CHAPTER 2:

Review of Literature
2.1. Photoreceptors regulate plant growth and development

Light is one of the very important parameters on which plants depend for their growth and development. In order to adjust to constant changing environmental conditions, plants have evolved an array of photoreceptors. These photoreceptors allow plants to utilize a broad spectrum of light, starting from ultraviolet (UV<400 nm) to far-red (>700 nm), to control plant growth and development. Three families of photoreceptors that perceive regulatory light signals have been identified: phytochromes (PHY), cryptochromes (CRY) and phototropins (PHOT). Light induced signal transduction starts with the perception of light by these specialised photoreceptors and culminates in the regulation of the expression of about 2500 genes in Arabidopsis thaliana (Tepperman et al., 2001, 2004).

2.1.1. Phytochromes

Plant phytochromes are dimeric proteins typically consisting of two identical apoproteins covalently linked with phytochromobilin, a linear tetrapyrrole bilin with compound that acts as a chromophore. The ability of a given phytochrome to absorb red and far-red light stems from its bound phytochromobilin, which undergoes a reversible isomerization at the C15 and C16 double bond in response to red light (666 nm) and far-red light (730 nm) (Abe et al., 1985). After initial assembly of phytochrome, the phytochromobilin assumes the C15-Z,anti conformation and is ready to absorb red light. This form of phytochrome is called Pr form and is considered to be the biologically inactive form, upon absorption of red light the C15-Z,anti conformation is converted to the C15-E,anti conformation. This form of phytochrome is called Pfr form.

The carboxy-terminal domain of PHY contains two putative dimerisation sites (PAS1 and PAS2), a Quail-box that is responsible for interaction with partner proteins, and a histidine kinase domain. PHYs are synthesised in the dark in the physiologically inactive (Pr) form. Upon absorption of red light, Pr undergoes photo-conversion to the active Pfr form; this process is reversible by far-red light, allowing the phytochrome to act as a switch that is turned on by red light and turned off by far-red.

The plant phytochromes are encoded by a small gene family of five PHY genes in Arabidopsis (Mathews, 2006). The various phytochromes show similar but different molecular properties. PHYA is the light labile and is predominate phytochrome in etiolated seedlings, whereas PHYB and other phytochromes are light stable and predominate in light grown plants.
The stability of PHYC is dramatically decreased in *phyB* mutants in both *Arabidopsis* and Rice, suggesting that PHYB controls the activity of PHYC by regulating its stability (Monte et al., 2003; Tokano et al., 2005). *Arabidopsis* PHYA dimerizes with itself, whereas all other *Arabidopsis* PHYs can form dimers with each other. These various phytochromes differ largely with respect to their spectral specificities. In *Arabidopsis*, majority of the responses PHYA is responsible for the very low fluence response (VLFR) and the far-red high irradiance response (FR-HIR) (Dehesh et al., 1993; Nagatani et al., 1993; Shinomura et al., 1996, Whitlam et al., 1993), whereas the other phytochromes are responsible for the red/far-red reversible low fluence response (LFR) (Reed et al., 1994 and 1993). However, PHYA can mediate red light signaling under very high irradiance red light and during dark-light transitions (Franklin et al., 2007; Franklin and Whitlam, 2007; Tepperman et al., 2006). Various phytochromes play overlapping but distinct roles. 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 low R:FR light. PHYA promoted flowering, whereas PHYB delays flowering (Franklin et al., 2007). PHYC promotes seedling de-etiolation and primarily leaf expansion in response to red light and delays flowering (Balasubramanian et al., 2006; Franklin et al., 2003 Monte et al., 2003), whereas, PHYD and PHYE promote seedling de-etiolation and suppress shade avoidance responses (Aukerman et al., 1997; Delvin et al., 1998; Delvin et al., 1999). When PHYD and PHYE are overexpressed in *phyB* mutant background under the control of PHYB promoter, they could rescue the seedling and leaf morphology phenotypes of *phyB* partially, and only PHYE could able to rescue the flowering phenotype of *phyB* but not PHYD. These results suggest that phytochromes have overlapping and distinct functions (Sharrock et al., 2003) and the functions of PHYC, D and E are dependent on PHYB.

