CHAPTER 2:

Review of Literature
2.1 Light – An important signal for plant growth and development

As sessile organisms, higher plants are characterised by a high degree of developmental plasticity in response to environmental cues, which have an extensive regulatory influence on their growth and development, whereas the plants optimize their developmental patterns in a way that maximizes their chances of survival and reproduction. Light is arguably the single most important environmental factors that regulate plant growth and development (Kendrick and Kronenberg, 1994). It is involved in controlling multiple responses in the plant life cycle, including seed germination, seedling de-etiolation, phototropism, shade avoidance, circadian rhythms and flowering time. Collectively, these light-dependent responses are known as photomorphogenesis. Therefore it is not surprising that plants have adopted the ability to sense multiple parameters of light signals like the presence or absence of light in addition to the duration, wavelength and intensity of incident light and interpreting these signals to produce the appropriate physiological and developmental response (Moller at al., 2002; Montgomery and Lagarias, 2002). The effect of light on the plants between seed germination and the formation of the first true leaves has been studied extensively (Quail, 2002). Arabidopsis seedlings are genetically endowed with the ability to follow two different strategies of development, skotomorphogenesis and photomorphogenesis, depending on the ambient light conditions (Mohr and Shropshire, 1983). Skotomorphogenesis is the developmental strategy followed in darkness and characterised by long hypocotyls, and undeveloped (small and unopened) cotyledons with apical hook, undifferentiated chloroplasts and retarded cell-type differentiation (e.g., no stomata). In contrast, photomorphogenesis is the developmental strategy followed in light, which is characterized by short hypocotyl, open and enlarged cotyledons, with developed chloroplasts and differentiated cell types (Figure1.1). The switch between dark- and light-grown development starts with the perception of light through an array of photoreceptors, each responding to specific regions of the light spectrum. At least four different types of photoreceptors have been identified in Arabidopsis, including the three classical photoreceptors (phytochromes, cryptochromes, and phototropins) and a newly recognised set of blue light photoreceptors (Zeitlupes), F-box proteins containing a light, oxygen, and voltage (LOV) domain and kelch repeats. Phytochromes predominantly absorb the red and far-red wavelengths (600 – 750 nm), whereas cryptochromes and phototropins perceive blue and UV-A wavelengths (320 – 500 nm), and unidentified UV-B photoreceptors absorb UV-B (282 – 320 nm)
Figure 1.1. The contrasting phenotypes of dark-vs light-grown Arabidopsis seedlings. (Arabidopsis Book, 2002).
2.2 Photoreceptors – Light signal Sensor in plants

2.2.1 Phytochrome Gene family

The discovery of physiological responses, such as the germination of lettuce seeds which is promoted by red (R) light and repressed by subsequent far-red (FR) light, led to the identification of phytochromes (Borthwick et al., 1952). Phytochromes, are by the far the most, studied of all the plant photoreceptors. Phytochromes are found in eukaryotes, including plants, green algae, and fungi and in prokaryotes, including both photosynthetic cyanobacteria and eubacteria (Sharrock, 2008). Plants contain multiple forms of phytochrome, and in angiosperms, these falls into two functional groups, type-I and type-II. In Arabidopsis thaliana, there are five PHY genes (PHYA-PHYE) (Sharrock and Quail, 1989). Type-I phytochrome (photo-labile, which includes only PHYA) accumulate in dark grown etiolated seedlings and degrade rapidly upon light exposure, whereas type- II phytochromes, are relatively stable in the light, and includes PHYB to PHYE (Abe et al., 1985; Furuya M. 1989; Tokuhisa et al., 1985). Sequence analysis suggests that these phytochromes can be clustered into three subfamilies: phyA/phyC, phyB/phyD, and phyE. All the phytochromes are expressed throughout the plant. phyA is most abundant in dark-grown seedlings and light down-regulates PHYA both at the transcription and translational level (Clough et al., 1999; Clark at al., 1994; Hirschfeld et al., 1998). Arabidopsis PHYA and PHYB form homodimers (Jones and Quail, 1986; Wanger et al., 1996), and also dimerize with each other via sequences in their C-terminal regions (Edgerton and Jones, 1992; Cherry et al., 1993; Kim et al., 2006), whereas the other PHYs, PHYC and PHYE only heterodimerize with PHYB and PHYD (Ted et al., 2009). Formation of such heteromeric photoreceptors increases the potential complexity of R/FR light sensing and signaling mechanism in plants.

2.2.2 Phytochrome – Structure and spectral properties

Purified Phytochromes are soluble dimeric chromoproteins, with each monomer consisting of a ~125 kDa polypeptide with a covalently attached linear tetrapyrole chromophore, phytochromobilin, which is synthesized in chloroplast from heme (Davis et al., 1999; Duek et al., 2003; Kohchi et al., 2001; Nagy and Schafer 2002). Structurally phytochrome is divided into two domains, connected by a flexible hinge region an
amino-terminal photosensory domain and a carboxy-terminal consist of a regulatory, dimerization and signal output domain (Quail, 1997). The N-terminal domain comprises four sub domains: P1, P2/PAS, P3/GAF, and P4/PHY (named sequentially from N terminus), whereas the C-terminal domain is divided into two sub domains, the PAS-A and PAS-B domains and the histidine kinase-related domain (HKRD) (Figure 1.2) (Wu and lagarias, 2000). The PAS domain is named after three proteins in which it occurs: Per (period circadian protein), Arn (Ah receptor nuclear translocator protein), and Sim (Single-minded protein). The HKRD lacks critical histidine residue. The P1 domain mainly needed for the phytochrome stability and its Pfr conformation. The P2/PAS and P3/GAF domains form the core photosensory domain and contain bilin lyase activity, which is necessary for ligating the chromophore to a cysteine residue in the P3/GAF domain and plays a very critical role in both photosensing and light signaling (Lamparter et al., 2004). The P4/PHY domain is necessary for fine tuning the stability of the Pfr conformation by directly interact with the D-ring of the chromophore to maintain its extended linear conformation in the Pfr form and ensuring proper spectral properties, nuclear localization, and kinase activity (Montgomery and Lagarias 2002).

The ability of a given phytochrome to absorb red and far-red light depends on its bound phytochromobilin, which undergoes a reversible photoisomerization at the C15-C16 double bond in response to red light (666nm) and far-red light (730nm) (Abe et al., 1985). After initial assembly of the phytochrome, the phytochromobilin assumes the C15-Z, \textit{anti} conformation and is ready to absorb the red light and this form of phytochrome is called the Pr form, which is considered as the biologically inactive form. Absorption of red light (R) triggers the conversion of C15-Z \textit{anti} conformation to the C15-E, \textit{anti} conformation resulting in the far-red light (FR) absorbing Pfr form (Figure 1.3), thus phytochrome acts as a switch that is turned on by red light and turned off by far-red light (Brothwick et al., 1952; Rockwell et al., 2006).

Spectral responses of the phytochrome can be classified into different classes based on the radiation energy of light that is required to obtain the response. These include low fluence responses (LFRs), very low fluence responses (VLFRs), and high irradiance responses (HIRs). LFRs are the classical phytochrome responses with R/Fr reversibility. VLFRs are not reversible and are sensitive to a broad spectrum of light between 300nm to 780nm. HIRs requires prolonged or high-frequency of intermittent illumination and it depends on the fluence rate of light (Casal et al., 2002; 2003; Nagy and Schafer 2002; Shinomura et al., 2000). Genetic studies on \textit{Arabidopsis} phytochrome
Figure 1.2. A. Domain structure of phytochrome and their associated functions. PAS, Per (period circadian protein) Arn (Ah receptor nuclear translocator protein), and Sim (single-minded protein); HKRD, histidine kinase-related domain; GAF, cGMP-stimulated phospho diesterase, Anabena adenylate cyclases, and Escherichia coli FhlA. (Annu. Rev. Plant BioI. 2008) B. Photoconversion of phytochrome. Red light (R) triggers a "Z" to "E" isomerization of the linear tetrapyrole, which is accompanied by rearrangement of the apoprotein backbone. This results in the photoconversion of phytochrome from the Pr form to the Pfr form. Far-red (FR) light converts the Pfr form back to the Pr form. (The Arabidopsis Book 2002)

Figure 1.3. Structure of blue light receptors.

