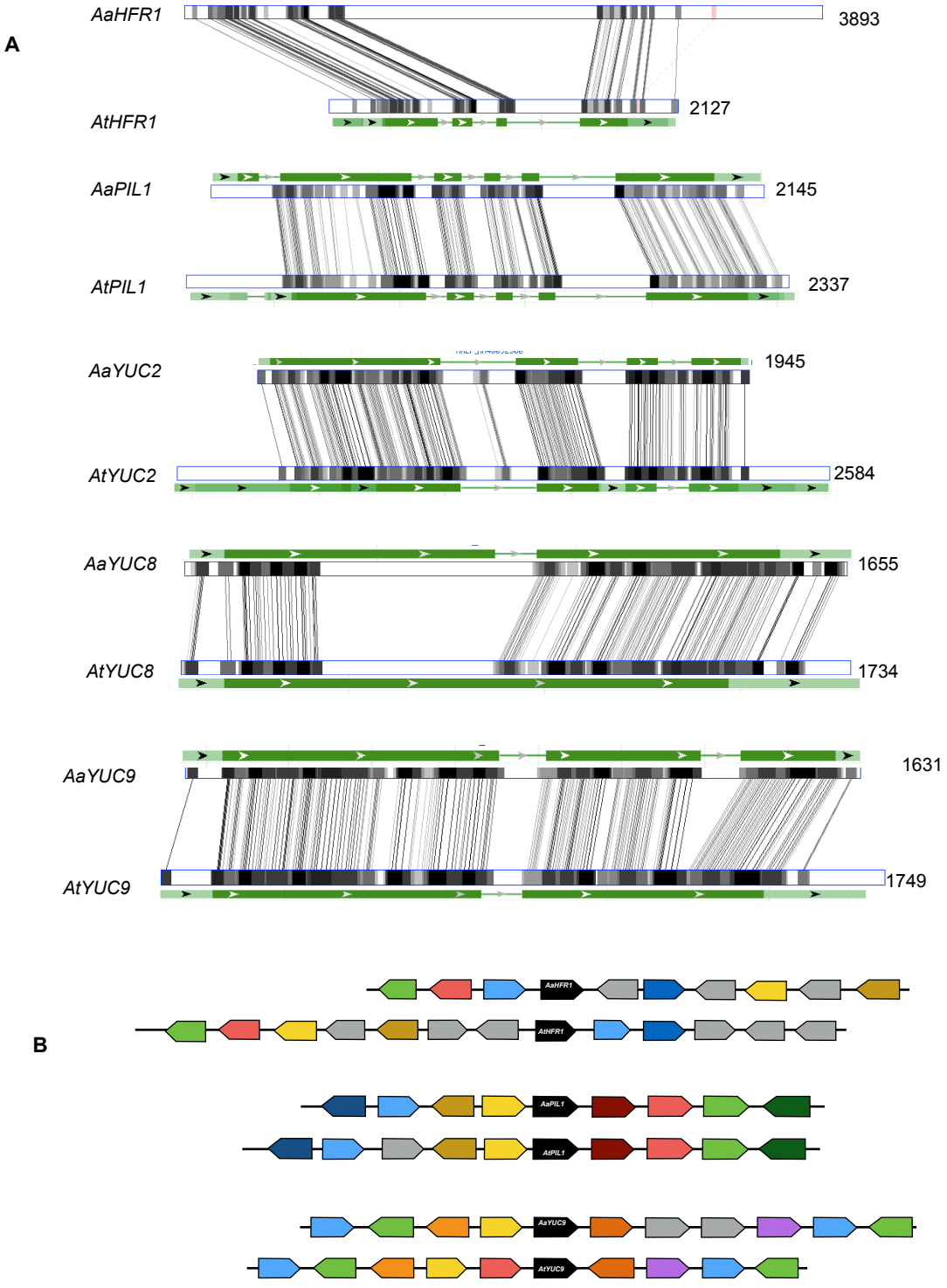


Figure S4. Comparison of genomic organization (A) and synteny (B) of *AaHFR1*, *AaPIL1*, *AaYUC9*, *AaYUC2* and *AaYUC8* with their counterparts in Arabidopsis.

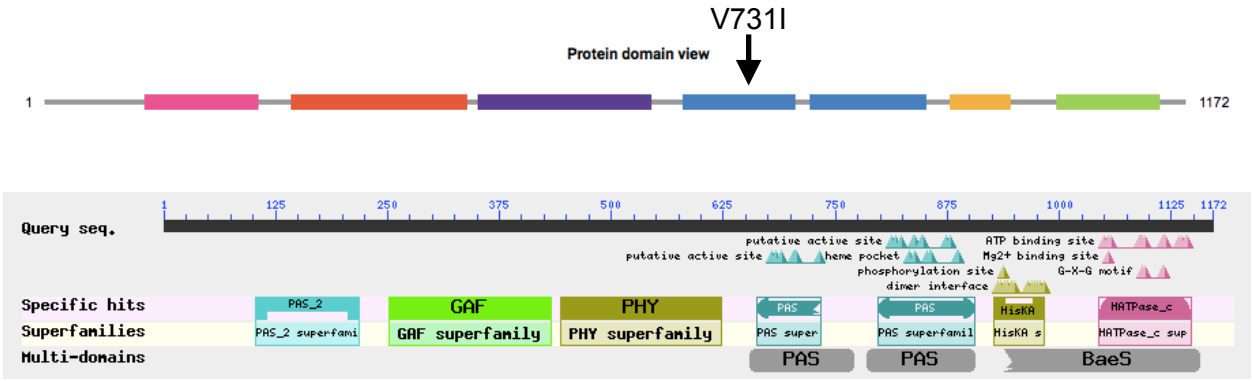


Figure S5. Protein sequence of AaPHYB in Tot, generated by SMART. Arrow indicates amino acid change of AaPHYB in Tot in comparison to Paj.

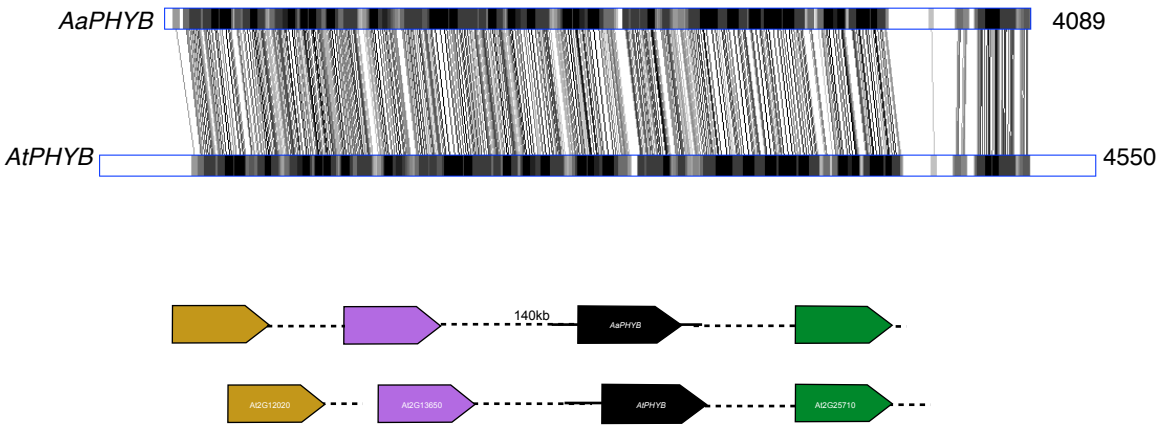


Figure S6. Comparison of genomic organization of AaPHYB with AtPHYB, and the conserved synteny.

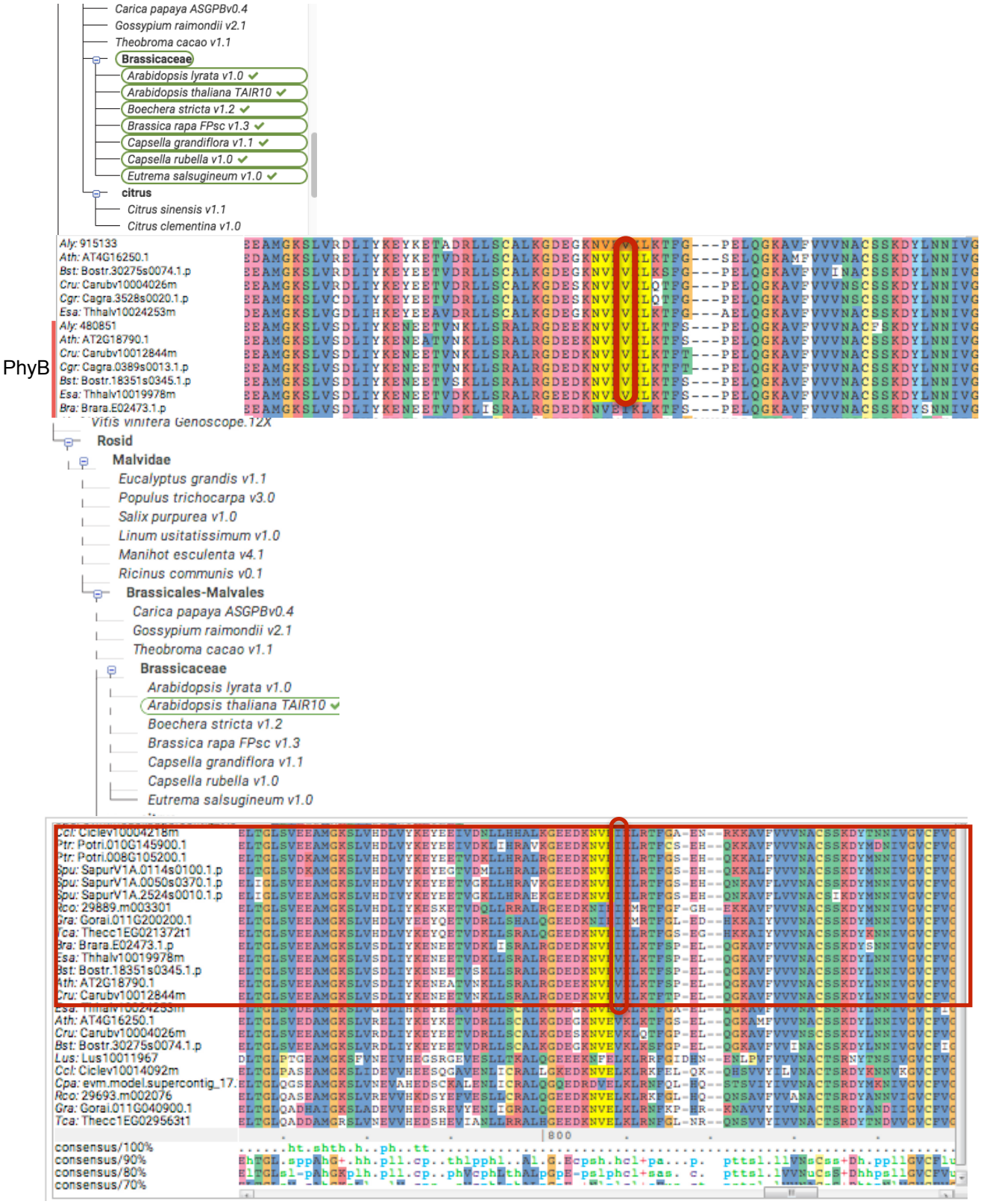


Figure S7. Protein sequence alignment of AaPHYB in Tot with other species in Brassicaceae family. The amino acid change V to I, marked with red lines, is very common in other species.