2.1.2. Cryptochromes

In *Arabidopsis*, blue light and UV-A responses are primarily mediated by four blue light photoreceptors in *Arabidopsis*: CRY1 and CRY2, PHOT1 and PHOT2. Major blue light responses mediated by blue light photoreceptors CRY1 and CRY2 include 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 (Borthwick et al., 1952), anthocyanin accumulation (Boylan and Quail, 1991; Bohlenius et al., 2006) and regulation of flowering time (Casal et al., 2002;
Chen et al., 2003; Chen et al., 2006). The hy4/CRY1 mutants grown under blue light show elongated hypocotyls with less expanded cotyledons. CRY1 and CRY2 are both found in the nucleus, CRY1 was reported to undergo nucleus/cytoplasm shuttling in response to light, but no such subcellular trafficking has been reported for CRY2 (Cashmore et al., 1999; Guo et al., 1999; Kleiner et al., 1999; Yang et al., 2001). Nuclear localised cryptochrome closely interacts with the chromatin. Very recently it has been reported that CRY2 is ubiquitinated in the nucleus and further targeted for degradation by the 26S proteasome in response to blue light (Yu et al., 2007). Another cryptochrome (Lin and Shalitin, 2003), CRY3 is very closely related to cryptochrome isolated from cyanobacteria and is significantly different from both CRY1 and CRY2 (Brudler et al., 2003; Kleine et al., 2003). CRY3 has dual targeting signal and mediates its transport to chloroplast and mitochondria, indicating a potential role in regulating transcription in organelles.

CRY1 and CRY2 have an N-terminal photolysase-related (PHR) domain (CNT) and a less-conserved, intrinsically unstructured C-terminal DAS domain (CCT), which is not present in CRY3 (Brudler et al., 2003; Partch et al., 2005). The PHR domain non-covalently binds to two chromophores, a flavin adenine dinucleotide (FAD) and a pterin. CCT 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).

2.1.3. Photoropins

PHOT1 and PHOT2 work together to mediate phototropism, blue light induced chloroplast movements, leaf flattening, leaf positioning and blue light dependent regulation of stomatal opening and rapid inhibition of hypocotyl growth (Clark et al., 1994; Christie, et al., 1998; Sakamoto and Briggs, 2002; Correl and Kiss, 2005; Mao et al., 2005; Christie, 2007; Inoue et al., 2008). Studies have shown that CRY and PHOT perform overlapping roles. For example, PHOT functions at an early stage to regulate photomorphogenic development, including rapid inhibition of hypocotyl elongation (Dehesh et al., 1993) and enhancement of cotyledon expansion (Deng et al., 1991). CRY and PHOT function together to enhance phototropism under low fluence rate of blue light.
Phototropins are plant-specific blue light receptor protein kinases with two, light-oxygen-voltage (LOV1 and LOV2) domains in the N-terminal half, serve as photosensors, and a C-terminal half with serine/threonine kinase function (Briggs and Christie, 2002). These LOV domains are found in proteins regulating responses to light, oxygen, or voltage (Khurana and Poff, 1989; Khurana et al., 1998; Taylor and Zhulin, 1999).

Both PHOT1 and PHOT2 of Arabidopsis are largely associated with the plasma membrane, although following activation by light, a fraction of PHOT1 is released to the cytoplasm (Chen et al., 2004). Phototropins also have well established kinase activity. The blue light triggered autophosphorylation of these receptors initiates the transduction of the light signal (Christie et al., 1998; Salomon et al., 2000) involving several downstream signaling pathways. The activation of phytochromes and cryptochromes, can significantly affect transcription through signal transduction pathways and in a few cases by direct effects on the transcription factors (Jiao, et al., 2007).