A. Cryptochrome. CNT/PHR- CRY1/CRY2 N-terminal photolyase-related (PHR) domain; CCT/DAS - C-terminal-DAS (DQXVP-acidic-STAES) domain; FAD-Flavin adenine nucleotide. B. Phototropin. FMN-Flavin mononucleotide; LOV- (light, Oxygen, Voltage) domain. (Nature review Genetics 2007)
mutants, demonstrated that, PHYA is responsible for the very low fluence responses (VLFR) to a broad spectrum of light and for high irradiance responses to continuous FR, whereas the PHYB is the predominant type-II phytochrome responsible for the red/far-red photoreversible low fluence response (LFR) and shade-avoidance/neighbour-sensing responses to the ratio of R to FR (Mathew, 2006; Rockwell et al., 2006).

Various phytochromes play overlapping but distinct roles. With exception of seed germination and shade-avoidance response, which are solely controlled by phytochromes, other physiological processes, including seedling development and floral induction are controlled by interconnected networks of both phytochromes and cryptochromes. In *Arabidopsis* both PHYA and PHYB promote seed germination and de-etiolation in response to far-red and red light respectively. PHYB inhibits shade avoidance response under a high-ratio of R: FR light, whereas PHYA inhibits excessive shade avoidance responses under a low ratio of R: FR light. PHYA promotes flowering, whereas PHYB delays flowering (Franklin et al., 2007). Analysis of *phyAphyBphyD* and *phyAphyBphyE* triple mutants revealed that the PHYE is required for seed germination along with PHYA under far-red light (Hennig et al., 2002). Studies have shown that functional PHYB and PHYD are necessary for normal cotyledon size in white light (Aukerman et al, 1997). PHYC promotes seedling de-etiolation, primary leaf expansion and delays flowering in response to red light (Balasubramanian et al., 2006; Franklin et al., 2003; Monte et al., 2003). PHYD and PHYE promote seedling de-etiolation and suppress shade avoidance responses (Aukerman et al., 1997; Develin et al., 1998; 1999). Overexpression of PHYD and PHYE under the control of *PHYB* native promoter in *phyB* mutants partially rescues the seedling and leaf morphology of *phyB* mutant, whereas the PHYE could able to rescue the flowering phenotype of *phyB* but not PHYD (Sharrock et al 2003).

Red light induced photo-conversion of Pr to Pfr form leads to exposure of the P3/GAF, PAS-A, and PAS-B domains, and initiates the translocation of the Pfr form of phytochrome into nucleus, where it initiates many downstream signaling events. The C-terminal domain of PHYB constitutively localizes to the nucleus irrespective of the light conditions and form nuclear speckles (Usami et al., 2007), where as PHYA and PHYB localize to nucleus and form nuclear speckles upon light irradiation and it has been demonstrated that nuclear translocation is necessary for the majority of the biological function of phyA and phyB (Kricher et al., 1999; Yamaguchi et al., 1999; Huq et al., 2003; Matsushita et al., 2003; Hiltbrunner et al., 2006; Rosler et al., 2007).
nucleus, phytochromes localize to speckles or nuclear bodies and trigger the transcription cascade that leads to the regulation of light-responsive genes (Kricher et al., 2002). In contrast, PHYC, PHYD, and PHYE constitutively localize to the nucleus in light/dark grown seedlings, but their nuclear speckle formations are either light-dependent (PHYC and PHYE) or light-independent (PHYD).

2.2.3. Role of Heme Oxygenase in phytochrome bio-synthesis.

Plants employ a complex web of signaling pathways directed by several photoreceptor families to perceive and respond to light. Phytochromes (phys) are one of the dominant photoreceptors, directing growth and photomorphogenic response to red (R) and far-red (FR) light (Smith, 1995; Quail, 2002). Phytochromes can assume one of the two stable conformers, a R-absorbing Pr form and a FR-absorbing Pfr form, which are repeatedly photointerconvertible by R and FR, respectively. The coordinated action of two spatially separate pathways are required to synthesize photochemically active phys; one in the chloroplast that produces chromophore (3E)-phytochromobilin (PΦB) and another in the cytosol that synthesizes the phy apoproteins, which are encoded by small families of nuclear genes (PHYA-E in Arabidopsis thaliana; Quail, 2002). The synthesis of PΦB is directed by an enzymatic cascade in the plastid that begins with 5-aminolevulinic acid (Terry et al., 1995; Terry, 1997). The early steps in the PΦB pathway are shared with those required to synthesize chlorophyll and heme. The committed step in the synthesis of phytochrome chromophore is the oxidative cleavage of a portion of the heme pool by a heme oxygenase (HO) to form biliverdin IXα (BV). In plants, this reaction requires both O₂ and the electron donor, ferredoxin, and releases carbon monoxide (CO) and Fe²⁺ (Muramoto et al., 2002). BV is further reduced in plants to (3Z)-PΦB by the ferredoxin-dependent PΦB synthase (Frankenberg et al., 2001; Kohchi et al., 2001). Finally, (3Z)-PΦB is isomerized to create PΦB; whether this 3Z to 3E conversion is enzymatic or occurs spontaneously remains to be determined. Presumably, PΦB is then exported to the cytoplasm where it binds to the newly synthesized apo-phys.

In Arabidopsis HOs are encoded by a small gene families which includes HY1/HO1, HO2, HO3 and HO4. The encoded HO proteins fall into two subfamilies based on amino acid sequence alignments. One subfamily includes HY1, HO3 and HO4, which have the canonical HO active site, that harbours a conserved histidine which functions as the proximal heme ligand and the second sub-family includes just HO2, in which the histidine is replaced by an arginine. Gene expression studies have revealed
that in *Arabidopsis* all the four members of the HO family are transcriptionally active with substantially overlapping pattern of expression. Transcript profiling studies revealed that *HY1/HO1* is clearly the highest expressed; followed by *HO2*, with both *HO3* and *HO4* expressed at low levels. In *Arabidopsis* *HY1* encodes a 282-amino-acid protein, localized to stroma of the chloroplast (Davis et al., 1999; Muramoto et al., 1999). The *HO1*-deficient *hyl* mutant of *Arabidopsis* seedlings shows elongated hypocotyls in white light and almost completely 'blind' to red and far-red light and contain no detectable holophytochrome (Davis et al., 2001; Koornneef et al., 1980; Parks and Quail., 1991), indicating that at seedling developmental stage *HO1* is primarily responsible for PΦB synthesis. Mutants deficient in *HO2* do show a small decrease in holophytochrome accumulation and a parallel (small) decrease in light responsivity, suggesting that *HO2* does have a role in PΦB synthesis in seedlings and expression analysis have revealed that both *HO1* and *HO2* is expressed throughout the seedlings (Davis et al., 2001). One of the characteristics of all chromophore-deficient mutants is that they 'recover' as they mature (Terry et al., 1997). One possible reason is that HOs other than *HO1* play increasingly important role later in development. Another possibility is that *HO3* and *HO4* may have tissue-specific roles. It was noted that both *HO1* and *HO2* were strongly expressed in root tips of etiolated seedlings, a region in which phytochrome genes are highly expressed (Davis et al., 2001). One example where the role of HOs in phytochrome chromophore synthesis is well defined comes from the recent identification of *HO* genes on the same operons as gene encoding bacteriophytochromes (Bhoo et al., 2001).