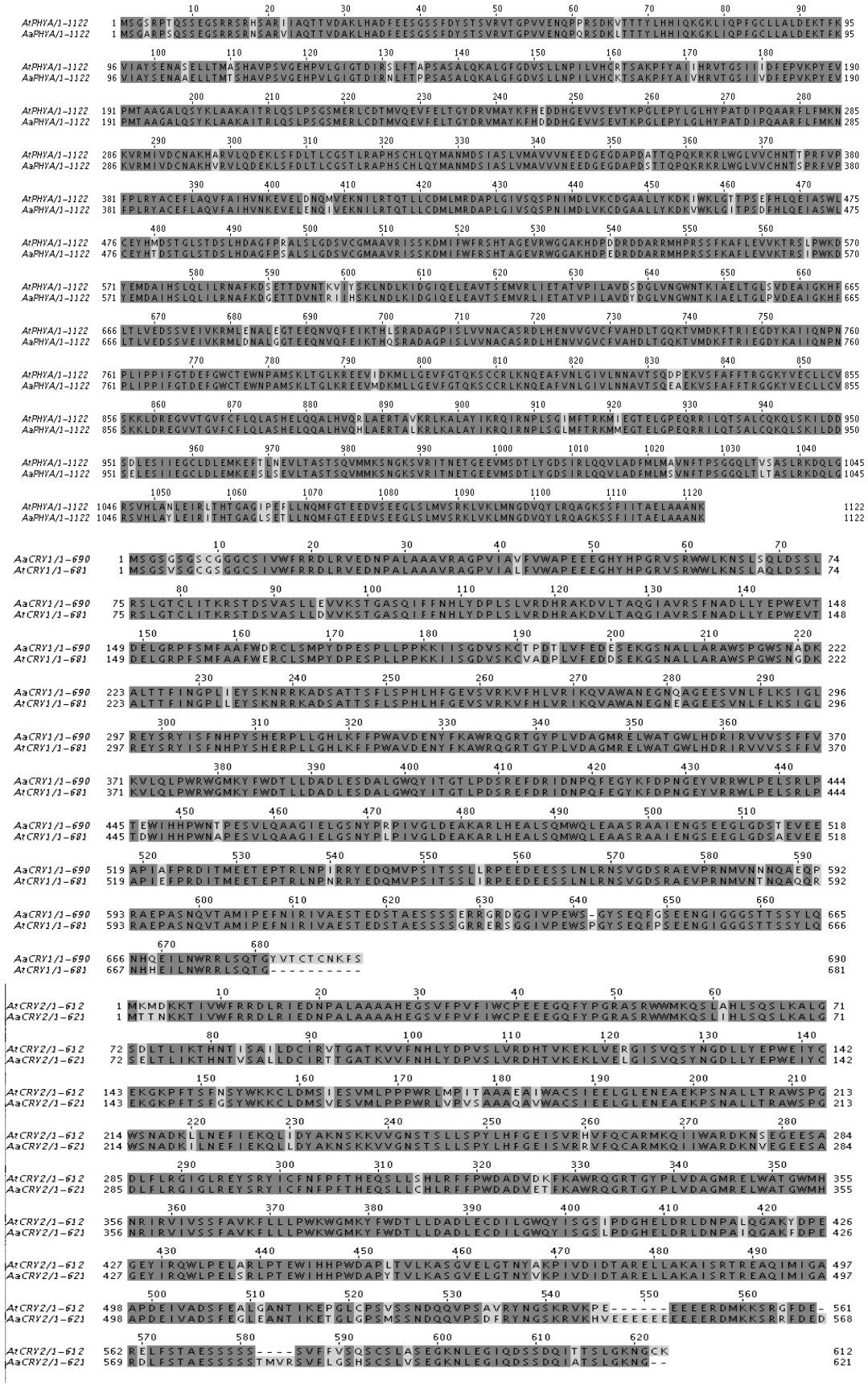
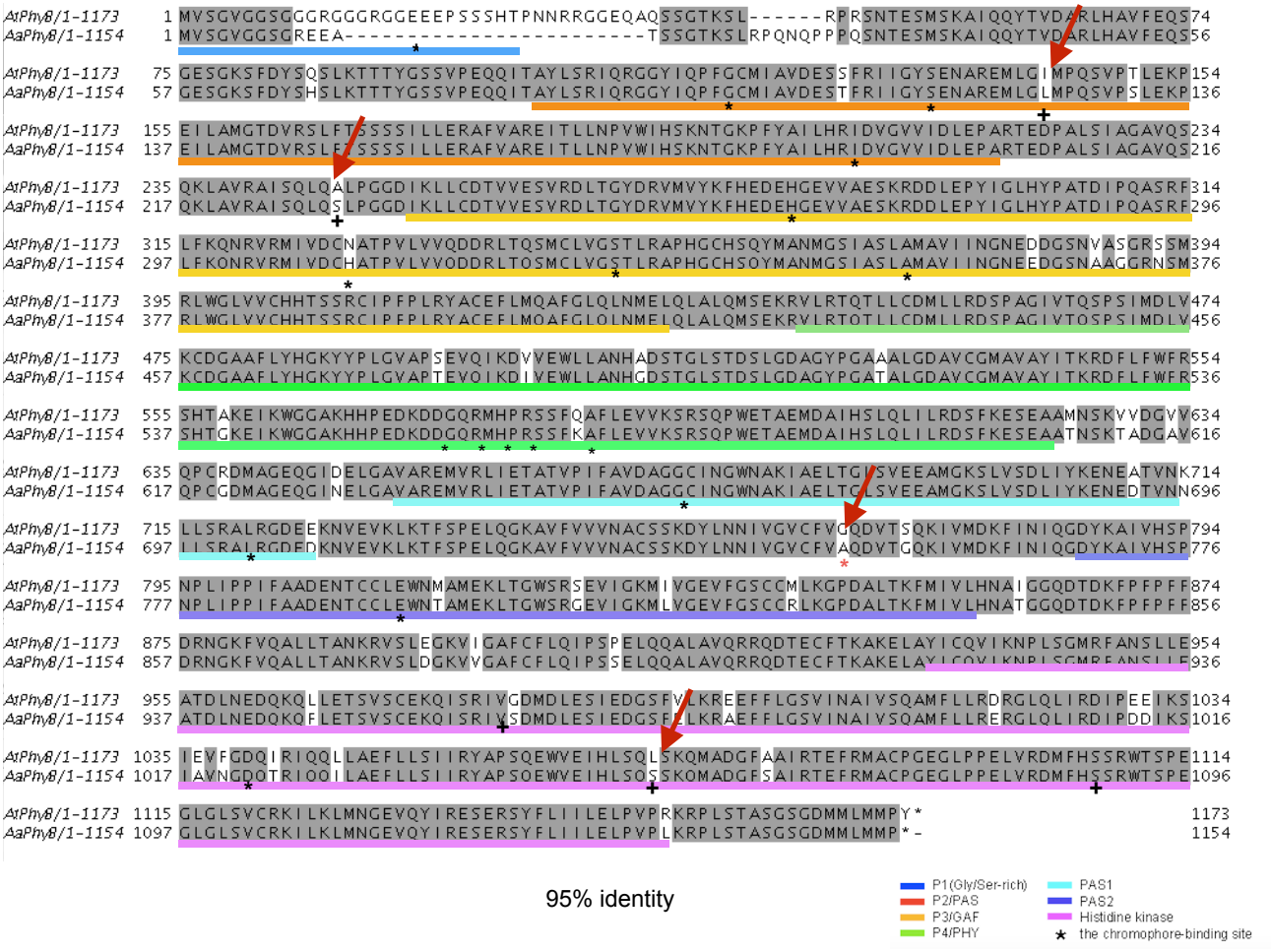


Figure S8. Protein sequence alignment of phyA (96%), cry1 (96%) and cry2 (93%) in *A. alpina* with their corresponding counterparts in Arabidopsis.



*, predicted conserved residues in active PHYBs (see also Bae and Choi, 2008),
+,single amino acid changes previously linked to the PHYB function (Filiault et al., 2008)

Figure S9. Protein sequence alignment of AaPHYB with AtPHYB.

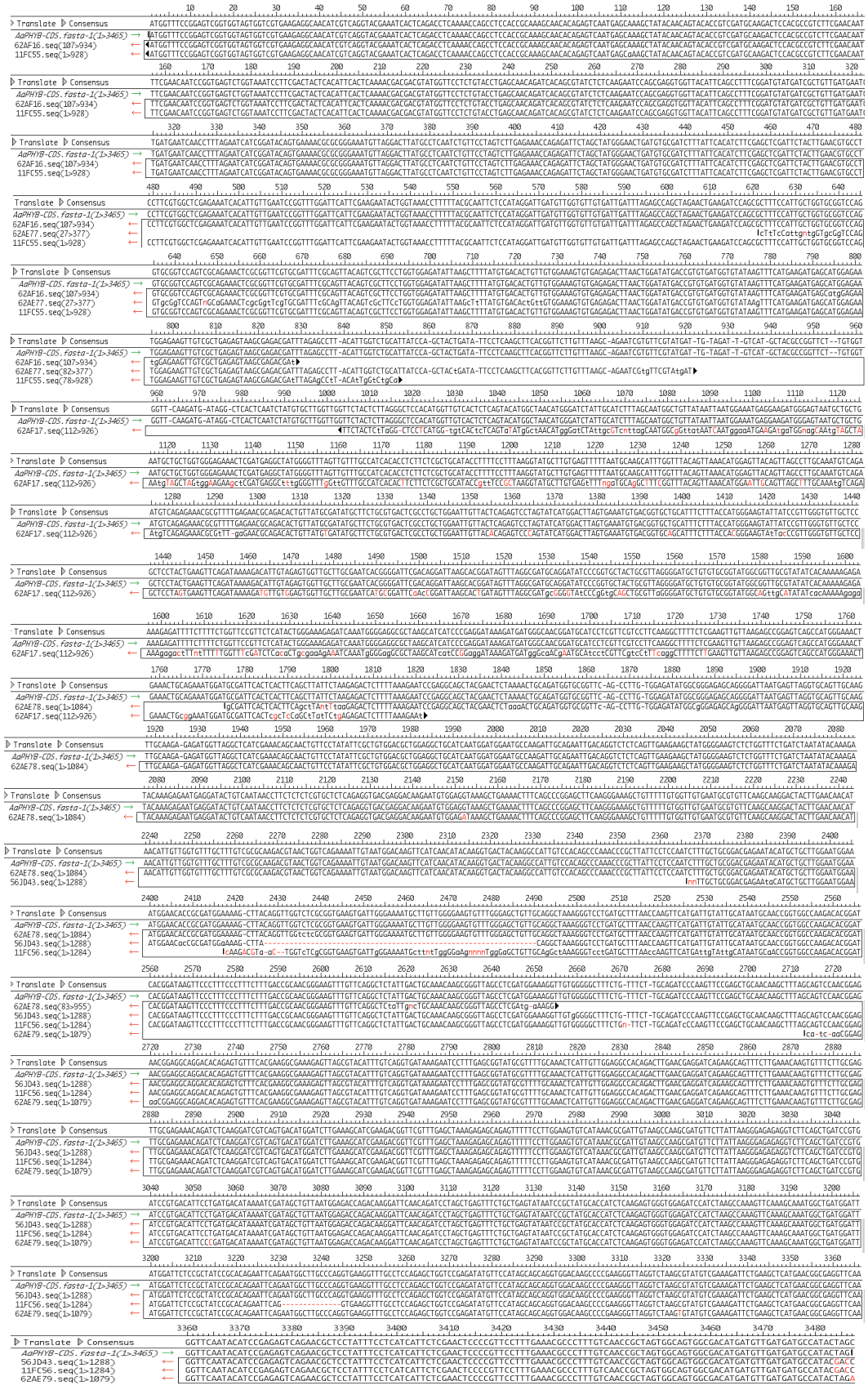


Figure S10. The amplified CDS sequence of *AaPHYB* in Wca, verified by GATC sequencing with different primers and aligned to the predicted Paj *AaPHYB* sequence.

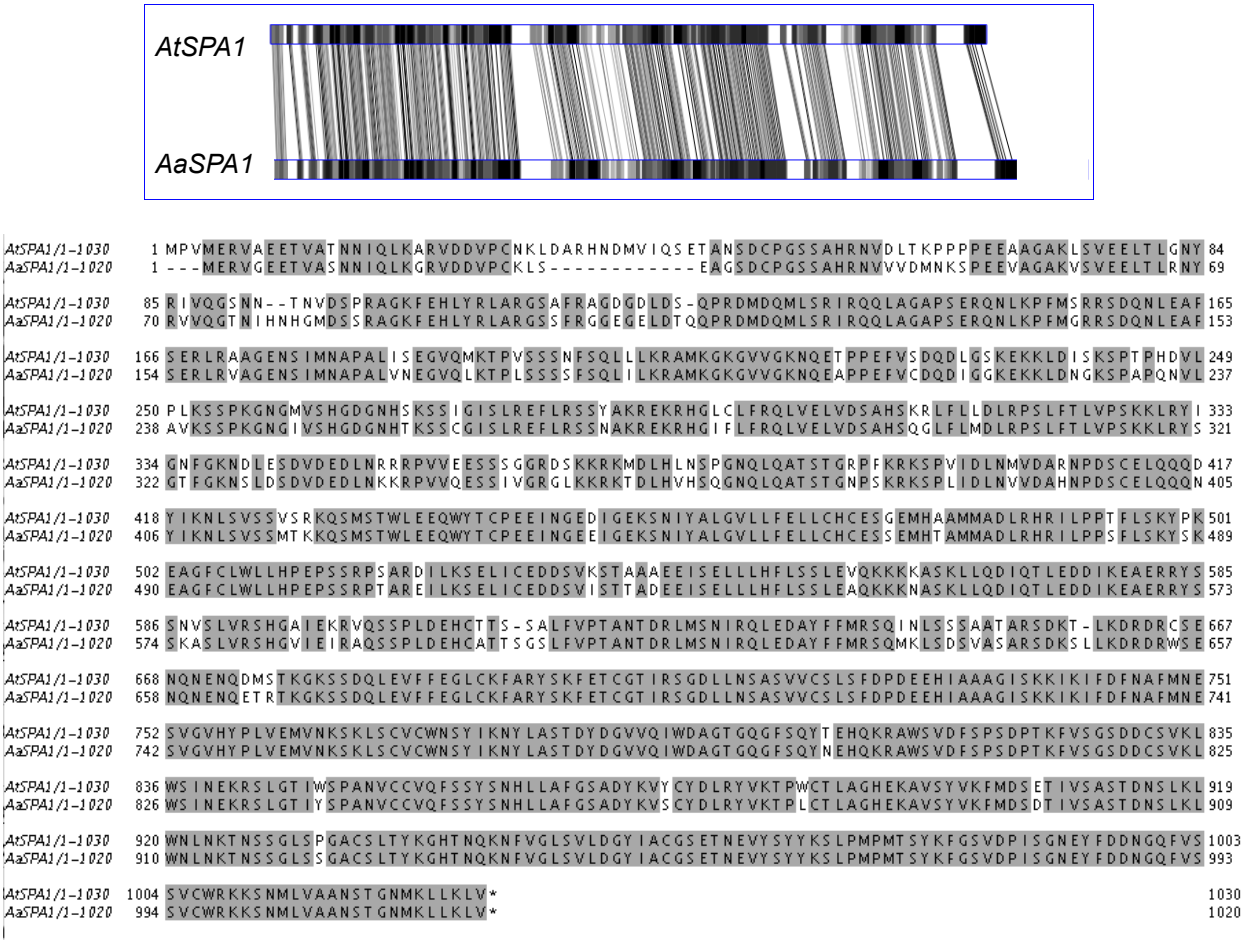
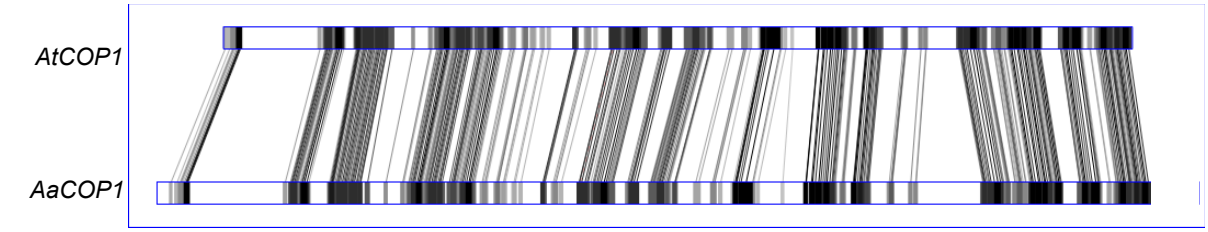


Figure S11. Genomic organization and protein sequence alignment of AaSPA1 with AtSPA1.