### 2.2. bHLH transcription factors and light regulated elements

The basic helix-loop-helix (bHLH) proteins are a superfamily of transcription factors that have been well characterised in eukaryotes (Atchley and Fitch, 1997). This family is defined by the bHLH signature domain, which consists of ~60 amino acids with two functionally distinct regions. The basic region, located at the N-terminal end of the domain, is involved in DNA binding and consists of ~15 amino acids with a large number of basic residues. The bHLH region at the C-terminal end functions as dimerization domain and is constituted mainly of hydrophobic residues that form two amphipathic α-helices separated by a loop region of variable sequence and length (Nair and Burley, 2000). The core DNA sequence motif recognised by the bHLH protein is consensus hexanucleotide sequence known as the E-box (5'-CANNTG-3'). There are different types of E-boxes depending on the identity of the two central bases, one of the most common is the palindromic G-box (5'-CACGTG-3'). Certain conserved aminoacids within the basic region of the protein provide recognition of the core consensus site, whereas other residues in the domain dictate specificity for a given type of E-box (Robinson et al., 2000).

Arabidopsis genome encodes 147 proteins as members of the bHLH family (Toledo-Ortiz et al., 2003). According to their phylogenetic relationships, DNA binding motifs and functional properties, known bHLH proteins from animals have been divided into six main groups; i.e., group A to F (Atchley and Fitch, 1997). Group A and B include
bHLH proteins that bind to hexameric DNA sequence referred to as E-box (CANNTG) respectively CACCTG or CAGCTG (group A) and CACGTG or CATGTTG (group B) (Murre et al., 1989; Deng et al., 1992). Member of the group C contain an additional protein-protein interaction region (PAS domain) and binds to sequence (NACGTG or NGCGTG) that is unlike the E-box. Proteins in group D have the HLH region but lack the basic region, they can form heterodimers with bHLH, and thus they are functionally related to bHLH proteins. Member of group E protein binds to the sequence CACGNG preferentially (Fisher and Caudy, 1998). Group F consists of COE-bHLH proteins, they have divergent sequence compared with other groups and another domain for dimerization and the DNA binding (Ledent and Vervoot, 2001). Compared to animals, only a small number of bHLH proteins have been functionally characterised in plants.

Sequence analysis of the plant bHLH proteins suggests that they belong to group B (Atchley and Fitch, 1997). Recent report from Ramsay and Grover, 2005, demonstrate that some plant bHLH proteins can interact with proteins that lack a bHLH domain. In particular, protein complexes with MYB, bHLH and WD40 proteins were proposed to regulate guard cell and root hair differentiation. The basic region of the bHLH domain determines the DNA binding activity of the protein (Massari and Murre, 2000). Therefore, the presence or absence of basic residues in the first 17 positions of the bHLH domain is the basis for defining the first two major categories of AtbHLH proteins in terms of the DNA binding properties, DNA binding and non-DNA binding.

A total of 120 proteins are predicted to bind to DNA, because they have an average of 6 residues in the first 17 positions, whereas 27 proteins are predicted not to bind DNA, because they have a less basic region (an average of 3.8 basic residues in the first 17 positions). The DNA binding bHLH category can be subdivided further into two subcategories based on the predictive DNA binding sequence i.e., E-box binders and non-E-box binders. This subdivision is based on the presence or absence of two specific residues in the basic region: Glu13 and Arg16 based on the alignment (Toledo-Ortiz et al., 2003). The analysis of the crystal structure have shown that Glu-13 is critical because it contacts the first CA in the E-box DNA binding motif (CANNTG). Meanwhile the role of Arg16 is to fix, stabilise and position the critical Glu13, therefore it plays an indirect role in DNA binding (Ellenberg et al., 1994; Fuji et al., 2000). In Arabidopsis, 89 proteins (60% of the total number and 81% of the proteins predicted to bind DNA) have the conserved His/Lys-9, Glu-13, and Arg-17 residues and therefore would be predicted to be G-box binders.
2.2.1. Dimerization of bHLH proteins

bHLH proteins are well known to dimerize (Ciarapica et al., 2003), dimerization is affected by multiple parameters including hydrophobic interfaces, interaction between charged amino acids in the bHLH region, and partner availability, but no complete explanation for partner recognition specificity has been documented (Ciarapica et al., 2003).