### 2.2.4. Cryptochromes

Cryptochromes are the major receptors for blue and ultraviolet (UV-A) light. CRY1 and CRY2 are the two well characterised cryptochromes in *Arabidopsis*. The third cryptochrome CRY3 is the most divergent form, and functionally not characterised up to date and the sequence of CRY3 shows more similarity with *Synechosystis* CRY DASH (for *Drosophila-Arabidopsis-Synechosystis-Human*) (Quail, 2002; Kleine et al., 2003; Brudler et al., 2003). Most cryptochromes, with the exception of CRY-DASH proteins, are composed of two domains, an amino-terminal domain photolyase-related (PHR) region and a carboxy-terminal domain of varying size. The PHR region of cryptochrome contains two chromophores that absorb light; one chromophore has flavin adenine dinucleotide (FAD) and the other 5, 10-methenyltetrahydrofolate (pterin or
MTHF). The cryptochrome N-terminal PHR (photolyase related) domain shares sequence similarity with photolyase, a family of flavoproteins which catalyzes the repair of UV-light damaged DNA, and a distinguishing C-terminal domain that is absent in photolyase and has no strong sequence similarity with any known protein domain (Cashmore et al., 1999; Lin and Shalitin, 2003). In case of CRY3 it has no carboxy-terminal extension but has a transient peptide sequence targeting it to both chloroplast and mitochondria (Kleine et al., 2003).

CRY1 is the primary photoreceptor for high blue light fluence, and shuttles between the nucleus and cytosol, as it acts in both places (Cashmore et al., 1999; Wu and Spalding, 2007; Yu et al., 2007). Originally cry1 was identified as hy4 mutant of Arabidopsis deficient in response to blue/UV-A light. Under red and far-red light conditions cry1 mutants behave like wild-type seedlings (Ahmand, M 1999; Koornneef et al., 1980). CRY2, a major player in low fluence of blue light is the second member of the cryptochrome family and constitutively localized in the nucleus, where it completes its posttranslational life cycle (Yu et al. 2007). cry2 mutant is allelic to fha a late-flowering mutant (Koornneef et al., 1991). Major blue light responses mediated by CRY1 and CRY2 includes inhibition of hypocotyl elongation (Furuya, 1993; Quail et al., 1994; Barnes et al., 1996; Ahmad and Cashmore, 1996; Fankhauser and Chory, 1997; Deng and Quail, 1999; Quail, 2002; Schepens et al., 2004; Bauer et al., 2004; Bohlenius et al., 2006), enhancement of cotyledon expansion (Brothwick et al., 1952), anthocyanin accumulation (Boylan and Quail, 1991; Bohlenius et al., 2006) and regulation of flowering time and resetting the circadian clock (Casal et al., 2002; chen et al., 2003; 2006). The C-terminal domain of Arabidopsis CRY1 or CRY2 (CCT1 or CCT2) mediates a constitutive light response through direct interaction with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) (Yang et al., 2000; Wang et al., 2001; Yang et al., 2001). Autophosphorylation of CRY1 and CRY2 are reported to be important for their function (Lin and Shalitin, 2003; Shalitin et al., 2002; Yu et al., 2007). The light activation of the N terminus of CRY1 (CNT) induces a conformational change in its C terminus (CCT), allowing its autophosphorylation and dimerization and possible interaction with the downstream partner proteins (Sang et al., 2005). CRY2 and phyB interact in the nuclear bodies in a light-dependent and interdependent manner (Gue et al., 1998; Mas et al., 2000).
2.2.5. Phototropins

Phototropins are ubiquitous in higher plants and conserved among different plant species. Initially identified as NPH1 (nonphototropic hypocotyl) which codes for a plasma membrane-associated protein from dark-grown pea epicotyls that become phosphorylated upon blue light irradiation (Gallagher et al., 1988). Model Plant Arabidopsis contains two membrane associated phototropins designated as phot1 and phot2, which gets autophosphorylated upon irradiation by blue light and a fraction of PHOT1, is released to the cytoplasm (Briggs and Christie, 2002; Celeya and Liscum, 2005).

Protein structure of plant phototropins can be separated into two segments: a N-terminal photosensory domain composed of two domains (LOV1 and LOV2) and a C-terminal Ser/Thr kinase domain belonging to the AGC family (cAMP-dependent protein kinase, cGMP-dependent protein kinase, and phospholipid-dependent protein kinase C). LOV domains are closely related to a subset of proteins within the PAS domain superfamily that are regulated by external signals such as light, oxygen, or voltage, hence the acronym LOV (Khurana and Poff, 1989; Khurana et al., 1998; Bogre et al., 2003). In dark LOV domains noncovalently binds to cofactor flavin mononucleotide (FMN) chromophore. Upon blue light exposure this LOV domains undergoes autophosphorylation, and results in enhanced kinase activity (Christie, 2007; Tokutomi., 2008). An amphipathic α-helix called the Ja-helix connects the LOV2 domain with the kinase domain. In dark this α-helix is structured and docked on the LOV2 surface. Upon light excitation, the helix unfolds and liberates the kinase domain from the inhibitory effect of LOV2 (Harper et al., 2003; Jones et al., 2007). Together with cryptochromes, phototropins mediate the effects of UV-A/blue light (320-500 nm). Both phot1 and phot2 act to regulate the blue light induced hypocotyl phototropism, leaf flattening, leaf positioning, stomatal opening and blue light induced chloroplast movement (Christie et al., 1998; Sakamoto and Briggs, 2002; Correl and Kiss, 2005; Mao et al., 2005; Christie, 2007; Inoue et al., 2008). Under low intensity of blue light, Phot1 play a major role in accumulating chloroplasts to the upper surface of the leaf to harvest light for photosynthesis, whereas at high intensity of blue light phot2 plays major role in dispensing the chloroplasts away from high irradiation and thus prevent the damage to photosynthetic apparatus (Sakai et al., 2001; Jarillo et al., 2001; Kagawa et al., 2001).
2.3. Downstream signal transducers of photomorphogenesis

Approximately 2500 genes in *Arabidopsis thaliana* (10% of the genome) are regulated by phytochromes under prolonged light exposure, whereas ~ 250 genes are regulated at least two-fold under continuous red light within 1 h (Tepperman et al., 2006). Numerous loci's are involved in transducing the light signal from the photoreceptors to downstream effectors that govern the physiological changes that will eventually result in photomorphogenesis. These molecules may belong to either transcription factors that act downstream at the DNA level or may act as a signal transducers at the intermediate level that may play either positive or negative regulators of the photomorphogenesis. Although some molecules/factors acts at a particular wavelength of light, few respond to a multiple wavelength of light, which shows the presence of some common upstream common signal transducers and shared signaling components. Further signal received by various photoreceptors and downstream molecules converge and transducer the signal to a common set of effectors molecules which in turn translate the perceived signal at the genome level (Figure 1.4).