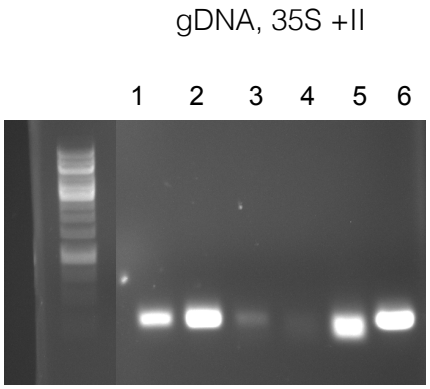



```
AtCOP1/1-676    1 MEEISTDPVVPAVKPDPRTSSVGEGANRHENDDGGS---GGSEIGAPDLDKDLLCPICMQIIKDAFLTACGHS 70
AaCOP1/1-674    1 MEEI---STVPAVKPDPRTTSVGEGVHRHENDDGGSGGDGGSEIGALDLDKDLLCPICMQIIKDAFLTACGHS 70

AtCOP1/1-676   71 FCYMCIITHLRNKSDCPCCSQHLTNNQLYPNFLLDKLLKKTSARHVSKTASPLDQFREALQRGCDVSIKEVDN 143
AaCOP1/1-674   71 FCYMCIITHLRNKSDCPCCSQHLTNNQLYPNFLLDKLLKKTSARHVSKSASPLDQFREALQRGCDVSIKEVDN 143

AtCOP1/1-676  144 LLTLLAERKRKMEQEEAERNMQILLDFLHCLRKQKVDELNEVQTDLQYIKEDINAVERHRIDLYRARDRYSVK 216
AaCOP1/1-674  144 LLSLLAEKKRKMEQEEAERNMQILLDFLHCLRKQKVDELNEVQTDLQYIKEDINAVERHRIDLYRARDRYSVK 216

AtCOP1/1-676  217 LRMLGDDPSTRNAWPHEKNQIGFNSNSLSIRGGNFVGNYQNKKVEGKAQGSSHGLPKKDALSGSDSQSLNQST 289
AaCOP1/1-674  217 LRMLGDDPSARNAWPLEKNHTGFNSNSLSMRGGNSF-NFQNKKVEGKAQGSSHGLQKKDALSGSDSQSLNQST 288

AtCOP1/1-676  290 VSMARKKRIHAQFNDLQECYLQKRRQLADQPNSKQENDKSVVRREGYSNGLADFQSVLTTFTRYSRLRVIAEI 362
AaCOP1/1-674  289 VSMARKKRIHAQFNDLQECYLQKRRQLVDQPLIKQEGDKSVVRREGYSNGLADFQSVLTTFTRYSRLRVIAEI 361

AtCOP1/1-676  363 RHGDIFHSANIVSSIEFDRDDELFATAGVSRCIKVFDFSSVVNEPADMQCPIVEMSTRSKLSCLSWNKHEKNH 435
AaCOP1/1-674  362 RHGDLFHSANIVSSIEFDRDDELFATAGVSRCIKVFDFSSVVNEP-DTQCPIVEMSTRSKLSCLSWNKHEKNH 433

AtCOP1/1-676  436 IASSDYEGIVTVWDVTTRQSLMEYEEHEKRAWSVDFSRTEPSMLVSGSDDCKVKVWCTRQEASVINIDMKANI 508
AaCOP1/1-674  434 IASSDYEGIVTVWDVTTRQSLMEYEEHEKRAWSVDFSRTEPSMLVSGSDDCKVKVWCTRQEASVLNIDMKANI 506

AtCOP1/1-676  509 CCVKYNPGSSNYIAVGSADHHIHYYDLRNISQPLHVFSGHKKAVSYVKFLSNNELASASTDSTLRLWDVKDNL 581
AaCOP1/1-674  507 CCVKYNPGSSNYIAVGSADHHIHYYDLRNISQPLHVFSGHKKAVSYVKFLSNNELASASTDSTLRLWDVKDNL 579

AtCOP1/1-676  582 PVRTFRGHTNEKNFVGLTVNSEYLACGSETNEVYVYHKEITRPVTSHRFGSPDMDDAEEEAGSYFISAVCWKS 654
AaCOP1/1-674  580 PVRTFRGHTNEKNFVGLTVNSEYLACGSETNEVYVYHKEITRPVTSHRFGAPEMDDAEEEAGSYFISAVCWKS 652

AtCOP1/1-676  655 DSPTMLTANSQGTIKVLVLAA* 676
AaCOP1/1-674  653 DSPTMLTANSQGTIKVLVLAA* 674
```

Figure S12. Comparison of genomic organization and protein sequence alignment of AaCOP1 with AtCOP1.

Figure S13. Protein sequence alignment of AaSPA2 (85%), AaSPA3 (90.6%) and AaSPA4 (86%) with their counterparts in Arabidopsis.

A



B

1	UBI10	Undetermined	1	SPA1	Undetermined
2	UBI10	19.6983	2	SPA1	31.3159
3	UBI10	16.2219	3	SPA1	25.0123
4	UBI10	16.8915	4	SPA1	25.0511
5	UBI10	16.1335	5	SPA1	24.1908
6	UBI10	16.8216	6	SPA1	24.4283
8	UBI10	16.2863	8	SPA1	24.0366
9	UBI10	15.3112	9	SPA1	23.318
10	UBI10	16.4517	10	SPA1	24.666
11	UBI10	16.2975	11	SPA1	24.9111
12	UBI10	16.551	12	SPA1	23.9956
13	UBI10	16.5344	13	SPA1	24.12

Figure S14. Transformants of amiRNA-mediated silencing of *AaSPA1* (A) analyzed with primer pair 35S+II, and the expression level of *AaSPA1* in transformants by RT-PCR (B).

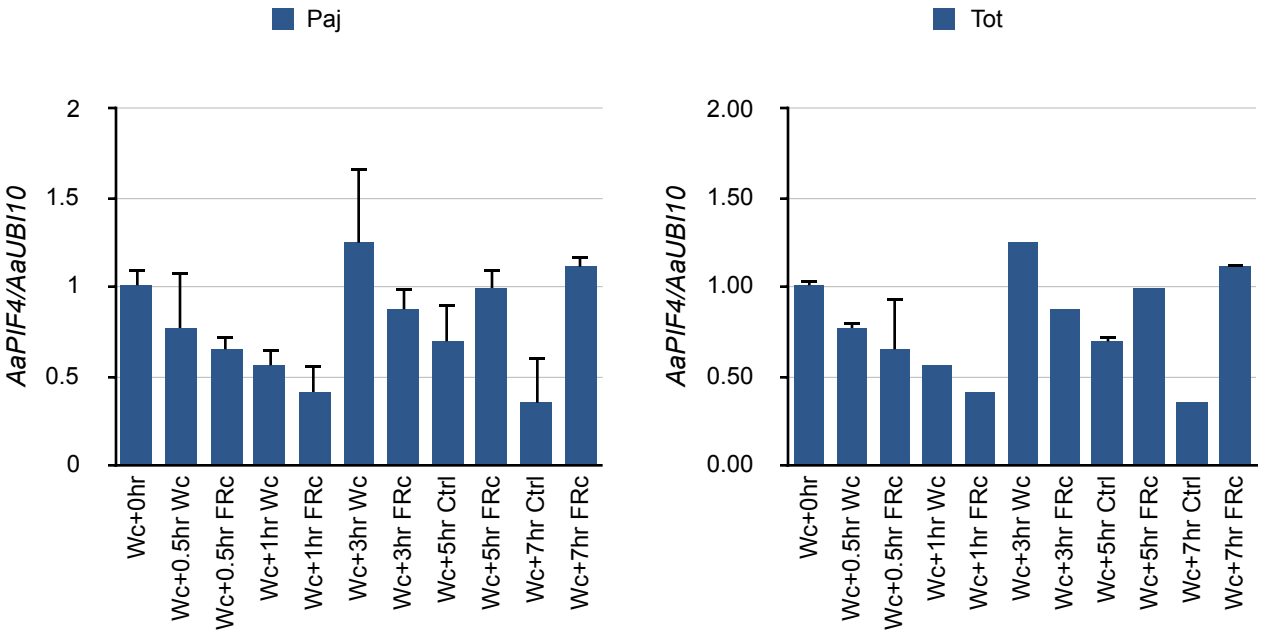


Figure S15. The expression of *AaPIF4* in *A. alpina* Paj and Tot .

VII. References

Adrian, J., Farrona, S., Reimer, J. J., Albani, M. C., Coupland, G., & Turck, F. (2010). cis-Regulatory elements and chromatin state coordinately control temporal and spatial expression of *FLOWERING LOCUS T* in Arabidopsis. The Plant Cell, 22(5), 1425–1440.

Ahmad, M., & Cashmore, A. (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature, 363, 210–211.

Ahmad, M., Jarillo, J. A., Cashmore, A. R., The, S., Cell, P., Feb, A., Ahmad,M., Jarillo, J., Cashmore, A. R. (1998). Chimeric Proteins between cry1 and cry2 Arabidopsis Blue Light Photoreceptors Indicate Overlapping Functions and Varying Protein Stability. The Plant Cell, 10(2), 197–207.

Albani, M. C., Battey, N. H., & Wilkinson, M. J. (2004). The development of ISSR-derived SCAR markers around the *SEASONAL FLOWERING LOCUS* (*SFL*) in *Fragaria vesca*. Theor Appl Gene, 109(3), 571–579.

Albani, M. C., Castaings, L., Wötzel, S., Mateos, J. L., Wunder, J., Wang, R., Reymond, M., Coupland, G. (2012). *PEP1* of Arabis alpina Is Encoded by Two Overlapping Genes That Contribute to Natural Genetic Variation in Perennial Flowering. PLoS Genetics, 8(12), e1003130.

Albani, M. C., & Coupland, G. (2010). Comparative analysis of flowering in annual and perennial plants. Current Topics in Developmental Biology, 91, 323–348.

Alonso-blanco, C., Aarts, M. G. M., Bentsink, L., Keurentjes, J. J. B., & Reymond, M. (2009). What Has Natural Variation Taught Us about Plant Development, Physiology, and Adaptation. The Plant Cell, 21, 1877–1896.

Anderson, J. T., Lee, C., & Mitchell-olds, T. (2012). Life history QTLs and natural selection on flowering time in *Boechera stricta*, a perennial relative of Arabidopsis. Evolution, 65(3), 771–787.

Andrés, F., & Coupland, G. (2012). The genetic basis of flowering responses to seasonal cues. Nature Reviews. Genetics, 13(9), 627–39.

Angel, A., Song, J., Dean, C., & Howard, M. (2011). A Polycomb-based switch underlying quantitative epigenetic memory. Nature, 476, 105–108.

Aukerman, M. J., Hirschfeld, M., Wester, L., Weaver, M., Clack, T., Amasino, R. M., & Sharrock, R. A. (1997). A deletion in the *PHYD* gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. The Plant Cell, 9(8), 1317–26.

Aukerman, M. J., & Sakai, H. (2003). Regulation of Flowering Time and Floral Organ Identity by a MicroRNA and Its *APETALA2* -Like Target Genes. The Plant Cell, 15, 2730–2741.

Bae, G., & Choi, G. (2008). Decoding of light signals by plant phytochromes and their interacting proteins. Annual Review of Plant Biology, 59, 281–311.

Bagnall, D. J., King, R. W., Whitelam, G. C., Boylan, M. T., Wagner, D., & Quail, P. H. (1995). Flowering responses to altered expression of phytochrome in mutants and transgenic lines of *Arabidopsis thaliana* (*L.*) Heynh. Plant Physiology, 108(4), 1495–1503.

Bailey, C. D., Koch, M. A., Mayer, M., Mummenhoff, K., O'Kane, S. L., Warwick, S. I., Windham, M.D. & Al-Shehbaz, I. A. (2006). Toward a global phylogeny of the Brassicaceae. Molecular Biology and Evolution, 23(11), 2142–2160.

Balasubramanian, S., Sureshkumar, S., Agrawal, M., Michael, T. P., Wessinger, C., Maloof, J. N., Clark, R., Warthmann, N., Chory, J. & Weigel, D. (2006). The *PHYTOCHROME C*

photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. Nature Genetics, 38, 711–715.