In plants, heterodimerization between two members of the bHLH family, PIF3 and HFR1 have been reported (Fairchild et al., 2000). The dimer formed by HFR1 and PIF3 could act a regulatory type of heterodimer either by preventing PIF3 from binding to an E-box or by targeting the dimer to a different type of DNA recognition motif. Also, two closely related proteins PIF3 and PIF4 interact strongly in yeast two-hybrid and in vitro pulldown assays (Toledo-Ortiz et al., 2003).

The molecular properties of bHLH proteins involves the generation of a high degree of complexity and diversity in transcriptional regulatory activity through variation in the DNA sequence motif recognised by individual DNA binding proteins (Ciarapica et al., 2003). The capacity to combinatorially amplify the spectrum of possible specific DNA-protein interactions, through selective heterodimerization between bHLH proteins with different DNA sequence recognition specificity and the capacity to interact with a network of transcriptional co-activators, co-repressors and signaling molecule through protein-protein interactions (Ciarapica et al., 2003; Levens et al., 2003).

2.2.2. Light responsive elements

Most light regulated developmental processes are targeted by alterations in gene expression through the regulation of transcription specific genes (Tobin and Kehoe 1994; Terzaghi and cashmore., 1995). Some of these genes such as CAB (chlorophyll a/b binding proteins of PS II light harvesting complex) and RBCS (small subunit of ribulose bisphosphate carbaxylase) are expressed to very high levels upon exposure to light, whereas some of the genes are negatively regulated by light (PHY A and NADPH photochlorophyllide reductase) (Silverthrone and Tobin, 1987; Quail., 1991). Studies on light control of transcription by deletion and mutagenesis analyses of promoter reporter constructs of different light regulated promoters such as CAB, RBCS and CHS has led to the identification of a number of light responsive elements (LREs) (Gilmartin et al., 1990; Anderson et al., 1994; Kehoe et al., 1994). These LREs are defined as small DNA
sequences of 6-10 bp long present upstream of the transcription start site and sufficient to confer light regulated expression of the promoter. The light responsive elements G, GATA, Z-box and GT1, which are commonly present in different minimal region of the light regulated promoters have been demonstrated to be essential for light controlled transcriptional (Milar and Kay, 1996; Terzaghi and cashmore, 1995; Tobin and Kehoe, 1994) regulation.

G-box element has the core sequence CACGTG, found in the promoters of many genes such as CAB, RBCS, CHS, (Block et al., 1990; Weishaar et al., 1991; Arias et al., 1993; Orozco and Ogren, 1993; Foster et al., 1994; Menkens et al., 1995). GATA (I box) elements have the core sequence GATAA, and found in light regulated promoters of RBCS genes, which have a two GATA elements 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 non-light regulated promoters (Lam and Chua, 1990). A Z-DNA-forming sequence, Z-box element has the core sequence ATACGTGT and is 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). GT1 has the core sequence GGTTAA, and is found in the promoters of a number of genes such as RBCS-3A, PHYA, CAB, RCA, PETA, and CHS15 (Green et al., 1989; Gilmartin et al., 1992; Sarokin and chua, 1992; Dehesh et al., 1990; Orozco and Ogren, 1993). Even though G, GATA and GT1 LREs have been studied in some detail with respect to identification of specific transacting factors and regulation of these LREs by specific light signaling components, corresponding information with the Z-box is not available thus far.