2.3.1. Phytochrome signaling components

Many genetic and genomic analyses suggest the existence of various regulators downstream to different photoreceptors (Ahmad and Cashmore, 1996; Soh et al., 1998; Hoecker et al., 1999; Bolle et al., 2000; Hsieh et al., 2000; Quail, 2002; Wang and Deng, 2003). Many *Arabidopsis* mutants like *jhy1, jhy3, fin2, fin5, fin219, far1, patl laf1, laf3, laf6* and *hfr1/rep1/rsf1*, show less sensitivity to continuous far-red light, indicating that their wild type gene products acts a positive regulators of phyA signaling (Whitelam et al., 1993; Soh et al., 1998; Cho et al., 2003; Hudson et al., 1999; Hsieh et al., 2000; Bolle et al., 2000, Fankhauser and Chory, 2000; Soh et al., 2000), whereas seedlings mutant for *SPAI* and *EIDI* show increased sensitivity to the FR light signal indicating that these molecules negatively regulate the phyA signaling pathway. Mutants specific for phyB signaling includes *red1, pef2, pef3, gi, elf3, elf4* and *srr1*. These mutants show many red light specific phenotype including long hypocotyls under red light, early flowering in short days, and elongated petioles suggesting that their wild type products positively regulates the phyB signaling (Huq et al., 2000; Liu et al., 2001; Staiger et al., 2003; Doyle et al., 2002; Khanna et al., 2003). At the same time, many genetic and molecular approaches have also identified potential signaling components that act downstream of both phytochrome A and phytochrome B. This group includes *PEF1,*
Figure 1.4. Transcriptional networks for seedling photomorphogenesis
(Nature review Genetics 2007)
**COG1**, **PFT1**, **PRR7** **PSI2**, **PKS1** and **NDPK2** share the signaling events in both phyA and phyB pathway (Ahmad and Cashmore, 1996; Park et al., 2003; Cerdan and Chory, 2003; Kaczorowski and Quail, 2003; Genoud et al., 1998; Fankhauser et al., 1999; Choi et al., 1999). Several biochemical studies have implied the involvement of trimeric G-protein, calmodulin, and cGMP in phytochrome signaling (Romero and Lam, 1993; Bowler et al., 1994; Neuhaus et al., 1997). Therefore it’s evident that each phytochrome may have both unique and shared signaling components that work together to transduce the light signal.

Recent studies suggests that one mode of phytochrome signaling is initiated by the direct interaction of the biologically active form of phytochrome with members of the basic-helix-loop-helix (bHLH) transcription factor superfamily called the Phytochrome Interacting Factors. The PIF1/PIL5, PIF3, PIF4, PIF5/PIL6, PIF6/PIL2 and PIF7 bHLH proteins are principally negative regulators of photomorphogenesis, and preferentially binds to the Pfr form of the phytochromes and in most cases degraded **in-vivo** in phy­dependent ways after transfer from dark to R (Ni et al., 1998; 1999; Zhu et al., 2000; Huq and Quail, 2002; Khanna et al., 2004; Oh et al., 2004; Al-Sady et al., 2006; Leivar et al., 2008). PIFs proteins have a signature bHLH domain consisting of two distinct regions: a ~15 amino acid basic region involved in binding to the target DNA, and an ~60 amino acid HLH region involved in dimerization. PIF1, PIF3 and PIF4 bind specifically to a subtype of E-box called G-box (5’-CAGGTG-3’) (Huq and Quail, 2002; Huq et al., 2004; Martinez-Garcia et al., 2000). PIF3 can homodimerize and also heterodimerize with PIF4 and both PIF3-PIF3 homodimers and PIF3-PIF4 heterodimer bind to the G-box DNA elements (Toledo-Ortiz et al., 2003). Sequence alignments shows that PIFs share in common a conserved sequence motif at their N-terminal region, designated as active phytochrome-binding (APB) motif and this motif is necessary and sufficient for binding to the biologically active Pfr form of phyB (Khanna et al., 2004).

### 2.3.2. Cryptochrome signaling components

Many genetic studies have identified a number of light signaling components including HY5, HYH, AtPP7, HFR1, SUB1, HRB, OBP3, MYC2/ZBF1, SHB1 and GBF1/ZBF2, that play important role in cryptochrome mediated blue signaling (Koornneef et al., 1980; Ang and Deng, 1994; Pepper and Chory, 1997; Fairchild et al., 2000; Guo et al., 2001; Holm et al., 2002; Moller et al., 2003; Kang et al., 2005., Yadav et al., 2005;
Mallapa et al. 2006; Hong et al., 2008). Among these regulatory proteins HYH, AtPP7, MYC2 and GBF1 specifically involved in blue light-mediated signaling.

HYH, a member of bZIP transcription factor, shows close homology with HY5, and promotes blue-light specific photomorphogenesis. Blue light grown hyh/hy5 double-mutant shows enhanced hypocotyl growth than the seedlings carrying hyh or hy5 mutation alone, suggesting that hyh mutation augments the hypocotyl phenotype of hy5, specifically in blue light. Over expression of HYH largely suppress the dramatic phenotypes of hy5, suggesting a functional overlap between these two proteins. And further accumulation of HYH protein and not its mRNA is dependent on the presence of HY5. Both HY5 and HYH can, respectively, acts as heterodimers and homodimers, thus mediating light-regulated expression of overlapping as well as distinct target genes (Holm et al., 2002). AtPP7 encodes a Ser/Thr protein phosphatase and atpp7 seedlings shows loss of hypocotyl growth inhibition and cotyledon expansion and reduction in the expression of light regulated genes in response to blue light, thus AtPP7 acts as a positive regulator of cryptochrome signaling (Moller et al., 2003). MYC2 is a bHLH transcription factor interacts with both Z- and G- box LREs of light-regulated promoters and negatively regulates the blue light mediated photomorphogenic growth and blue and far-red light mediated gene expression, whereas MYC2 positively regulates the lateral root formation (Yadav et al., 2005). ZBF2/GBF1, a bZIP transcription factor functions in blue light mediated signaling and it negatively regulates hypocotyl growth and RBCS1A expression, whereas positively regulates cotyledon expansion, CAB1 expression and lateral root formation (Mallapa et al., 2006).

2.3.3. Positive Regulators

ELONGATED HYPOCHOTYL5 (HY5) is the first genetically characterised positive regulator of photomorphogenesis. Mutations in HY5 results in an elongated hypocotyl in all light conditions suggesting, that HY5 acts down-stream to all the photoreceptors. The phenotype of hy5 seedlings includes defects in light inhibition of hypocotyl elongation, light-induced chlorophyll accumulation, and extensive root abnormalities. In addition hy5 mutants shows several pleiotropic phenotypes like defect in cell elongation, cell proliferation, and chloroplast development and the root phenotypes suggests that HY5 controls cell proliferation positively in the secondary thickening and negatively regulates the lateral root formation (Koornneef et al., 1980; Ang and Deng, 1994; Pepper and Chory, 1997; Ang et al., 1998; Ulm et al., 2004; Lee et al., 2007; Oyama et al., 1997).
DNA protein interaction studies have shown that HY5 specifically binds to the G-box (CACGTG), ACE box (ACGT-containing element) and Z-box containing promoters of various light inducible genes (Ang et al., 1998, Chattopadhyay et al., 1998; Yadav et al., 2002; Shin et al., 2007;). Recently a modified chromatin immunoprecipitation technique in combination with a whole-genome tilling array (CHIP-chip) revealed that HY5 binds to promoter region of a large number of annotated genes (Lee et al., 2007). Most of the genes subject to HY5 regulation are included among the genes regulated by light and constitute ~20% of all light-regulated genes (Ma et al., 2001). Interestingly lee et al., (2007) found that >60% of the early-induced genes by phyA and phyB (Tepperman et al., 2001, 2004) are HY5 binding targets, which suggests that HY5 is high in the hierarchy of the transcriptional cascade during photomorphogenesis. It was shown that HY5 along with phytochrome interacting factor PIF3, positively regulates the anthocyanin biosynthesis gene, flavonone 3-hydroxylase (F3H), where PIF3 and HY5 binds to E-boxes and ACGT-containing elements respectively (Shin et al., 2007). Recent studies have shown that HY5 along with CCA1 binds to the G-box present in the Lhcb promoter and act in concert to regulate the expression of Lhcb genes (Andronis et al., 2008). HY5 binds to the promoter of LZF1, a C2C2-CO B-box containing transcriptional regulator that positively regulates anthocyanin accumulation and chloroplast development (Chang et al., 2008).