Balcerowicz, M., Fittinghoff, K., Wirthmueller, L., Maier, A., Fackendahl, P., Fiene, G., Koncz, C. & Hoecker, U. (2011). Light exposure of Arabidopsis seedlings causes rapid de-stabilization as well as selective post-translational inactivation of the repressor of photomorphogenesis *SPA2*. Plant Journal, 65(5), 712–723.

Bäurle, I., & Dean, C. (2006). The Timing of Developmental Transitions in Plants. Cell, 125(4), 655–664.

Bergonzi, S., Albani, M. C., Ver Loren van Themaat, E., Nordström, K. J. V, Wang, R., Schneeberger, K., Moerland, P. D. & Coupland, G. (2013). Mechanisms of Age-Dependent Response to winter Temperature in Perennial Flowering of *Arabis alpina*. Science, 340(6136), 1094–7.

Böhlenius, H., Huang, T., Laurence, C.-C., Brunner, A. M., Jansson, S., Strauss, S. H., & Ove Nilsson1. (2006). CO/FT Regulatory Module ControlsTiming of Flowering and Seasonal Growth Cessation in Trees. Science, 312(2006), 1040–1043.

Boss, P. K., Bastow, R. M., Mylne, J. S., & Dean, C. (2004). Multiple Pathways in the Decision to Flower: Enabling, Promoting, and Resetting. The Plant Cell, 16, 18–31.

Botto, J. F., Sanchez, R. A., Whitelam, G. C., & Casal, J. J. (1996). Phytochrome A Mediates the Promotion of Seed Germination by Very Low Fluences of Light and Canopy Shade Light in Arabidopsis. Plant Physiology, 110(2), 439–444.

Botto, J. F., & Smith, H. (2002). Differential genetic variation in adaptive strategies to a common environmental signal in Arabidopsis accessions: Phytochrome-mediated shade avoidance. Plant, Cell and Environment, 25(1), 53–63.

Briggs, W. R., & Christie, J. M. (2002). Phototropins 1 and 2: Versatile plant blue-light receptors. Trends in Plant Science, 7(5), 204–210.

Briggs, W. R., & Olney, M. a. (2001). Photoreceptors in plant photomorphogenesis to date. Five phytochromes, two cryptochromes, one phototropin, and one superchrome. Plant Physiology, 125(1), 85–88.

Cao, K., Cui, L., Zhou, X., Ye, L., Zou, Z., & Deng, S. (2016). Four Tomato *FLOWERING LOCUS T*-Like Proteins Act Antagonistically to Regulate Floral Initiation. Front. Plant Sci., 6, 1–13.

Casal, J. J. (2012). Shade avoidance. The Arabidopsis Book / American Society of Plant Biologists, 10, e0157.

Casal, J. J. (2013). Photoreceptor Signaling Networks in Plant Responses to Shade. Annu Rev Plant Biol, 64(8), 1–25.

Casal, J. J., Candia, A. N., & Sellaro, R. (2014). Light perception and signalling by phytochrome A. Journal of Experimental Botany, 65(11), 2835–2845.

Casal, J. J., Sánchez, R. A., & Botto, J. F. (1998). Modes of action of phytochromes. Journal of Experimental Botany, 49(319), 127–138.

Casal, J. J., & Yanovsky, M. J. (2005). Regulation of gene expression by light. International Journal of Developmental Biology, 49(5-6), 501–511.

Casal, J. J., Yanovsky, M. J., & Luppi, J. P. (2000). Two photobiological pathways of phytochrome a activity, only one of which shows dominant negative suppression by phytochrome B. Photochemistry and Photobiology, 71(4), 481–486.

Castillon, A., Shen, H., & Huq, E. (2007). Phytochrome Interacting Factors: central players in phytochrome-mediated light signaling networks. Trends in Plant Science, 12(11), 514–521.

Cerdán, P. D., & Chory, J. (2003). Regulation of flowering time by light quality. Nature, 423(6942), 881–885.

Chailakhyan, M. K. (1968). Internal factors of plant flowering. Plant Physiology, 2(1), 1–36.

Chen, D., Xu, G., Tang, W., Jing, Y., Ji, Q., Fei, Z., & Lin, R. (2013). Antagonistic Basic Helix-Loop-Helix/bZIP Transcription Factors Form Transcriptional Modules That Integrate Light and Reactive Oxygen Species Signaling in Arabidopsis. The Plant Cell, 25(5), 1657–73.

Chen, M., Chory, J., & Fankhauser, C. (2004). Light signal transduction in higher plants. Annual Review of Genetics, 38, 87–117.

Chen, S., Lory, N., Stauber, J., & Hoecker, U. (2015). Photoreceptor Specificity in the Light-Induced and COP1-Mediated Rapid Degradation of the Repressor of Photomorphogenesis SPA2 in Arabidopsis. PLoS Genetics, 11(9), 1–22.

Chen, S., Wirthmueller, L., Stauber, J., Lory, N., Holtkotte, X., Leson, L., Schenkel, C., Ahmad, M. & Hoecker, U. (2016). The functional divergence between SPA1 and SPA2 in Arabidopsis photomorphogenesis maps primarily to the respective N-terminal kinase-like domain. BMC Plant Biology, 16(165), 1–12.

Cheng, Y., Dai, X., & Zhao, Y. (2006). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. Genes and Development, 20(13), 1790–1799.

Cheng, Y., Dai, X., & Zhao, Y. (2007). Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. The Plant Cell, 19(8), 2430–9.

Cho, L., Yoon, J., & An, G. (2016). The control of flowering time by environmental factors. International Journal of Laboratory Hematology, 38(1), 42–49.

Clack, T., Mathews, S., & Sharrock, R. A. (1994). The phytochrome apoprotein family in Arabidopsis is encoded by five genes: the sequences and expression of *PHYD* and *PHYE*. Plant Molecular Biology, 25(3), 413–427.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C. & Coupland, G. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science, 316(5827), 1030–3.

Cruz-Mazo, G., Buide, M. L., Samuel, R., & Narbona, E. (2009). Molecular phylogeny of *Scorzoneroides* (Asteraceae): Evolution of heterocarpy and annual habit in unpredictable environments. Molecular Phylogenetics and Evolution, 53(3), 835–847.

D'Aloia, M., Bonhomme, D., Bouché, F., Tamseddak, K., Ormenese, S., Torti, S., Coupland, G. & Périlleux, C. (2011). Cytokinin promotes flowering of Arabidopsis via transcriptional activation of the *FT* paralogue *TSF*. Plant Journal, 65(6), 972–979.

Datson, P. M., Murray, B. G., & Steiner, K. E. (2008). Climate and the evolution of annual/perennial life-histories in Nemesia (Scrophulariaceae). Plant Systematics and Evolution, 270(1-2), 39–57.

de Wit, M., Keuskamp, D. H., Bongers, F. J., Hornitschek, P., Gommers, C. M. M., Reinen, E., Martínez-Cerón, C., Fankhauser, C. & Pierik, R. (2016). Integration of Phytochrome and Cryptochrome Signals Determines Plant Growth during Competition for Light. Current Biology, 26, 1–7.

de Wit, M., Ljung, K., & Fankhauser, C. (2015). Contrasting growth responses in lamina and petiole during neighbor detection depend on differential auxin responsiveness rather than different auxin levels. New Phytologist, 208(1), 198–209.

Delker, C., Sonntag, L., James, G., Janitza, P., Ibañez, C., Ziermann, H., Peterson, T., Denk, K., Mull, S., Ziegler, J., Davis, S., Schneeberger, K. & Quint, M. (2014). The DET1-COP1-HY5

Pathway Constitutes a Multipurpose Signaling Module Regulating Plant Photomorphogenesis and Thermomorphogenesis. Cell Reports, 9(6), 1983–1989.

Deng, X., Caspar, T., & Quail, P. H. (1991). *Cop1* : a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. Genes & Development, 5, 1172–1182.

Deng, X. W., & Quail, P. H. (1999). Signalling in light-controlled development. Cell & Developmental Biology, 10(2), 121–129.

Deng, X.-W., & Quail, P. H. (1992). Genetic and phenotypic characterization of *cop1* mutants of *Arabidopsis thaliana*. The Plant Journal, 2(1), 83–95.

Ding, J., & Nilsson, O. (2016). Molecular regulation of phenology in trees-because the seasons they are a-changin'. Current Opinion in Plant Biology, 29, 73–79.

Djakovic-Petrovic, T., Wit, M. De, Voesenek, L. A. C. J., & Pierik, R. (2007). DELLA protein function in growth responses to canopy signals. Plant Journal, 51(1), 117–126.

Doi, K., Izawa, T., Fuse, T., Yamanouchi, U., Kubo, T., Shimatani, Z., Yano, M. & Yoshimura, A. (2004). *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of *Hd1*. Genes and Development, 18(8), 926–936.

Duanmu, D., Bachy, C., Sudek, S., Wong, C.-H. C.-H., Jimenez, V., Rockwell, N. C., Martin, S. S., Ngan, C. Y., Reistetter, E. N., van Baren, M. J., Price, D. C., Wei, C.-L., Reyes-prieto, A., Ωagarias, J. C. & Worden, A. Z. (2014). Marine algae and land plants share conserved phytochrome signaling systems. Proceedings of the National Academy of Sciences, 111(44), 15827–32.

Dubcovsky, J., Loukoianov, A., Fu, D., Valarik, M., Sanchez, A., & Yan, L. (2006). Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2*. Plant Molecular Biology, 60(4), 469–480.

Eklöf, J. M., & Brumer, H. (2010). The XTH gene family: an update on enzyme structure, function, and phylogeny in xyloglucan remodeling. Plant Physiology, 153(2), 456–466.

El-Assal, E. S., Alonso-Blanco, C., Peeters, A. J., Raz, V., & Koornneef, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of *CRY2*. Nature Genetics, 29(4), 435–440.

Endo, M., Tanigawa, Y., Murakami, T., Araki, T., & Nagatani, A. (2013). *PHYTOCHROME-DEPENDENT LATE-FLOWERING* accelerates flowering through physical interactions with phytochrome B and CONSTANS. Proc. Natl. Acad. Sci. U. S. A., 110(44), 18017–22.

Fankhauser, C. (2001). The Phytochromes, a Family of Red/Far-red Absorbing Photoreceptors. Journal of Biological Chemistry, 276(15), 11453–11456.

Filiault, D. L., & Maloof, J. N. (2012). A genome-wide association study identifies variants underlying the *Arabidopsis thaliana* shade avoidance response. PLoS Genetics, 8(3).

Filiault, D. L., Wessinger, C. A., Jose, R., Lutes, J., Borevitz, J. O., & Weigel, D. (2008). Amino acid polymorphisms in Arabidopsis phytochrome B cause differential responses to light. Proc. Natl. Acad. Sci. U. S. A., 105(24), 8482–8482.

Fittinghoff, K., Laubinger, S., Nixdorf, M., Fackendahl, P., Baumgardt, R. L., Batschauer, A., & Hoecker, U. (2006). Functional and expression analysis of Arabidopsis SPA genes during seedling photomorphogenesis and adult growth. Plant Journal, 47(4), 577–590.

Franklin, K. A. (2008). Shade avoidance. New Phytologist, 179, 930–944.

Franklin, K. A., Allen, T., & Whitelam, G. C. (2007). Phytochrome A is an irradiance-dependent red light sensor. Plant Journal, 50(1), 108–117.

Franklin, K. A., Larner, V. S., & Whitelam, C. C. (2005). The signal transducing photoreceptors of plants. International Journal of Developmental Biology, 49(5-6), 653–664.

Franklin, K. A., & Quail, P. H. (2010). Phytochrome functions in Arabidopsis development. Journal of Experimental Botany, 61(1), 11–24.

Fraser, D. P., Hayes, S., & Franklin, K. A. (2016). Photoreceptor crosstalk in shade avoidance. Current Opinion in Plant Biology, 33, 1–7.

Frewen, B. E., Chen, T. H. H., Howe, G. T., Davis, J., Antje Rohde, Boerjan, W., & Bradshaw, H. D. (2000). Quantitative trait loci and candidate gene mapping of bud set and bud flush in *populus*. Genetics, 154(2), 837–845.

Friedman, J., & Rubin, M. J. (2015). All in good time: Understanding annual and perennial strategies in plants. American Journal of Botany, 102(4), 497–499.

Fujiwara, S., Oda, A., Yoshida, R., Niinuma, K., Miyata, K., Tomozoe, Y., … Mizoguchi, T. (2008). Circadian Clock Proteins LHY and CCA1 Regulate SVP Protein Accumulation to Control Flowering in Arabidopsis. The Plant Cell, 20(11), 2960–2971.

Galstyan, A., Cifuentes-Esquivel, N., Bou-Torrent, J., & Martinez-Garcia, J. F. (2011). The shade avoidance syndrome in Arabidopsis: A fundamental role for atypical basic helix-loop-helix proteins as transcriptional cofactors. Plant Journal, 66(2), 258–267.

Gao, X., Nagawa, S., Wang, G., & Yang, Z. (2008). Cell polarity signaling: Focus on polar auxin transport. Molecular Plant, 1(6), 899–909.

Garner, W. W., & Allard, H. a. (1920). Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in Plants. Journ. Agr. Research, 18(11), 415.

Genoud, T., Schweizer, F., Tscheuschler, A., Debrieux, D., Casal, J. J., Schäfer, E., Hiltbrunner, A. & Fankhauser, C. (2008). *FHY1* mediates nuclear import of the light-activated phytochrome A photoreceptor. PLoS Genetics, 4(8), e1000143.

Golembeski, G.S., Kinmonth-Schultz, H.A., Song, Y.H., Imaizumi, T. (2014). Photoperiodic flowering regulation in *Arabidopsis thaliana*. Adv Bot Res., 72(4), 1–28.