It is a common theme in eukaryotes transcriptional regulation that a single promoter cis-element can respond to a particular stimulus, and combination of different cis-elements in a single promoter allows it to respond to multiple signals sometimes in synergistic manner (Hill and Treisman, 1995). However in plants, several lines of evidences have shown that a minimum of two different LREs in specific combinations are required to confer proper light induction (Degenhardt and Tobin, 1996; Puente et al., 1996). Promoters with paired LREs are able to respond to a wide spectrum of light signals involving multiple photoreceptors phyA, phyB and cry1 (chattopadhyay et al., 1998a) and single LRE promoters respond to particular wavelength of light. Downstream regulatory components like COP1 and HY5 have been demonstrated to be involved in the regulation of different Z-box containing promoters in a contrasting manner using synthetic as well as
native promoters (Yadav et al., 2002). Also, it has been reported the interdependent functions of phyA and phyB in the regulation of gene expression by using different Z-box containing promoters.

2.3. Signalling intermediates and molecular mechanisms involved in light-signal transduction

2.3.1. Downstream signalling molecules
Several transcription factors, which include both positive and negative regulators, have been genetically identified as acting downstream of specific photoreceptors or a set of photoreceptors in photomorphogenesis. Although some transcription factors predominately respond to one type of light, others respond to two or more, indicating the presence of shared signaling components. Light signals perceived by photoreceptors and the complex array of light sensing and early signaling processes have been shown in some cases to converge to common downstream regulators that in turn controls cellular developmental decisions.

2.3.1.1. Positive regulators
ELONGATED HYOCOTYL5 (HY5) is the first genetically defined molecule to be reported as positive regulator of photomorphogenesis based on the light insensitivity of hy5 mutants (Koornneef et al., 1980; Ang and Deng, 1994; Pepper and Chory, 1997). This was identified by loss of function mutants exhibiting partial etiolated morphology under light growth conditions. Mutations at HY5 locus leads to defects in light inhibition of hypocotyls elongation, light induced chlorophyll accumulation, and extensive root abnormalities (Oyama et al., 1997) in far-red, red, blue, white and UV-A light conditions, indicating that HY5 is required for mediating developmental responses to phytochromes and cryptochromes. Very recently it has been demonstrated to be involved and plays positive role in UV-B mediated stress pathway and photomorphogenic growth (Oravecz et al., 2006; Ulm et al., 2004). HY5 is responsible for the regulation of fundamental developmental processes of the plant cell: cell elongation, cell proliferation and chloroplast development (Oyama et al., 1997; Ang et al., 1998).

Molecular cloning of HY5 gene has revealed that the gene encodes a 168 amino acid protein with a bZIP motif, one of the motifs found in transcriptional regulators (Oyama et al., 1997; Ang et al., 1998). HY5 binds to G-box DNA sequences containing an ACGT core motif, which are present in the promoters of various light inducible genes.
in plants (Ang et al., 1998; Chattopadhyay et al., 1998). Similar nuclear localized bZIP protein, HY5 HOMOLOG (HYH) has been reported to be specifically involved in blue light signalling acting as a weak positive regulator. HYH protein levels decreases in dark but its mRNA levels increases in responses to red and or blue light compared to darkness but not in response to far-red (Holm et al., 2002). A Ca++ binding protein, CAM7, acts as a positive regulator and works downstream to all the known photoreceptors. Overexpression of CAM7 leads to reduced hypocotyl growth irrespective of wavelengths of light (Kushwaha et al., 2008).

Mutations in bHLH protein HFR1 (long hypocotyl under far-red) lead to an etiolated phenotype both in the far-red light and blue light signaling (Fairchild et al., 2000; Soh et al., 2000; Spiegelman et al., 2000; Duek et al., 2003; Jang et al., 2007). HFR1 mRNA level expresses in darkness but increases in far-red and blue light. Under red light its mRNA level decreases (Duek and Frankhauser, 2003). HFR1 does not bind to phytochromes but it binds PIF3 forming a heterodimer that in turn binds the Pfr form of phytochromes (Fairchild et al., 2000; Toledo-Ortiz et al., 2003). LONG AFTER FAR-RED LIGHT (LAF1), a MYB protein, constitutively localized within the nucleus and has been reported to be functioning in far-red light mediated signalling and it is also know to form heterodimers by interacting with HFR1 (Ballesteros et al., 2001, Jang et al., 2007). FHY (far-red elongated hypocotyls) 3 and FAR (far-red impaired response) 1 acts as positive regulator in far-red light signalling (Hudson et al., 1999; Zeidler et al., 2004, Wang et al., 2002). Expression of FHY3 and FARI are induced by far-red light, which are reported to be necessary for the export of phyA into the nuclear speckles. Further, the expression of FHY3 and FARI are decreased with prolonged treatment of far-red, indicating negative feedback regulation by phyA signaling and suggest 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).