CAM7 belongs to a group of seven CAM genes in Arabidopsis, which in turn are grouped into four protein isoforms (CAM1/CAM4, CAM2/CAM3/CAM5, CAM6 and CAM7) based on the homology of their amino acid sequences. In-vitro and in-vivo experiments shows that CAM7 binds to Z-/G- box LRE’s present in the CAB1 and RBCS1A promoter and positively regulate their expression. Overexpression of CAM7 shows hyperphotomorphogenic growth irrespective of all the light conditions and along with HY5. CAM7 acts synergistically for hypocotyl growth and function in an independent and interdependent manner for light gene expression (Kushwaha et al., 2008). HYH (HY5 HOMOLOG) codes for a 17kD, nuclear localised bZIP factor that acts as a weak positive regulator specific to blue light, and its plays additive role along with HY5 for the hypocotyl growth and the accumulation of HYH protein depends on the functional HY5, this suggests that HY5 stabilizes the HYH at the protein level(Holm et al., 2002).

Mutations HFR1/REPI/RSF1 lead to etiolated phenotype in both far-red light and blue light condition (Fairchild et al., 2000; Soh et al., 2000; Spiegelman et al., 2000;
Duek et al., 2003). HFR1 forms a heterodimer with PIF3 and binds to Pfr form of phytochromes (Fairchild et al., 2000; Toledo-Ortiz et al., 2003). LONG AFTER FAR-RED LIGHT (LAF1), a constitutively nuclear localised, two MYB domain containing bHLH proteins, acts a transcription activator specific to far-red light signaling. LAF1 interacts with HFR by forming heterodimers to stabilise the HFR1 level (Ballesteros et al., 2001; Jang et al., 2007). FHY (far-red elongated hypocotyls) 3 and FAR (far-red impaired response) 1 are two essential signal transducers for phyA-mediated FR-HIR responses and play both overlapping and distinct roles in phyA signaling. Both the proteins are targeted to nucleus and form homodimers and heterodimers with each other and (Hudson et al., 1999; Wang and Deng, 2002). Expression of FHY3 and FAR1 are induced by far-red light, which are reported to necessary for the phyA into the nuclear speckles. Further, the expression of FHY3 and FAR1 are decreased with prolonged treatment of far-red, indicating negative feedback regulation by phyA signaling and suggests that FHY3 and FAR1 act at a focal point of a feedback loop that maintains the homeostasis of phyA signaling (Lin and Wang, 2004; Lin et al., 2007).

LZF1/STH3 a B-box containing transcriptional regulator positively regulates anthocyanin, chlorophyll accumulation and chloroplast development and physically interacts with HY5 in a far-red light specific manner (Chang et al., 2008; Dutta et al., 2008). STH2, another B-box containing factor, shows interaction with COP1 and HY5 and positively regulates anthocyanin accumulation and the inhibition of seedling hypocotyl elongation in red and blue light, whereas it negatively regulates lateral root formation (Dutta et al., 2007). HRBl (HYPERSENSITIVE TO RED AND BLUE1) a ZZ type Zinc finger protein, positively regulates leaf expansion and CAB3, CHS and PIF4 gene expression in red and blue light dependent manner (Kang et al., 2005). Blue Insensitive Trait 1 (BIT1) a MYB transcription factor acts as a positive regulator of blue-light dependent seedling development and positively regulate the expression of PsbS and CHS in blue light dependent manner, and it interacts with COP1 and undergoes proteosomal degradation in blue light dependent manner (Hong et al., 2008).

Two Myb-related transcription factors-CCA1 (CIRCADIAN CLOCK ASSOCIATED 1) AND LHY (LATE ELONGATED HYPOCOTYL) and a pseudo-response regulator TOC1 (TIMING OF CAB EXPRESSION 1) acts as a components of core oscillators in circadian rhythms (Wang and Tobin, 1998; Schaffer et al., 1998; Alabadi et al., 2001). OBF4 binding protein (OBP) 3, a nuclear localised Dof
transcription factor, acts as a positive regulator of red and white light mediated inhibition of hypocotyl elongation (Ward et al., 2005).

2.3.4. Negative regulators

Several molecules act as negative regulators of light signaling by down regulating the expression of genes that promote photomorphogenesis and thus keep the photomorphogenic growth under check in the absence of light. Analysis of mutants that display a complete light-grown phenotype in darkness have defined a total of 11 genes (COP1, DET1, COP8-10, FUS5, FUS6, FUS8, FUS11, FUS12, COP16) which are collectively called COSTITUENTLY PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA (COP/DET/FUS) loci (Chory et al., 1989; Deng et al., 1991; Misera et al., 1994; Kwok et al., 1996). Transcript products of these genetic loci’s act as negative regulators of the light control of plant development (Hoecker, 2005). Dark-grown mutant seedlings of this group display several pleiotropic phenotypes, including open and expanded cotyledons, short hypocotyl, chloroplast development, stomatal maturation, expression of light-inducible genes, and anthocyanin accumulation (Schwechheimer and Deng, 2000; Yi and Deng, 2005). The pleiotropic phenotype of these mutants implies that these genes constitute the developmental switch between the photomorphogenic and skotomorphogenic programs (McNellis and Deng, 1995) and mutants for all these loci are lethal in adult stage, indicating that the functional product of these genes have an essential role in plant development. Biochemically, these COP/DET/FUS proteins belong to three groups of protein complexes: the COP1 complex (es), the COP9 signalosome (CSN) and the CDD complex (COP10, DDB1, and DET1).

The COP9 signalosome (CSN) is an evolutionary-conserved multi-protein complex composed of eight subunits, of which six subunits contain the conserved PCI (Proteasome, COP9 signalosome, eukaryotic initiation factor) domain and the remaining two subunits contain the conserved MPN (MOV34, PAD N-terminal) domain (Wei and Deng, 2003; Schwechheimer, 2004). The PCI domain has a α-helical structure, localized at the C terminus of the protein, and considered to be important for proper complex assembly (Tsuge et al., 2001) The MPN domain is predicted to have an α/β structure, positioned at N terminus of the protein and plays role in protein-protein interaction (Fu et al., 2001). This eight-subunit COP9 signalosome (CSN), a nuclear enriched protein complex shows homology with the lid sub-complex of the 26S proteosome and acts to deconjugate NEDD8/Rub1 from the cullin subunit of SCF(SKP1/Cullin/F-box)-type E3
ligases (Lyapinia et al., 2001; Schwechheimer et al., 2001; Serino and Deng, 2003; Wei and Deng, 2003). The phenotype of loss-of-function of CSN subunits includes, altered photomorphogenic response, altered response to plant hormones with severe morphological defects, defects in leaf morphology and symmetry, altered cotyledon number and floral abnormalities (Peng et al., 2001a; 2001b; Schwechheimer et al., 2001). These defects suggest that CSN cellular activity affects multiple pathways in the plant life cycle. The CDD (COP10-DDB1-DET1) complex, so far reported only in plants, is able to enhance E2 ubiquitin-conjugating activity in-vitro (Yanagawa et al., 2004) and plays supportive role along with COP1 to ubiquitylate photomorphogenesis-promoting factors, possible by acting as an E2 enhancer (Suzuki et al., 2002).