Gommers, C.M.M, Visser, E.J.W., St Onge, K.R., Voesenek, L.A.C.J., Pierik, R. (2012). Shade tolerance : when growing tall is not an option. Trends in Plant Science, 18(2), 1360–1385.

Greenham, K., & McClung, C. R. (2015). Integrating circadian dynamics with physiological processes in plants. Nature Reviews. Genetics, 16(10), 598–610.

Grillo, M. A., Li, C., Fowlkes, A. M., Briggeman, T. M., Zhou, A., Schemske, D. W., & Sang, T. (2009). Genetic architecture for the adaptive origin of annual wild rice, *Oryza nivara*. Evolution, 63(4), 870–883.

Halliday, K. J., Koornneef, M., & Whitelam, G. C. (1994). Phytochrome B and at Least One Other Phytochrome Mediate the Accelerated Flowering Response of *Arabidopsis thaliana* L. to Low Red/Far-Red Ratio. Plant Physiology, 104, 1311–1315.

Hanano, S., & Goto, K. (2011). Arabidopsis *TERMINAL FLOWER1* is involved in the regulation of flowering time and inflorescence development through transcriptional repression. The Plant Cell, 23(9), 3172–84.

Hanumappa, M., Pratt, L. H., Cordonnier-Pratt, M. M., & Deitzer, G. F. (1999). A Photoperiod-Insensitive Barley Line Contains a Light-Labile Phytochrome B. Plant Physiology, 119(3), 1033–1040.

Hayama, R., & Coupland, G. (2004). The Molecular Basis of Diversity in the Photoperiodic Flowering Responses of Arabidopsis and Rice. Plant Physiology, 135(2), 677–684.

Hayes, S., Velanis, C. N., Jenkins, G. I., & Franklin, K. A. (2014). UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. Proc. Natl. Acad. Sci. U. S. A., 111(32), 11894–118999.

Helliwell, C. A., Wood, C. C., Robertson, M., James Peacock, W., & Dennis, E. S. (2006). The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. Plant Journal, 46(2), 183–192.

Hempel, F. D., Weigel, D., Mandel, M. a, Ditta, G., Zambryski, P. C., Feldman, L. J., & Yanofsky, M. F. (1997). Floral determination and expression of floral regulatory genes in Arabidopsis. Development, 124(19), 3845–3853.

Heo, J. B. & Sung, S. (2011). Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science, 331(6013), 76–79.

Hersch, M., Lorrain, S., de Wit, M., Trevisan, M., Ljung, K., Bergmann, S., & Fankhauser, C. (2014). Light intensity modulates the regulatory network of the shade avoidance response in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A., 111(17), 2–7.

Hiltbrunner, A., Tscheuschler, A., Viczián, A., Kunkel, T., Kircher, S., & Schäfer, E. (2006). FHY1 and FHL act together to mediate nuclear accumulation of the phytochrome A photoreceptor. Plant and Cell Physiology, 47(8), 1023–1034.

Hisamatsu, T., & King, R. W. (2008). The nature of floral signals in Arabidopsis. II. Roles for *FLOWERING LOCUS T (FT)* and gibberellin. Journal of Experimental Botany, 59(14), 3821–3829.

Ho, W. W. H., & Weigel, D. (2014). Structural features determining flower-promoting activity of Arabidopsis *FLOWERING LOCUS T*. The Plant Cell, 26(2), 552–64.

Hoecker, U. (2005). Regulated proteolysis in light signaling. Current Opinion in Plant Biology, 8(5), 469–476.

Hoecker, U., & Quail, P. H. (2001). The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in Arabidopsis. J. Biol. Chem, 47475(312), 1–28.

Hoecker, U., Xu, Y., & Quail, P. H. (1998). *SPA1*: a new genetic locus involved in phytochrome A-specific signal transduction. The Plant Cell, 10, 19–33.

Hoecker, U., Tepperman, J.M, Quail, P. (1999). *SPA1*, a WD-Repeat Protein Specific to Phytochrome A Signal Transduction. Science, 284(5413), 496–499.

Holtkotte, X., Dieterle, S., Kokkelink, L., Artz, O., Leson, L., Fittinghoff, K., Hayama, R. Ahmad, M. & Hoecker, U. (2016). Mutations in the N-terminal kinase-like domain of the repressor of photomorphogenesis *SPA1* severely impair *SPA1* function but not light responsiveness in Arabidopsis. Plant Journal, 88(2), 205–218.

Hornitschek, P., Kohnen, M. V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J. M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I. & Fankhauser, C. (2012). Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. Plant Journal, 71(5), 699–711.

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O., & Fankhauser, C. (2009). Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. The EMBO Journal, 28(24), 3893–902.

Hsu, C.-Y., Adams, J. P., Kim, H., No, K., Ma, C., Strauss, S. H., Drnevich, J., Vandervelde, L., Ellis, J. D., Rice, B. M., Wickett, N., Gunter, L. E., Tuskan, G. A., Brunner, A. M., Page, G. P., Barakat, A., Carlson, J. E. DePamphilis, C. W. Luthe, D. S. & Yuceer, C. (2011). *FLOWERING LOCUS T*

duplication coordinates reproductive and vegetative growth in perennial poplar. Proc. Natl. Acad. Sci. U. S. A., 108(26), 10756–61.

Hsu, C.-Y., Liu, Y., Luthe, D. S., & Yuceer, C. (2006). Poplar *FT2* Shortens the Juvenile Phase and Promotes Seasonal Flowering. The Plant Cell, 18(8), 1846–1861.

Huala, E., Oeller, P. W., Liscum, E., Han, I.-S., Larsen, E., & Briggs, W. R. (1997). Arabidopsis NPH1: A Protein Kinase with a Putative Redox-Sensing Domain. Science, 278(5346), 2120 – 2123.

Huijser, P., & Schmid, M. (2011). The control of developmental phase transitions in plants. Development, 138(19), 4117–4129.

Hyun, Y., Kim, J., Cho, S. W., Choi, Y., Kim, J. S., & Coupland, G. (2015). Site-directed mutagenesis in *Arabidopsis thaliana* using dividing tissue-targeted RGEN of the CRISPR/Cas system to generate heritable null alleles. Planta, 241(1), 271–284.

Imaizumi, T., & Kay, S. A. (2006). Photoperiodic control of flowering: not only by coincidence. Trends in Plant Science, 11(11), 550–558.

Inoue, K., Nishihama, R., Kataoka, H., Hosaka, M., Manabe, R., Nomoto, M., … Kohchi, T. (2016). Phytochrome signaling is mediated by *PHYTOCHROME INTERACTING FACTOR* in the liverwort. Plant Cell, 28(6), 1406–1421.

Ishikawa, M., Kiba, T., & Chua, N. H. (2006). The Arabidopsis *SPA1* gene is required for circadian clock function and photoperiodic flowering. Plant Journal, 46(5), 736–746.

Ito, S., Song, Y. H., Josephson-Day, a. R., Miller, R. J., Breton, G., Olmstead, R. G., & Imaizumi, T. (2012). *FLOWERING BHLH* transcriptional activators control expression of the photoperiodic flowering regulator *CONSTANS* in Arabidopsis. Proceedings of the National Academy of Sciences, 109(9), 3582–3587.

Jang, I. C., Yang, J. Y., Seo, H. S., & Chua, N. H. (2005). HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. Genes and Development, 19(5), 593–602.

Jang, I.-C., Henriques, R., Seo, H. S., Nagatani, A., & Chua, N.-H. (2010). Arabidopsis PHYTOCHROME INTERACTING FACTOR proteins promote phytochrome B polyubiquitination by COP1 E3 ligase in the nucleus. The Plant Cell, 22(7), 2370–83.

Jang, S., Marchal, V., Panigrahi, K. C. S., Wenkel, S., Soppe, W., Deng, X.-W., Valverde, F. & Coupland, G. (2008). Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. The EMBO Journal, 27, 1277–1288.

Jiao, Y., Lau, O. S., & Deng, X. W. (2007). Light-regulated transcriptional networks in higher plants. Nature Reviews. Genetics, 8(3), 217–230.

Jiao, Y., Ma, L., Strickland, E., & Deng, X. W. (2005). Conservation and divergence of light-regulated genome expression patterns during seedling development in rice and Arabidopsis. The Plant Cell, 17(12), 3239–56.

Jiao, Y., Yang, H., Ma, L., Sun, N., Yu, H., Liu, T., … Deng, X. (2003). A Genome-Wide Analysis of Blue-Light Regulation of Arabidopsis Transcription Factor Gene Expression during Seedling Development. Plant Physiology, 133, 1480–1493.

Jin, S., Jung, H. S., Chung, K. S., Lee, J. H., & Ahn, J. H. (2015). FLOWERING LOCUS T has higher protein mobility than *TWIN SISTER of FT*. Journal of Experimental Botany, 66(20), 6109–6117.

Johanson, U., West, J., Lister, C., Michaels, S., & Amasino, R. (2000). Molecular Analysis of *FRÍGIDA* , a Major Determinant of Natural Variation in Arabidopsis Flowering Time. Science, 290, 344–348.

Johnson, E., Bradley, M., Harberd, N. P., & Whitelam, C. C. (1994). Photoresponses of Light-Crown phyA Mutants of. Plant Physiol, 105, 141–149.

Johnston, J. S., Pepper, A. E., Hall, A. E., Chen, Z. J., Hodnett, G., Drabek, J., Lopez, R. & Price, H. J. (2005). Evolution of genome size in Brassicaceae. Annals of Botany, 95(1), 229–235.

Jung, J. H., Seo, P. J., Kang, S. K., & Park, C. M. (2011). miR172 signals are incorporated into the miR156 signaling pathway at the *SPL3/4/5* genes in Arabidopsis developmental transitions. Plant Molecular Biology, 76(1-2), 35–45.

Jung, J. H., Domijan, M., Klose, C., Biswas, S., Ezer, D., Gao, M., Box, M. S., Charoensawan, V., Cortijo, S., Locke, J. C., Schäfer, E., Jaeger, K. E. & Wigge, P. A. (2016). Phytochromes function as thermosensors in Arabidopsis. Science, 354(6314), 886–889.

Jung, J. H., Seo, Y. H., Seo, P. J., Reyes, J. L., Yun, J., Chua, N. H., & Park, C. M. (2007). The *GIGANTEA*-regulated microRNA172 mediates photoperiodic flowering independent of *CONSTANS* in *Arabidopsis*. The Plant Cell, 19(9), 2736–48.

Kang, C. Y., Lian, H. L., Wang, F. F., Huang, J. R., & Yang, H. Q. (2009). Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in Arabidopsis. The Plant Cell, 21(9), 2624–2641.

Kardailsky, I., Shukla, V. K., Ahn, J. H., Dagenais, N., Christensen, S. K., Nguyen, J. T., Chory, J., Harrison, M. J. & Weigel, D. (1999). Activation tagging of the floral inducer *FT*. Science, 286, 1962–1965.

Karlgren, A., Gyllenstrand, N., Källman, T., Sundström, J. F., Moore, D., Lascoux, M., & Lagercrantz, U. (2011). Evolution of the *PEBP* gene family in plants: functional diversification in seed plant evolution. Plant Physiology, 156(4), 1967–77.

Karve, A. A., Jawdy, S. S., Gunter, L. E., Allen, S. M., Yang, X., Tuskan, G. A., Wullschleger, S. D. & Weston, D. J. (2012). Initial characterization of shade avoidance response suggests functional diversity between *Populus phytochrome B* genes. New Phytologist, 196(3), 726–737.

Keller, M. M., Jaillais, Y., Pedmale, U. V., Moreno, J. E., Chory, J., & Ballaré, C. L. (2011). Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades. Plant Journal, 67(2), 195–207.

Keuskamp, D. H., Pollmann, S., Voesenek, L. A. C. J., Peeters, A. J. M., & Pierik, R. (2010). Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. Proc. Natl. Acad. Sci. U. S. A., 3(52), 22740–22744.

Keuskamp, D. H., Sasidharan, R., Vos, I., Peeters, A. J. M., Voesenek, L. A. C. J., & Pierik, R. (2011). Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in Arabidopsis seedlings. Plant Journal, 67(2), 208–217.

Khan, M. R. G., Ai, X. Y., & Zhang, J. Z. (2014). Genetic regulation of flowering time in annual and perennial plants. Wiley Interdisciplinary Reviews. RNA, 5(3), 347–359.

Kircher, S., Gil, P., Kozma-Bognár, L., Fejes, E., Speth, V., Husselstein-Muller, T., Bauer, D., Adám, E., Schäfer, E. & Nagy, F. (2002). Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. The Plant Cell, 14(7), 1541–55.

Kircher, S., Kozma-bognar, L., Kim, L., Adam, E., Harter, K., & Schafer, E. (1999). Light Quality-Dependent Nuclear Import of the Plant Photoreceptors Phytochrome A and B. The Plant Cell, 11(8), 1445–1456.

Kleine, T., Kindgren, P., Benedict, C., Hendrickson, L., & Strand, A. (2007). Genome-wide gene expression analysis reveals a critical role for CRYPTOCHROME1 in the response of Arabidopsis to high irradiance. Plant Physiology, 144(3), 1391–1406.

Koch, M. a., Bishop, J., & Mitchell-Olds, T. (1999). Molecular Systematics and Evolution of Arabidopsis and Arabis. Plant Biology, 1(5), 529–537.

Koch, M. A., Kiefer, C., Ehrich, D., Vogel, J., Brochmann, C., & Mummenhoff, K. (2006). Three times out of Asia Minor: The phylogeography of *Arabis alpina* L. (Brassicaceae). Molecular Ecology, 15(3), 825–839.