Among these COP/DET/FUS proteins, COP1 is at the point of convergence downstream to multiple light signals (Von Arnim and Deng, 1994; Osterlund and Deng, 1998) and widely considered as a central repressor of photomorphogenesis. COP1 was the first in the COP family to be cloned and characterised at the molecular level (Deng et al., 1992; Wei et al., 1994; Chamovitz et al., 1996; Staub et al., 1996). COP1 encodes a protein with a novel combination of three structurally recognised domains, namely an N-terminal RING-finger domain, a putative coiled-coil (Coil) region and C-terminal WD-40 repeats (Deng et al., 1992; Mcnellis et al., 1994a). The RING-finger domain comprises eight metal ligands with a consensus of C3HC4, and binds two zinc atoms in a unique tetrahedral 'cross-brace' thus forming one integrated structural unit (Von Arnim and Deng, 1993; Borden and Freemont, 1996; Saurin et al., 1996). The Coil region is predicted to be a α-helical structure capable of forming a superhelix (Lupas, 1996). The WD-40 motif is ~40 amino acids in length and contains a highly conserved tryptophan-aspartate (WD) sequence which is characteristic of β subunit of trimeric G-protein (Deng et al., 1992; Neer et al., 1994; Torii et al., 1998). The core domain of COP1 has a bipartite nuclear localization signal, which helps in the nuclear import of COP1 and a cytoplasmic localization signal (CLS), which is necessary to shuttle back COP1 to cytoplasm. Genetic and molecular studies have indicated that COP1 acts as an autonomous repressor of photomorphogenesis. Overexpression of full-length COP1 causes reduced light responsiveness whereas overexpression of truncated COP1 fragment containing RING-finger and Coil domain alone shows a hyperphotomorphogenic phenotype both in dark and light conditions indicating that COP1 N-terminal fragment alone can sustain the basal function during development of Arabidopsis (McNellis et al., 1994; 1996; Stacey et al., 2000), whereas overexpression of
WD-40 repeat alone reduces seedling responses to light. GUS-COP1 fusion protein studies have shown that in dark COP1 accumulates in nucleus to suppress photomorphogenesis, whereas light inactivates COP1 which is indicated by reduced COP1 abundance in the nucleus (Von Arnim and Deng 1996, Osterlund and Deng, 1998; Stacey et al., 1999). Studies have shown that three photoreceptors phyA, phyB and cry1, trigger nuclear depletion of COP1 under their respective light conditions, by regulating the balance between the competing CLS and NLS activities, which further supports the notion that COP1 acts downstream of multiple photoreceptors (Osterlund and Deng, 1998; Stacey et al., 1999).

Arabidopsis SPA1 (suppressor of phytochrome A-105), a negative regulator of phyA signaling, is a nuclear localised protein (Hoecker et al., 1998). SPA1 is a member of a small family of four structurally related proteins: SPA1, SPA2, SPA3 and SPA4. Structurally SPA proteins contain an N-terminal kinase like domain, followed by a coiled-coil domain and seven WD-40 repeats in the C-terminal, thus SPA proteins show homology with the COP1 protein at the WD-40 domain. Yeast two-hybrid and in-vitro interaction studies have shown that SPA1 protein physically interacts with COP1 through their mutual coiled-coil domains (Laubinger and Hoecker, 2003; Laubinger et al., 2004). Seedlings of quadruple spa mutants display constitutive photomorphogenic phenotype similar to cop1 mutants, suggesting the functional redundancy among these negative regulators and SPA1 may function in a parallel pathway along with COP1 in far-red light. In addition, SPA1, like COP1 physically interacts with HY5 and HFR1, two substrate proteins targeted for degradation by COP1. These observations suggest that SPA1 may function in concert with COP1 to target proteins (such as HY5 and HFR1) for degradation, thereby repressing photomorphogenesis in light (Saijo et al., 2003; Yang et al., 2005). A very recent report has shown that COP1 and the four partially redundant SPA proteins are interdependent in forming a heterogeneous group of functional SPA-COP1 complexes and each complexes may contribute equally to the regulated proteolysis of HY5, thus contributing to the fine-tuning of the light control of plant development (Zhu et al., 2009).

Several phytochrome interacting factors also functions as a negative regulators of light signaling. PIF3 negatively regulates the phy mediated inhibition of hypocotyl elongation, where as, positively promotes the cotyledon expansion and anthocyanin accumulation. COP1 stabilises PIF3 in darkness whereas it is degraded by phytochromes (Kim et al., 2003; Monte et al., 2004; Shin et al., 2007; Bauer et al.,
PIF1, a bHLH protein negatively regulates several aspects of phyA-phyB mediated photomorphogenesis. PIF1 negatively regulates light induced seed germination and chlorophyll biosynthesis as well as plays a minor role in suppression of hypocotyl elongation and cotyledon expansion. PIF1 also regulates gibberellic acid metabolism by repressing the expression of GA biosynthetic genes (GA3ox1 and GA3ox2) and upregulates the expression of GA catabolic gene (GA2ox2) and thus suppress seed germination (Oh et al., 2006, 2007). PIF1 is known to be phosphorylated, polyubiquitinated and degraded by phytochromes under both red and far-red light conditions (Shen et al., 2008). PIF4 is involved in the phyB mediated seedlings deetiolation and selectively binds to Pfr form of phyB through its APB motif (Khanna et al., 2004). Very recently PIF7, a bHLH factor shown to be involved in red light mediated hypocotyl growth inhibition, and together with PIF3, PIF4 promotes the degradation of PHYB in an additive manner (Leivar et al., 2008).

EID1 (Empfindlicher Im Dunkelroten Lict) is a F-box protein that acts as a negative regulator in phyA mediated signaling (Buche et al., 2000). EID1 forms SCF$^{EID}$ ligase which degrades the positive regulators of phyA signaling pathway (Dieterle et al., 2001). COG1, a Dof family transcription factor acts a negative regulator under both red and far-red light (Park et al., 2003). MYC2/JAI1/JIN1, a bHLH factor acts a negative regulator for blue and far-red mediated photomorphogenesis and specifically binds to Z-/G-box LREs present in the CAB1 and RBCS1A promoter and negatively regulates its expression whereas MYC2 positively regulates flowering and lateral root formation (Yadav et al., 2005). GBF1/ZBF2 a bZIP factor binds to both Z and G-box LREs present in CAB1 and RBCS1A promoter, and acts a repressor in blue light mediated seedling deetiolation and positive regulator for cotyledon expansion and lateral root formation (Mallapa et al., 2006). Short Hypocotyl under White light (SHW) 1 acts as a negative regulator of hypocotyl growth in white light and dark and positively regulates the expression of CAB and RBCS gene expression (Bhatia et al., 2008).

2.4. COP1 interacts with different photomorphogenesis promoting factors

Regulated proteolysis has an essential role in the development of all organisms. Targeted protein degradation via the 26S proteasome is one of the most important means utilised by all the eukaryotic organisms to control transcription, signal transduction, cell cycle progression, and metabolic activities. For many substrates ubiquitin attachment is one of the important signals for proteasome-mediated degradation. The attachment of the
76-amino-acid ubiquitin to a degradation substrate is catalysed by the sequential activities of an ubiquitin activating enzyme (E1), an ubiquitin-conjugating enzyme (E2), and an ubiquitin ligase enzyme (E3). It is generally believed that E3 confers the substrate specificity by direct recognition of the substrate proteins (Pickart, 2001) and the E3 ubiquitin ligases comprises of a large and diverse family of proteins or protein complexes containing either a HECT domain or a RING/U-box domain. The RING domain E3s is further divided into single subunit RING/U-box E3s, which includes Constitutive Photomorphogenesis1 (COP1), SEVEN IN ABSENTIA IN ARABIDOPSIS THALIANA 5 (SINAT5), and Arm Repeat-containing1 (ARC1), and the multi-subunit RING E3s includes SCF, CUL3-BTB, and APC complexes (Moon et al., 2001).

COP1 belongs to a group of pleotropic CONSTITUTIVE PHOTOMORPHOGENIC (COP/DET/FUS) proteins that has been identified as repressors of photomorphogenic development in plants (Osterlund et al., 1999; Wei and Deng, 1999). COP1 contains three structural domains: an N-terminal RING (Really Interesting New Gene) finger domain, a central coiled-coil domain and a C-terminal WD-repeat domain. Micro array analysis found that >20% of the Arabidopsis genome, representing more than 28 pathways, is regulated by COP1 in dark and the most of the light-activated gene expression is repressed directly or indirectly by COP1 activity (Ma et al., 2002). COP1 acts as a E3 ligase to target several photomorphogenesis-promoting transcription factors, such as ELONGATED HYPOCOTYL5 (HY5) (Osterlund et al., 2000), LONG AFTER FAR-RED LIGHT1 (LAF1) (Seo et al., 2003), LONG HYPOCOTYL IN FAR-RED1 (HFR1) (Duck et al., 2004; Jang et al., 2005; Yang et al., 2005; Lin and Wang, 2007). In addition, the far-red light photoreceptor phyA (Seo et al., 2004) and the blue light photoreceptor cry2 (Wang et al., 2001; Shalitin et al., 2002) are also likely targets of COP1.

HY5 a bZIP protein, most extensively studied transcription factor involved in promoting photomorphogenesis by multiple photoreceptors as well as in the regulation of lateral root development (Koornneef et al., 1980; Oyama et al., 1997; Ang et al., 1998). In-vitro DNA-protein interaction studies revealed that HY5 binds specifically to the G-box present in the promoters of several light-inducible genes, such as CHS and RBCSI A (Ang et al., 1998; Chattopadhyay et al., 1998). COP1 the master repressor of photomorphogenic pathway, interact both genetically and physically with HY5 and negatively regulates its activity and both proteins co-localise to form sub-nuclear speckles in living plant cells (Ang and Deng, 1994; Ang et al., 1998). Studies have
shown that COP1 interacts with HY5 through its coiled-coil domain and in turn the N-terminal 77 amino acid of HY5 is necessary for its interaction with COP1. HY5 is phosphorylated by a light-regulated casein kinase II (CKII) at the COP1 binding domain and this phosphorylation abolishes the interaction of HY5 with COP1 and its subsequent degradation (Hardtke et al., 2000).

HYH, another G-box binding bZIP protein, involved in blue light-signaling interacts with COP1 WD-40 domain, through a COP1 interaction motif present in the HYH protein and degraded in the dark involving the CSN complex (Holm et al., 2002). LAF1, a nuclear localised MYB transcription activator that participates in the transmission of phyA signals to downstream responses is also ubiquitinated by COP1 by its interaction with ring finger domain of COP1 and further this ubiquitination is stimulated by SPA1, a negative regulator of phyA signaling (Ballesteros et al., 2001; Seo et al., 2003). HFR1 a bHLH transcription factor that plays role in far-red light specific signaling is also known to co-localize with COP1 in the nuclear bodies and the N-terminal region of HFR1 interacts with the COP1 WD-40 domain and ubiquitinated by COP1 E3 ligase and marked for post-translational degradation during photomorphogenesis (Jang et al., 2005). FHY1 a genetically characterised positive signal transducer of the phyA pathway is regulated by far-red light and phyA through the 26S proteasome-mediated degradation (Shen et al., 2005). PIF3, a negative regulator in phytochrome pathway undergoes ubiquitin mediated proteolysis by the COP1-E3 ligase activity in a light dependent manner (Bauer et al., 2004). Recently it has been reported that PIF1 is regulated by light mediated degradation through the ubiquitin-26S proteosome pathway (Shen et al., 2005). Photoreceptor phyA, co-localise with COP1 and both the Pr and Pfr form of phyA as well as the PHYA apoprotein interacts and ubiquitinated by COP1 (Seo et al., 2004).

Therefore, light mediated signal transduction pathways get desensitized or stopped by COP1-E3 ligase mediated proteolysis of positive as well as negative regulators of photomorphogenesis, or directly the photoreceptor itself, in presence or absence of light.

2.5. Light regulated gene expression

Plants not only use light as an energy source, but also as an informational signal to control developmental processes for optimal growth under the prevailing light environment (kendrik and Kronenberg, 1994; Mcnells and Deng, 1995). Many of the
light-controlled developments are triggered by alterations in gene expression through the
regulated transcription of specific genes in defined cell types and developmental stages
(Gilmartin et al., 1990; Thompson and White, 1991; Tobin and Kehoe, 1994; Teraaghi
and Cashmore, 1995). Some genes such as nuclear-encoded photosynthesis related genes
for chlorophyll a/b binding proteins (CAB) and ribulose 1, 5-bisphosphate carboxylase
small subunit (RBCS), are induced by light. On the other hand, some genes, such as
PHY A, NADPH-protochlorophyllide reductase and asparagine synthase are down-
regulated by light (Donald and Cashmore, 1990; Gilmartin et al., 1990; Ha and An,

2.5.1. Light responsive elements and their interacting protein partners
Characterisation of the promoter elements involved in light regulation has been widely
used as a convenient starting point for understanding the light control of gene expression
(Gilmartin et al., 1990; Manzara et al., 1991; Anderson et al., 1994; Conley et al., 1994).
Studies on the light control of transcription by deletion and mutagenesis analysis of the
promoter reporter constructs of different light regulated promoters such as CAB, RBCS
and CHS has led to identification of a number of light responsive elements (LREs).
These LREs are defined as small sequences of 6-10 bp long present upstream of the
transcription start site and sufficient to confer light regulated expression of the promoter.
Several consensus sequence elements, including G, GATA, GT1, and Z-box are
commonly present in the light regulated minimal promoter regions and necessary for
high promoter activity in the light (Kehoe et al., 1994; Terzaghi and Cashmore, 1995).

GT1 has the core sequence GGTTAA. GT1 sites are usually found in tandem,
and spacing between two sites is critical. These are found in a number of genes such as
RBCS3A, PHYA, CAB, RCA, PETA, and CHS15 (Green et al., 1989; Gilmartin et al.,
1992; Sarokin et al., 1992; Dehesh et al., 1990; Orozco and Ogren, 1993). GATA (I box)
elements have the core sequence GATA, and found in many light regulated promoters
of both monocot and dicot plants (Borello et al., 1993; Gidoni et al., 1989; Guiliano et
al., 1988). RBCS genes have a single GATA element near G-box whereas CAB has two
or three GATA elements arranged in tandem and separated by few base pairs and found
near the TATA–box (Batschauer et al., 1994; Borello et al., 1993; Carrasco et al., 1993;
Gidoni et al., 1989). GATA element is also present in the non-light regulated promoters
(Lam and Chua, 1990). G-box element has the core sequence CACGTG, found in the
promoters of many genes such as CAB, RBCS, CHS and RCA (Foster et al., 1994;
Menkens et al., 1995; Arias et al., 1993; Block et al., 1990; Orozco and Ogren, 1993; Weishaar et al., 1991). G-box binding factors have been identified and cloned from many different plant species (Carrasco et al., 1993; Foster et al., 1994; Lubbertstedt et al., 1994; Menkens et al., 1994; Schindler et al., 1992; Schulze-Lefert et al., 1989). Z-box element has the core sequence ATACGTGT and found in light regulated promoter of CAB gene. Deletion analyses of Arabidopsis CAB1 promoter have demonstrated that the Z-box is essential for the light dependent developmental expression of CAB1 gene (Ha and An, 1988). Very recently three factors MYC2/ZBF1, GBF1/ZBF2, and CAM7/ZBF3 has been shown to interact with Z-box LREs present in several light regulated genes such as CAB1 and RBCS1A promoters.