Koch, M., Haubold, B., & Mitchell-Olds, T. (2000). Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase in Arabidopsis, Arabis, and related genera (Brassicaceae). Molecular Biology and Evolution, 17, 1483–1498.

Kohnen, M., Schmid-Siegert, E., Trevisan, M., Allenbach Petrolati, L., Sénéchal, F., Müller-Moulé, P., Maloof, J. N., Xenarios, I. & Fankhauser, C. (2016). Neighbor Detection Induces Organ-specific Transcriptomes, Revealing Patterns Underlying Hypocotyl-specific Growth. The Plant Cell, 28, 1–53.

Kojima, S. (2002). *Hd3a*, a Rice Ortholog of the Arabidopsis *FT* Gene, Promotes Transition to Flowering Downstream of *Hd1* under Short-Day Conditions. Plant and Cell Physiology, 43(10), 1096–1105.

Koornneef, M., Alonso-Blanco, C., & Vreugdenhil, D. (2004). Naturally occurring genetic variation in *Arabidopsis thaliana*. Annual Review of Plant Biology, 55(1), 141–172.

Koornneef, M., Hanhart, C. J., & van der Veen, J. H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Molecular & General Genetics : MGG, 229(1), 57–66.

Kozuka, T., Kobayashi, J., Horiguchi, G., Demura, T., Sakakibara, H., Tsukaya, H., & Nagatani, A. (2010). Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiology, 153(4), 1608–18.

Kumar, S. V., Lucyshyn, D., Jaeger, K. E., Alós, E., Alvey, E., Harberd, N. P., & Wigge, P. a. (2012). Transcription factor *PIF4* controls the thermosensory activation of flowering. Nature, 484(7393), 242–245.

Kwok, S. F., Piekos, B., Miséra, S., & Deng, X.-W. (1996). A Complement of Ten Essential and Pleiotropic Arabidopsis *COP/DET/FUS* Genes Is Necessary for Repression of Photomorphogenesis in Darkness. Plant Physiol, 110(3), 731–742.

Lau, O. S., & Deng, X. W. (2012). The photomorphogenic repressors COP1 and DET1: 20 years later. Trends in Plant Science, 17(10), 584–593.

Laubinger, S., Fittinghoff, K., & Hoecker, U. (2004). The SPA quartet: a family of WD-repeat proteins with a central role in suppression of photomorphogenesis in Arabidopsis. The Plant Cell, 16(9), 2293–2306.

Laubinger, S., & Hoecker, U. (2003). The SPA1-like proteins SPA3 and SPA4 repress photomorphogenesis in the light. Plant Journal, 35(3), 373–385.

Laubinger, S., Marchal, V., Le Gourrierec, J., Wenkel, S., Adrian, J., Jang, S., Kulajta, C., Braun, H., Coupland, G. & Hoecker, U. (2006). Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development, 133(16), 3213–3222.

Lazaro, A., Mouriz, A., Piñeiro, M., & Jarillo, J. A. (2015). Red light-mediated degradation of CONSTANS by the E3 ubiquitin ligase HOS1 regulates photoperiodic flowering in Arabidopsis. The Plant Cell, 27, 2437–2454.

Lee, J., He, K., Stolc, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I. & Deng, X. W. (2007). Analysis of Transcription Factor HY5 Genomic Binding Sites Revealed Its Hierarchical Role in Light Regulation of Development. The Plant Cell, 19(3), 731–749.

Lee, J., Oh, M., Park, H., & Lee, I. (2008). SOC1 translocated to the nucleus by interaction with AGL24 directly regulates *LEAFY*. Plant Journal, 55(5), 832–843.

Lee, R., Baldwin, S., Kenel, F., McCallum, J., & Macknight, R. (2013). *FLOWERING LOCUS T* genes control onion bulb formation and flowering. Nature Communications, 4, 2884–2893.

Leivar, P., & Monte, E. (2014). PIFs: systems integrators in plant development. The Plant Cell, 26(1), 56–78.

Li, F. W., Melkonian, M., Rothfels, C. J., Villarreal, J. C., Stevenson, D. W., Graham, S. W., … Mathews, S. (2015). Phytochrome diversity in green plants and the origin of canonical plant phytochromes. Nat Communacations, 6, 7852–7864.

Li, J., Li, G., Gao, S., Martinez, C., He, G., Zhou, Z., Wong, G. K., Pryer, K. M. & Deng, X. W. (2010). Arabidopsis transcription factor *ELONGATED HYPOCOTYL5* plays a role in the feedback regulation of phytochrome A signaling. The Plant Cell, 22(11), 3634–3649.

Li, L., Ljung, K., Breton, G., Schmitz, R. J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B. J., Ivans, L. J., Pedmale, U. V., Jung, H. S. Ecker, J. R., Kay, S. A. & Chory, J. (2012). Linking photoreceptor excitation to changes in plant architecture. Genes and Development, 26(8), 785–790.

Lian, H. L., He, S. B., Zhang, Y. C., Zhu, D. M., Zhang, J. Y., Jia, K. P., Sun, S. X., Li, L. & Yang, H. Q. (2011). Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. Genes and Development, 25(10), 1023–1028.

Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J. P. & Eshed, Y. (2006). The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc. Natl. Acad. Sci. U. S. A., 103(16), 6398–6403.

Liljegren, S. J., Gustafson-Brown, C., Pinyopich, A., Ditta, G. S., & Yanofsky, M. F. (1999). Interactions among APETALA1, LEAFY, and TERMINAL FLOWER1 specify meristem fate. The Plant Cell, 11(6), 1007–1018.

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J., & Cashmore, A. R. (1998). Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proc. Natl. Acad. Sci. U. S. A., 95(5), 2686–2690.

Lin, M.-K., Belanger, H., Lee, Y.-J., Varkonyi-Gasic, E., Taoka, K.-I., Miura, E., Xoconostle-Cázares, B., Gendler, K., Jorgensen, R. A., Phinney, B. Lough, T. J. & Lucas, W. J. (2007). FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. The Plant Cell, 19(5), 1488–1506.

Liu, B., Zuo, Z., Liu, H., Liu, X., & Lin, C. (2011). Arabidopsis cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. Genes and Development, 25(10), 1029–1034.

Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y. & Yu, H. (2012). FTIP1 is an essential regulator required for florigen transport. PLoS Biology, 10(4), e1001313.

Liu, L.-J., Zhang, Y.-C., Li, Q.-H., Sang, Y., Mao, J., Lian, H.-L., Wang, L. & Yang, H.-Q. (2008). COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in Arabidopsis. The Plant Cell, 20(2), 292–306.

Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D., Lin, C. (2008b). Photoexcited CRY2 Interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science, 322, 1535–1539.

Lorrain, S., Allen, T., Duek, P. D., Whitelam, G. C., & Fankhauser, C. (2008). Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant Journal, 53(2), 312–323.

Lu, X. D., Zhou, C. M., Xu, P. B., Luo, Q., Lian, H. L., & Yang, H. Q. (2015). Red-light-dependent interaction of phyB with SPA1 promotes COP1-SPA1 dissociation and photomorphogenic development in Arabidopsis. Molecular Plant, 8(3), 467–478.

Lucas, M., Davière, J.-M., Rodríguez-Falcón, M., Pontin, M., Iglesias-Pedraz, J. M., Lorrain, S., Fankhauser, C., Blázquez, M. A., Titarenko, E. & Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature, 451, 480–484.

Lysak, M. A., & Lexer, C. (2006). Towards the era of comparative evolutionary genomics in Brassicaceae. Plant Systematics and Evolution, 259(2-4), 175–198.

Ma, D., Li, X., Guo, Y., Chu, J., Fang, S., Yan, C., Noel, J. P. & Liu, H. (2015). Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. Proc. Natl. Acad. Sci. U. S. A., 113, 224–229.

Mancinelli, A.L. (1994) The physiology of phytochrome action. In Photomorphogenesis in Plants. Edited by Kendrick, R.E. and Kronenberg, G.M.H. pp. 211–269. Kluwer Academic Publishers, Dordrecht.

Marquardt, S., Boss, P. K., Hadfield, J., & Dean, C. (2006). Additional targets of the Arabidopsis autonomous pathway members, *FCA* and *FY*. Journal of Experimental Botany, 57(13), 3379–3386.

Martínez-García, J. F., Gallemi, M., Molina-Contreras, M. J., Llorente, B., Bevilaqua, M. R. ., & Quail, P. H. (2014). The Shade Avoidance Syndrome in Arabidopsis: The Antagonistic Role of Phytochrome A and B Differentiates Vegetation Proximity and Canopy Shade. PLoS ONE, 9(10), e109275.

Mascheretti, I., Turner, K., Brivio, R. S., Hand, A., Colasanti, J., & Rossi, V. (2015). Florigen-encoding genes of day-neutral and photoperiod-sensitive maize are regulated by different chromatin modifications at the floral transition. Plant Physiology, 168(4), 1351–1363.

Mathieu, J., Yant, L. J., Mürdter, F., Küttner, F., & Schmid, M. (2009). Repression of flowering by the miR172 target SMZ. Current Science, 7(7), 1435–1439.

McNellis, T. W., & Deng, X. W. (1995). Light control of seedling morphogenetic pattern. The Plant Cell, 7(11), 1749–1761.

McNellis, T. W., von Arnim, a G., & Deng, X. W. (1994). Overexpression of Arabidopsis *COP1* results in partial suppression of light-mediated development: evidence for a light-inactivable repressor of photomorphogenesis. The Plant Cell, 6(10), 1391–1400.

Michael, T. P., Breton, G., Hazen, S. P., Priest, H., Mockler, T. C., Kay, S. A., & Chory, J. (2008). A morning-specific phytohormone gene expression program underlying rhythmic plant growth. PLoS Biology, 6(9), 1887–1898.

Mockler, T., Yang, H., Yu, X., Parikh, D., Cheng, Y., Dolan, S., & Lin, C. (2003). Regulation of photoperiodic flowering by Arabidopsis photoreceptors. Proc. Natl. Acad. Sci. U. S. A., 100(4), 2140–2145.

Möglich, A., Yang, X., Ayers, R. A., & Moffat, K. (2010). Structure and function of plant photoreceptors. Annu Rev Plant Biol, 61, 21–47.

Molinero-Rosales, N., Latorre, A., Jamilena, M., & Lozano, R. (2004). Single Flower Truss regulates the transition and maintenance of flowering in tomato. Planta, 218(3), 427–434.

Moon, J., Suh, S. S., Lee, H., Choi, K. R., Hong, C. B., Paek, N. C., Kim, S. G. & Lee, I. (2003). The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant Journal, 35(5), 613–623.

Mouradov, A., Cremer, F., & Coupland, G. (2002). Control of flowering time: interacting pathways as a basis for diversity. The Plant Cell, 14 , 111–130.

Nagy, F., & Schäfer, E. (2002). Phytochromes control photomorphogensis by differentially regulated, interacting signaling pathways in higher plants. Annual Review of Plant Biology, 53(1), 329–355.

Navarro, C., Abelenda, J. a., Cruz-Oró, E., Cuéllar, C. a., Tamaki, S., Silva, J., Shimamoto, K. & Prat, S. (2011). Control of flowering and storage organ formation in potato by *FLOWERING LOCUS T*. Nature, 478(7367), 119–122.

Neff, M. M., & Chory, J. (1998). Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiology, 118(1), 27–35.

Neff, M. M., Fankhauser, C., & Chory, J. (2000). Light: an indicator of time and place. Genes & Development, 14(858), 257–271.

Ni, W., Xu, S.-L., Tepperman, J. M., Stanley, D. J., Maltby, D. A., Gross, J. D., Burlingame, A. L., Wang, Z. & Quail, P. H. (2014). A mutually assured destruction mechanism attenuates light signaling in Arabidopsis. Science, 344(6188), 1160–1164.

Nito, K., Kajiyama, T., Unten-Kobayashi, J., Fujii, A., Mochizuki, N., Kambara, H., & Nagatani, A. (2015). Spatial regulation of the gene expression response to shade in Arabidopsis seedlings. Plant and Cell Physiology, 56(7), 1306–1319.

Nozue, K., Tat, A. V., Kumar Devisetty, U., Robinson, M., Mumbach, M. R., Ichihashi, Y., Lekkala, S. & Maloof, J. N. (2015). Shade Avoidance Components and Pathways in Adult Plants Revealed by Phenotypic Profiling. PLoS Genetics, 11(4), 1–26.

Ohgishi, M., Saja, K., Okada, K., & Sakai, T. (2004). Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinational multiple mutants in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A., 101(8), 2223–2228.

Osterlund, M. T., Hardtke, C. S., Wei, N., & Deng, X. W. (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature, 405(6785), 462–466.

Osugi, a., Itoh, H., Ikeda-Kawakatsu, K., Takano, M., & Izawa, T. (2011). Molecular Dissection of the Roles of Phytochrome in Photoperiodic Flowering in Rice. Plant Physiology, 157(3), 1128–1137.

Park, E., Park, J., Kim, J., Nagatani, A., Lagarias, C., & Choi, G. (2012). Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. The Plant Journal, 72, 537–546.

Parks, B. M., Hoecker, U., & Spalding, E. P. (2001). Light-induced growth promotion by SPA1 counteracts phytochrome-mediated growth inhibition during de-etiolation. Plant Physiology, 126(3), 1291–1298.

Pedmale, U. V., Huang, S. S. C., Zander, M., Cole, B. J., Hetzel, J., Ljung, K., Reis, Pedro A. B., Sridevi, P., Nito, K., Nery, J. R., Ecker, J. R. & Chory, J. (2015). Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue Light. Cell, 164(1-2), 233–245.