Recent studies have demonstrated that combinatorial interactions of distinct LREs is an important factor for light regulated promoter activities and these paired LRE-containing promoters can respond to phytochrome activating low-fluence light pulses and can mimic the responsiveness of native light regulated promoters (Degennhardt and Tobin, 1996; Feldbrugge et al., 1997; Puenete et al., 1996). Synthetic promoters with paired LREs such as G-GATA and GT1-GATA are able to respond to a wide spectrum of light mediated by multiple photoreceptors including phyA, phyB and cry1, similar to native light inducible promoters, where as light-inducible single LRE (such as G-box, GATA) containing promoters respond only to a particular wavelength of light (Chattopadhyay et al., 1998; Yadav et al., 2002).

2.6. Cross-talk between light and hormone signaling pathways
Seedlings follow skotomorphogenic development, when seeds germinate in the dark, whereas the alternative developmental program, photomorphogenesis, is triggered if seeds germinate in presence light (Neff et al., 2000). Plant growth and development is a complex phenomenon which are regulated and co-ordinated through interactions between light and phytohormones. Hormones have profound effects on plant growth and development, and in many cases the signals from hormones and those derived from light interact either positively or negatively. De- etiolation is also controlled by endogenous cues such as hormones and various studies have shown that correct hormone homeostasis in etiolated seedlings is essential to properly control the transition between skotomorphogenesis to photomorphogenesis (Vandenbussche et al., 2005). Seedlings defective in either gibberellins (GA) or brassinosteroid metabolism or signaling are not able to fully repress photomorphogenesis after germination in darkness and the seedlings
lose their apical hook and have open cotyledons, and expression of light regulated genes are elevated (Achard et al., 2003; Alabadi et al., 2004; Vriezen et al., 2004). Exogenous application of either auxin or gibberellins can stimulate hypocotyl elongation in light-grown seedlings (Jensen et al., 1998; Saibo et al., 2003). Light inhibits the positive effects on hypocotyl elongation growth of auxin, brassinosteroids and gibberellins. Depending on the species, light regulates biosynthesis and/or signaling of gibberellins (Garcia-Martinez and Gil, 2001). Brassinosteroid biosynthesis genes are generally downregulated by light (Ma et al., 2001) and photomorphogenesis is modulated by inactivators of brassinosteroids phyB activation-tagged suppressor1 (BAS1) and suppressor of phyB-47 (SOB7) (Turk et al., 2003, 2005). Blue light and cytokinins participate to varying degrees in numerous plant growth and development responses. Etiolated seedlings treated with exogenous cytokinins have short hypocotyls and expanded cotyledons similar to that of light-grown seedlings (Chory et al., 1994). Activity of several IAA proteins modified through phosphorylation by photoreceptors. Recently phytochrome B (PHYB) and a cytokinin-related two-component signaling pathway have been suggested to converge (Heyl and Schmulling, 2003; Sweere et al., 2001; Salmone et al., 2006), through the demonstration of the RESPONSE REGULATOR 4 protein, a possible mediator of cytokinin action, directly interacts with phytochrome B and stabilizes its active form (Sweere et al., 2001). It was suggested that PHYB, ARR4 and the circadian oscillator may function as signalling intermediates to integrate light and cytokinin pathways (Zheng et al, 2006).

Light perceived by phytochromes, promotes seed germination partly by increasing GA biosynthesis. PIL5, a bHLH protein is one of the major component linking light signals to GA metabolism. PIL5 inhibits seed germination by lowering bioactive GA levels in the absence of light, partly by repressing the transcription of two GA3ox genes and activating the expression of GA catabolic gene GA3ox2 gene resulting the reduced level of bioactive GA in wild-type seeds (Oh et al., 2006). PIL5 activates the expression of two DELLLA protein-encoding genes RGA and GAI that function as are repressors of GA signaling (Oh et al., 2007). Recently it was shown that PIF3 and PIF4 are involved in the positive control of genes mediating cell elongation and these factors are negatively regulated by phyB in light and reported to interact with DELLAs. These DELLAs prevent PIF2 from binding to their cognate promoters and thereby antagonize PIF dependent transcriptional activation. In the presence of GA, these DELLAs are
targeted for degradation, thereby allowing PIF3 and PIF4 to exert their function (Lucas et al., 2008; Feng et al., 2008).

HY5, a bZIP protein, is one of the major positive regulators of photomorphogenesis, acts downstream to the entire photoreceptor network (Ang et al., 1998; Chattopadhyay et al., 1998). Seedlings mutant for HY5 shows various pleiotropic phenotypes, including inhibition of hypocotyl elongation, anthocyanin accumulation and lateral root formation, which are known to depend on the action of hormones. Studies have shown in hy5 mutant there is altered balance of auxin and cytokinin signaling and also is decreased expression of two negative regulators of auxin signaling, the AUXIN RESISTANT 2 (AXR2/IAA7 and SOLITARY ROOT (SLR)/IAA14 genes. Increased expression of AXR2 in hy5 partially rescues the elongated hypocotyl phenotype (Cluis et al., 2004). HY5 also mediates ABA response in seed germination, early seedling growth and root development by directly binding to the ABI5 promoter and this binding is necessary for transcription of ABI5 and ABI5 targeted late embryogenesis genes in seeds (Chen et al., 2008).

MYC2 a bHLH protein that acts as a negative regulator of photomorphogenesis in blue light signaling pathway as been shown to function as a point of cross-talk between light, ABA, jasmonic acid and JA-ethylene signaling pathways (Abe et al., 2003; Anderson et al., 2004; Boter et al., 2004; Lorenzo et al., 2004; Yadav et al., 2005). ABA and Drought positively regulates the MYC2 expression and transgenics overexpressing MYC2 shows ABA hypersensitivity and thus MYC2 acts a positive regulator of ABA signaling (Abe et al., 2003). MYC2 negatively regulates Trp and Trp-derived secondary metabolism such as indole-glucosinolate biosynthesis during JA-signaling, whereas MYC2 positively regulates JA-mediated resistance to insect pests, such as Helicoverpa armigera, and tolerance to oxidative stress. Also, MYC2 regulates JA responses via differential regulation of an intermediate spectrum of transcription factors with activating or repressing roles in JA signaling. Binding of hormone JA to its receptor induces SCF\(^{COI}\) - dependent proteasome degradation of JAZ proteins liberating MYC2 and allowing transcriptional activation of jasmonate responses, however since JAZ genes are transcriptional targets of MYC2, their rapid expression induced MYC2 leads to negative regulation of MYC2 expression (Chini et al., 2007). Similarly a JA activated MAPK KINASE 3(MKK3)-MAP KINASE 6 (MPK 6) negatively regulates MYC2 (Takahashi et al., 2007).