Pierik, R., & De Wit, M. (2014). Shade avoidance: Phytochrome signalling and other aboveground neighbour detection cues. Journal of Experimental Botany, 65(11), 2815–2824.

Pierik, R., Djakovic-Petrovic, T., Keuskamp, D. H., de Wit, M., & Voesenek, L. A. C. J. (2009). Auxin and Ethylene Regulate Elongation Responses to Neighbor Proximity Signals Independent of Gibberellin and DELLA Proteins in Arabidopsis. Plant Physiology, 149(4), 1701–1712.

Pin, P. a, Benlloch, R., Bonnet, D., Wremerth-Weich, E., Kraft, T., Gielen, J. J. L., & Nilsson, O. (2010). An antagonistic pair of *FT* homologs mediates the control of flowering time in sugar beet. Science, 330(6009), 1397–1400.

Pin, P. A., & Nilsson, O. (2012). The multifaceted roles of *FLOWERING LOCUS T* in plant development. Plant, Cell and Environment, 35(10), 1742–1755.

Possart, A., Fleck, C., & Hiltbrunner, A. (2014). Shedding (far-red) light on phytochrome mechanisms and responses in land plants. Plant Science, 217-218(316), 36–46.

Putterill, J., Robson, F., Lee, K., Simon, R., & Coupland, G. (1995). The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell, 80(6), 847–857.

Putterill, J., & Varkonyi-Gasic, E. (2016). *FT* and florigen long-distance flowering control in plants. Current Opinion in Plant Biology, 33, 77–82.

Ranjan, A., Dickopf, S., Ullrich, K. K., Rensing, S. A., & Hoecker, U. (2014). Functional analysis of *COP1* and *SPA* orthologs from Physcomitrella and rice during photomorphogenesis of transgenic Arabidopsis reveals distinct evolutionary conservation. BMC Plant Biology, 14(1), 178.

Reddy, R. K., & Sharma, R. (1998). Spatial distribution and temporal regulation of phytochromes A and B levels in maize seedlings. Plant Physiology and Biochemistry, 36(10), 737–745.

Reed, J. W., Nagatani, A., Elich, T. D., Fagan, M., & Chory, J. (1994). Phytochrome A and Phytochrome B Have Overlapping but Distinct Functions in Arabidopsis Development. Plant Physiology, 104(4), 1139–1149.

Richardt, S., Lang, D., Reski, R., Frank, W., & Rensing, S. A. (2007). PlanTAPDB, a Phylogeny-Based Resource of Plant Transcription-Associated Proteins. Plant Physiology, 143(4), 1452–1466.

Rinne, P. L. H., Welling, A., Vahala, J., Ripel, L., Ruonala, R., Kangasjärvi, J., & van Der Schoot, C. (2011). Chilling of Dormant Buds Hyperinduces FLOWERING LOCUS T and Recruits GA-Inducible 1,3-b-Glucanases to Reopen Signal Conduits and Release Dormancy in *Populus*. The Plant Cell, 23, 130–146.

Rizzini, L., Favory, J. J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schafer, E., Nagy, F., Jenkins, G. I. & Ulm, R. (2011). Perception of UV-B by the Arabidopsis UVR8 protein. Science, 332(6025), 103–106.

Rockwell, N. C., Su, Y.-S., & Lagarias, J. C. (2006). Phytochrome Structure and Signaling Mechanisms. Annual Review of Plant Biology, 57(1), 837–858.

Roig-Villanova, I., Bou, J., Sorin, C., Devlin, P. F., & Martínez-García, J. F. (2006). Identification of Primary Target Genes of Phytochrome Signaling. Early Transcriptional Control during Shade Avoidance Responses in Arabidopsis Plant Physiology, 141, 85–96.

Roig-Villanova, I., Bou-Torrent, J., Galstyan, A., Carretero-Paulet, L., Portolés, S., Rodríguez-Concepción, M., & Martínez-García, J. F. (2007). Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. The EMBO Journal, 26(22), 4756–67.

Roig-villanova, I., & Martínez-garcía, J. F. (2016). Plant Responses to Vegetation Proximity: A Whole Life Avoiding Shade. Front. Plant Sci., 7, 1–10.

Rolauffs, S., Fackendahl, P., Sahm, J., Fiene, G., & Hoecker, U. (2012). Arabidopsis *COP1* and *SPA* genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiology, 160(4), 2015–2027.

Ruonala, R., Rinne, P. L. H., Kangasjärvi, J., & van der Schoot, C. (2008). *CENL1* expression in the rib meristem affects stem elongation and the transition to dormancy in *Populus*. The Plant Cell, 20(1), 59–74.

Saijo, Y., Sullivan, J. a, Wang, H., Yang, J., Shen, Y., Rubio, V., Ma, L. & Hoecker, U. (2003). The COP1 − SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes & Development, (203), 2642–2647.

Salter, M. G., Franklin, K. a, & Whitelam, G. C. (2003). Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature, 426(6967), 680–683.

Samach, A., Onouchi, H., Gold, S. E., Ditta, G. S., Schwarz-, Z., Yanofsky, M. F. &Coupland, G. (2000). Distinct Roles of CONSTANS Target Genes in Reproductive devolpment of Arabidopsis. Science, 288(5471), 1613–1616.

Sasidharan, R., & Pierik, R. (2010). Cell wall modification involving *XTH*s controls phytochrome-mediated petiole elongation in *Arabidopsis thaliana*. Plant Signaling & Behavior, 5(11), 1491–1492.

Sawa, M., & Kay, S. A. (2011). *GIGANTEA* directly activates *Flowering Locus T* in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A., 2011(28), 11698–11703.

Sawa, M., Nusinow, D. A., Steve, K., & Zmaizumi, T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. Science, 318, 261–265.

Schwab, R., Ossowski, S., Riester, M., Warthmann, N., & Weigel, D. (2006). Highly Specific Gene Silencing by Artificial MicroRNAs in Arabidopsis. The Plant Cell, 18(5), 1121–1133.

Searle, I., & Coupland, G. (2004). Induction of flowering by seasonal changes in photoperiod. The EMBO Journal, 23(6), 1217–1222.

Searle, I., He, Y., Turck, F., Vincent, C., Fornara, F., Kröber, S., Amasino, R. A. & Coupland, G. (2006). The transcription factor *FLC* confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes and Development, 20(7), 898–912.

Seo, H. S., Watanabe, E., Tokutomi, S., Nagatani, A., & Chua, N. H. (2004). Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes and Development, 18(6), 617–622.

Seo, H. S., Yang, J.-Y., Ishikawa, M., Bolle, C., Ballesteros, M. L., & Chua, N.-H. (2003). LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. Nature, 423(6943), 995–999.

Sessa, G., Carabelli, M., Sassi, M., Ciolfi, A., Possenti, M., Mittempergher, F., Becker, J., Morelli, G. & Ruberti, I. (2005). A dynamic balance between gene activation and repression regulates the shade avoidance response in Arabidopsis. Genes & Development, 19(23), 2811–2815.

Shannon, S., & Meeks-Wagner, D. R. (1993). Genetic Interactions That Regulate Inflorescence Development in Arabidopsis. The Plant Cell, 5, 639–655.

Sharrock, R. A., & Clack, T. (2002). Patterns of expression and normalized levels of the five Arabidopsis phytochromes. Plant Physiology, 130(1), 442–56.

Sharrock, R. A., & Quail, P. H. (1989). Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes & Development, 3(11), 1745–1757.

Sheerin, D. J., Menon, C., zur Oven-Krockhaus, S., Enderle, B., Zhu, L., Johnen, P., Schleifenbaum, F., Stierhof, Y. D., Huq, E. & Hiltbrunner, A. (2015). Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex. The Plant Cell, 27(1), 189–201.

Sheldon, C. C., Burn, J. E., Perez, P. P., Metzger, J., Edwards, J. A., Peacock, W. J., & Dennis, E. S. (1999). The FLF MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell, 11(3), 445–458.

Shim, J.S., Kubota, A., Imaizumi, T. (2016). Circadian clock and photoperiodic flowering in Arabidopsis- CO is a hub for signal integration. Plant Physiology, 173(1), 5–15.

Shindo, C., Aranzana, M. J., Lister, C., Baxter, C., Nicholls, C., Nordborg, M., & Dean, C. (2005). Role of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of Arabidopsis. Plant Physiology, 138, 1163–1173.

Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., & Furuya, M. (1996). Action spectra for phytochrome A- and B-specific photoinduction of seed germination in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A., 93, 8129–8133.

Shrestha, R., Gómez-Ariza, J., Brambilla, V., & Fornara, F. (2014). Molecular control of seasonal flowering in rice, Arabidopsis and temperate cereals. Annals of Botany, 114(7), 1445–1458.

Simillion, C., Vandepoele, K., Van Montagu, M. C. E., Zabeau, M., & Van de Peer, Y. (2002). The hidden duplication past of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A., 99(21), 13627–13632.

Simon, R., Igeno, M. I., & Coupland, G. (1996). Activation of floral meristem identity genes in Arabidopsis. Nature, 384(6604), 59–62.

Simpson, G. G., Dijkwel, P. P., Quesada, V., Henderson, I., & Dean, C. (2003). FY is an RNA 3' end-processing factor that interacts with FCA to control the Arabidopsis floral transition. Cell, 113(6), 777–787.

Smith, H., Xu, Y., & Quail, P. H. (1997). Antagonistic but complementary actions of phytochromes A and B allow seedling de-etiolation. Plant Physiology, 114(2), 637–641.

Somers, D. E., Schultz, T. F., Milnamow, M., & Kay, S. A. (2000). ZEITLUPE Encodes a Novel Clock-Associated PAS Protein from Arabidopsis. Cell, 101(3), 319–329.

Somers, D., Sharrock, R., Tepperman, J., & Quail, P. (1991). The hy3 Long Hypocotyl Mutant of Arabidopsis Is Deficient in Phytochrome B. The Plant Cell, 3(12), 1263–1274.

Song, Y. H., Ito, S., & Imaizumi, T. (2013). Flowering time regulation: Photoperiod- and temperature-sensing in leaves. Trends in Plant Science, 18(10), 575–583.

Song, Y. H., Shim, J. S., Kinmonth-Schultz, H. a, & Imaizumi, T. (2015). Photoperiodic Flowering: Time Measurement Mechanisms in Leaves. Annual Review of Plant Biology, 66, 441–464.

Song, Y. H., Smith, R. W., To, B. J., Millar, A. J., & Imaizumi, T. (2012). FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. Science, 336(6084), 1045–1049.

Sorin, C., Salla-Martret, M., Bou-Torrent, J., Roig-Villanova, I., & Martínez-García, J. F. (2009). *ATHB4*, a regulator of shade avoidance, modulates hormone response in Arabidopsis seedlings. Plant Journal, 59(2), 266–277.

Stacey, M. G., Kopp, O. R., Kim, T., & von Arnim, A. G. (2000). Modular Domain Structure of Arabidopsis COP1. Reconstitution of Activity by Fragment Complementation and Mutational Analysis of a Nuclear Localization Signal in Planta 1. Plant Physiol., 124, 979–989.

Stepanova, a. N., Yun, J., Robles, L. M., Novak, O., He, W., Guo, H., Ljung, K. & Alonso, J. M. (2011). The Arabidopsis YUCCA1 Flavin Monooxygenase Functions in the Indole-3-Pyruvic Acid Branch of Auxin Biosynthesis. The Plant Cell, 23(11), 3961–3973.

Suárez-López, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F., & Coupland, G. (2001). *CONSTANS* mediates between the circadian clock and the control of flowering in Arabidopsis. Nature, 410, 1116–1120.

Sullivan, J. A., & Deng, X. W. (2003). From seed to seed: The role of photoreceptors in Arabidopsis development. Developmental Biology, 260(2), 289–297.

Swiezewski, S., Liu, F., Magusin, A., & Dean, C. (2009). Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature, 462(7274), 799–802.

Tank, D. C., & Olmstead, R. G. (2008). From annuals to perennials: Phylogeny of *subtribe Castillejinae* (Orobanchaceae). American Journal of Botany, 95(5), 608–625.

Tao, Y., Ferrer, J. L., Ljung, K., Pojer, F., Hong, F., Long, J. A., Li, L., Moreno, J. E., Bowman, M. E., Ivans, L. J., Cheng, Y., Lim, J., Zhao, Y., Ballaré, C. L., Sandberg, G. Noel, J. P. & Chory, J. (2008). Rapid Synthesis of Auxin via a New Tryptophan-Dependent Pathway Is Required for Shade Avoidance in Plants. Cell, 133(1), 164–176.

Taoka, K., Ohki, I., Tsuji, H., Furuita, K., Hayashi, K., Yanase, T., Yamaguchi, M., Nakashima, C., Purwestri, Y. A., Tamaki, S., Ogaki, Y., Shimada, C., Nakagawa, A. Kojima, C. & Shimamoto, K. (2011). 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature, 476(7360), 332–335.

Tepperman, J. M., Zhu, T., Chang, H.-S., Wang, X., & Quail, P. H. (2001). Multiple transcription-factor genes are early targets of phytochrome A signaling. Proceedings of the National Academy of Sciences of the United States of America, 98(16), 9437–9442.

Torii, K. U., McNellis, T. W., & Deng, X. W. (1998). Functional dissection of Arabidopsis COP1 reveals specific roles of its three structural modules in light control of seedling development. EMBO Journal, 17(19), 5577–5587.

Torti, S., Fornara, F., Vincent, C., Andres, F., Nordstrom, K., Gobel, U., Knoll, D., Schoof, H. & Coupland, G. (2012). Analysis of the Arabidopsis Shoot Meristem Transcriptome during Floral Transition Identifies Distinct Regulatory Patterns and a Leucine-Rich Repeat Protein That Promotes Flowering. The Plant Cell, 24(2), 444–462.

Tsuge, T., Inagaki, N., Yoshizumi, T., Shimada, H., Kawamoto, T., Matsuki, R., Yamamoto, N. & Matsui, M. (2001). Phytochrome-mediated control of *COP1* gene expression in rice plants. Molecular Genetics and Genomics MGG, 265(1), 43–50.

Valverde, F., Mouradov, A., & Soppe, W. (2004). Photoreceptor Regulation of CONSTANS Protein in Photoperiodic Flowering. Science, 629(2002), 1003–1006.

von Arnim, A., & Deng, X. (1996). Light Control of Seedling Development. Plant Physiol, 47, 215–43.

Wahl, V., Ponnu, J., Schlereth, A., Arrivault, S., Langenecker, T., Franke, A., Feil, R., Lunn, J. E., Stitt, M. & Schmid, M. (2013). Regulation of flowering by Trehalose-6-Phosphate signaling in *Arabidopsis thaliana*. Science, 339, 704–708.

Wang, H., Kang, D., Deng, X. W., & Ning, W. (1999). Evidence for functional conservation of a mammalian homologue of the light-responsive plant protein COP1. Current Biology, 9(13), 711–714.

Wang, H., Ma, L. G., Li, J. M., Zhao, H. Y., & Deng, X. W. (2001). Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. Science, 294(5540), 154–158.

Wang, J. W., Czech, B., & Weigel, D. (2009a). miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. Cell, 138(4), 738–749.

Wang, R., Albani, M. C., Vincent, C., Bergonzi, S., Luan, M., Bai, Y., Kiefer, C., Castillo, R. & Coupland, G. (2011). *AaTFL1* confers an age-dependent response to vernalization in perennial *Arabis alpina*. Plant Cell, 23(4), 1307–1321.

Wang, R., Farrona, S., Vincent, C., Joecker, A., Schoof, H., Turck, F., Alonso-Blanco, C., Coupland, G. & Albani, M. C. (2009). *PEP1* regulates perennial flowering in *Arabis alpina*. Nature, 459(7245), 423–427.

Warwick, S. I., Francis, A., & Al-Shehbaz, I. A. (2006). Brassicaceae: Species checklist and database on CD-Rom. Plant Systematics and Evolution, 259(2-4), 249–258.

Watanabe, S., Hideshima, R., Zhengjun, X., Tsubokura, Y., Sato, S., Nakamoto, Y., Yamanaka, N., Takahashi, R., Ishimoto, M., Anai, T., Tabata, S. & Harada, K. (2009). Map-based cloning of the gene associated with the soybean maturity locus E3. Genetics, 182(4), 1251–1262.

Wei, N., & Deng, X.-W. (1996). The role of the *COP/DET/FUS* genes in light control of Arabidopsis seedling development. Plant Physiol., 112, 871–878.

Wigge, P. a, Kim, M. C., Jaeger, K. E., Busch, W., Schmid, M., Lohmann, J. U., & Weigel, D. (2005). Integration of Spatial and Temporal Information During Floral Induction in Arabidopsis. Science, 309(5737), 1056–1059.

Wigge, P. A. (2011). *FT*, A mobile developmental signal in plants. Current Biology, 21(9), 374–378.

Willing, E.-M., Rawat, V., Mandáková, T., Maumus, F., James, G. V., Nordström, K. J. V, Becker, C., Warthmann, N., Chica, C., Szarzynska, B., Zytnicki, M., Albani, M. C., Kiefer, C., Bergonzi, S., Castaings, L., Mateos, J. L., Berns, M. C., Bujdoso, N., Piofczyk, T., de Lorenzo, L., Barrero-Sicilia, C., Mateos, I., Piednoël, M., Hagmann, J., Chen-Min-Tao, R., Iglesias-Fernández, R., Schuster, S. C., Alonso-Blanco, C., Roudier, F., Carbonero, P., Paz-Ares, J., Davis, S. J., Pecinka, A., Quesneville, H., Colot, V., Lysak, M. A., Weigel, D., Coupland, G. & Schneeberger, K. (2015). Genome expansion of *Arabis alpina* linked with retrotransposition and reduced symmetric DNA methylation. Nature Plants, 1(2), 14023.

Won, C., Shen, X., Mashiguchi, K., Zheng, Z., Dai, X., Cheng, Y., Kasahara, H., Kamiya, Y., Chory, J. Zhao, Y. (2011). Conversion of tryptophan to indole-3-acetic acid by tryptophan aminotransferases in Arabidopsis and YUCCAs in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A., 108(45), 18518–18523.

Wu, G., & Poethig, R. S. (2006). Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target *SPL3*. Development, 133(18), 3539–3547.

Xie, X., Kagawa, T., & Takano, M. (2014). The phytochrome B/phytochrome C heterodimer is necessary for phytochrome C-mediated responses in rice seedlings. PLoS ONE, 9(5), e97264.

Xue, W., Xing, Y., Weng, X., Zhao, Y., Tang, W., Wang, L., Zhou, H., Yu, S., Xu, C., Li, X. & Zhang, Q. (2008). Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nature Genetics, 40(6), 761–767.

Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M., & Araki, T. (2005). *TWIN SISTER of FT* (*TSF*) acts as a floral pathway integrator redundantly with FT. Plant and Cell Physiology, 46(8), 1175–1189.

Yamaguchi, N., Winter, C. M., Wu, M. F., Kanoo, Y., Yamaguchi, A., Seo, M., & Wagner, D. (2014). Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis. Science, 344, 638–641.

Yamamoto, Y. Y. (1998). Role of a COP1 Interactive Protein in Mediating Light-Regulated Gene Expression in Arabidopsis. The Plant Cell, 10(7), 1083–1094.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S . & Dubcovsky, J. (2006). The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc. Natl. Acad. Sci. U. S. A., 103(51), 19581–19586.

Yang, H. Q., Tang, R. H., & Cashmore, A. R. (2001). The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. The Plant Cell, 13(12), 2573–87.

Yang, J., Lin, R., Sullivan, J., Hoecker, U., Liu, B., Xu, L., Deng, X. W. & Wang, H. (2005). Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in Arabidopsis. The Plant Cell, 17(3), 804–21.

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamatoto, K., Umehara, Y., Nagamura, Y. & Sasaki, T. (2000). *Hd1*, a Major Photoperiod Sensitivity Quantitative Trait Locus in Rice, Is Closely Related to the Arabidopsis Flowering Time Gene *CONSTANS*. The Plant Cell, 12(12), 2473–2484.

Yanovsky, M. J., & Kay, S. a. (2003). Living by the calendar: how plants know when to flower. Nature Reviews. Molecular Cell Biology, 4(4), 265–275.

Yu, S., Galvão, V. C., Zhang, Y.-C., Horrer, D., Zhang, T.-Q., Hao, Y.-H., Feng, Y-Q., Wang, S., Schmid, M. & Wang, J. W. (2012). Gibberellin regulates the Arabidopsis floral transition through miR156-targeted *SQUAMOSA* promoter binding-like transcription factors. The Plant Cell, 24(8), 3320–3332.

Yu, X., Klejnot, J., Zhao, X., Shalitin, D., Maymon, M., Yang, H., Lee, J., Liu, X., Lopez, J. & Lin, C. (2007). Arabidopsis cryptochrome 2 completes its posttranslational life cycle in the nucleus. The Plant Cell, 19(10), 3146–56.

Yu, X., Liu, H., Klejnot, J., & Lin, C. (2010). The Cryptochrome Blue Light Receptors. The Arabidopsis Book, (8), 1–27.

Zhao, Y., Christensen, S. K., Fankhauser, C., Cashman, J. R., Cohen, J. D., Weigel, D., & Chory, J. (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science, 291(5502), 306–309.

Zheng, X., Wu, S., Zhai, H., Zhou, P., Song, M., Su, L., … Yang, J. (2013). Arabidopsis phytochrome B promotes SPA1 nuclear accumulation to repress photomorphogenesis under far-red light. The Plant Cell, 25(1), 115–133. h

Zhu, D., Maier, A., Lee, J.-H., Laubinger, S., Saijo, Y., Wang, H., Xi, Y., Li, Z., Cai, Y., Meng, F., Yang, L., Wang, H. & Deng, X. W. (2008). Biochemical characterization of Arabidopsis complexes containing *CONSTITUTIVELY PHOTOMORPHOGENIC 1* and *SUPPRESSOR OF PHYA* proteins in light control of plant development. The Plant Cell, 20(9), 2307–2323.

Zhu, Q. H., & Helliwell, C. A. (2011). Regulation of flowering time and floral patterning by miR172. Journal of Experimental Botany, 62(2), 487–495.

Zhu, Y., Liu, L., Shen, L., & Yu, H. (2016). NaKR1 regulates long-distance movement of *FLOWERING LOCUS T* in Arabidopsis. Nature Plants, 2(6), 16075.

Zuo, Z., Liu, H., Liu, B., Liu, X., & Lin, C. (2011). Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in arabidopsis. Current Biology, 21(10), 841–847.

VIII. Acknowledgements

I would like to express my deepest gratitude to Prof. Dr. Ute Höcker for her continuous support throughout my PhD, for her patience, motivation, valuable suggestions and immense knowledge. Her guidance and constant inspiration helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor than her.

My sincere thanks also goes to Prof. Dr. Maria Albani, for her insightful comments, fruitful discussion and continuous encouragement. The setting up of the *Arabis alpina* project in our group was only possible and efficiently done under her helpful suggestions and generous assistance. I am also sincerely thankful for her time to evaluate this thesis.

I am extremely grateful to Prof. Dr. Werr for being the head of my examination committee.

I deeply appreciate the wonderful working atmosphere, the discussions and general assistances from all the lab colleagues of AG Höcker. My special thanks to Xu for not only her generous share of materials and help with the experiments, but also as a caring sister in private life. I thank Stephen for fruitful discussions and inspiring dialogues. I am grateful for all the support from the lab members, it was great sharing the laboratories with all of you in the past three years. My thanks also goes to Leona for her help with the experiments during her Master project module. Thanks to Oliver for translating the German version of the Abstract. Thanks to Dr. Jathish Ponnu for his help in correction of this thesis, and for accepting to be the besitzer in my PhD exam.

I would like to extend my thanks to all the lab members of AG Albani at MPIPZ, for their friendliness and help with experiments performed there. Thanks to Eva Willing from MPIPZ for her help introducing me into basic bioinformatics. I thank Youbung Hyun for sharing necessary materials for establishing the CRSIPR/Cas9 system in our group. I am highly thankful to all the lab members who work on *Arabis alpina* in AG Coupland from MPIPZ, for all the discussions and their generosity in sharing materials.

I am in dearth of words to express my gratitude to the CEPLAS program. The monthly CEPLAS meetings and discussions with all the elite professors and excellent young researchers inspired me so much for my PhD research. I am highly thankful to the CEPLAS organizers, Celine, Juliane, Esther and Sira, for all the excellent events, in particular to Petra for her help with all necessary documents and her homely care when I newly moved to Cologne for my PhD. I also would like to convey my sincere gratitude towards all the GSFBS staffs, especially Isabell and Kathy.

I wish to express my sincere gratitude towards the professional greenhouse staffs and technical staffs of the institute, for their help and care-taking of the plants.

The funding from CEPLAS, University of Cologne is duly acknowledged.

Eduard, thank you for always being by my side through all the tough times. Thank you for being patient, kind and caring. Life in the past year would be have been totally colorless without you.

My profound gratitude and love go to my family, their constant support and faith in me strengthened and empowered me against no matter what. To my beloved mother, my life-coach, who passed away before her time, whom I owe it all to.

IX. Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen –, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.
Die von mir vorgelegte Dissertation ist von Prof. Dr. Ute Höcker betreut worden.

Panpan Jiang

Lebenslauf

Panpan Jiang
Botanical Institute, Zülpicher str.47b
D50674, Cologne
Geburtdatum: 17. 10. 1989
Geburtsort: Henan, China
Staatsangehoerigkeit: Chinesisch

Hochschulausbildung

2013-2017 Ph.D., Botanik, Universität zu Köln, Köln, Deutschland; Titel der Doktorarbeit: Light signaling in *Arabis alpina*, a perennial relative of *Arabidopsis thaliana*; Betreuerin: Prof. Dr. Ute Höcker

2010-2012 M.Sc. in Plant Production Science, Faculty of Agriculture and Forestry, University of Helsinki, Finland, Schwerpunkte: Molekularbiologie, Hortikultur

2006-2010 B.Sc. Pflanzenschutz, Institute of Plant Protection, Nanjing Agricultural University, Nanjing, China, Schwerpunkte: Phytopathologie

Teilnahme an Wissenschaftlichen Konferenzen

07/2016 *27th International Conference on Arabidopsis Research*, GyeongJu, South Korea

02/2016 *Plant model species, VISCEA Conference.* Ausgewählt für die Präsentation. Vienna, Austria

05/2014 *From model systems to crops, challenges for a new era in plant biology, ICREA Workshop*, Barcelona, Spain

Stipendien und Auszeichnungen

2013-2017 CEPLAS graduate programm, Germany

2012 Finnish National Research Grant 'Maatalous-metsätieteiden rahasto's, Finland

2006-2009 Scholarship for excellent undergraduates, HongKong Christian Council & Nanjing Amity Foundation, China