Two MYB proteins, LHY and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), are involved in circadian rhythm (Schaffer et al., 1998; Wang and Tobin, 1998). CCA1 is the part of network connecting light and clock signals to the control of gene expression. Hypersensitive to Red and Blue (HRB) 1 is a positive regulator for red and blue light mediated leaf expansion and gene expression (Kang et al., 2005). OBF 4 binding protein (OBP) 3, a Dof transcription factor, also acts as a positive regulator of red light mediated inhibition of hypocotyl elongation (Ward et al., 2005). Phytochrome interacting factor (PIF) 3, acts as a positive regulator of CHS transcript accumulation (Kim et al., 2003).
Recent report from Shin et al., 2007, suggests that PIF3 and HY5 positively regulate anthocyanin biosynthesis by activating the transcription of same biosynthetic genes. Salt Tolerant Homolog (STH) 2, a B-box protein in *Arabidopsis*, acts as a positive regulator of photomorphogenesis and reported to interact with both HY5 and COP1 and plays direct role in activating transcription in plants (Dutta et al., 2007). Also, very recently it is been reported that Blue Insensitive Trait (BIT) 1, a MYB transcription factor, functions positively in blue light dependent hypocotyl growth and *PsbS* gene expression, and also known to be interacting with COP1 and degrading by COP1 in blue light (Hong et al., 2008).

### 2.3.1.2. Negative regulators

Several genes acting as negative regulators of photomorphogenesis have been isolated from extensive genetic screens that lead to the identification of mutants that display characteristics of light grown seedlings in complete darkness. Mutant analysis has further demonstrated a panel of negative regulators, which keep chloroplast development suppressed in the cotyledons of dark grown seedlings. Mutations at each of 10 loci known as *Constitutively Photomorphogenic* (*COP1, COP8–COP11*), *De-etiolated* (*DET1*), and *Fusca* (*FUS4, 5, 11, and 12*) result in the absence of etioplasts and development of partial chloroplasts in complete darkness, accompanied by cotyledon expansion, arrest of hypocotyl elongation, and light-specific cell type differentiation. The suppression of photomorphogenic seedling development under defined light conditions by overexpression of COP1 confirmed that at least COP1 can act as a light inactivable repressor of photomorphogenesis, acting downstream to all the photoreceptors and is the master repressor of photomorphogenic development (Deng and Quail, 1999; Holm and Deng, 1999). *COP1* is the first in the *COP* family to be cloned and characterized at the molecular level. *COP9, DET1, and FUS6 (COP11)* have also been cloned and characterized at the molecular level (Deng et al., 1992; Wei et al., 1994; Castle and Meinke, 1994; Pepper et al., 1994). *COP9, DET1* and *FUS6* encode novel helical rich proteins that are constitutively localized in the nucleus (Pepper et al., 1994; Wei et al., 1994; Chamovitz et al., 1996; Staub et al., 1996). COP9 has been found to be a part of an eight subunit protein complex of 560 kDa consisting of COP9, FUS6, presumably COP8 and others which are now known as COP9 signalosome (CSN) (Wei et al., 1994; Chamovitz et al., 1996; Wei and Deng, 1999; Serino and Deng, 2003). Mutant analysis reveals that COP1, COP10 and DET1 are not included in CSN complex. Sequence
analysis indicated that COP1 is a novel protein which forms homodimer in vitro and in vivo, and it consists of four recognizable structural domains: an N-terminal ring finger zinc binding domain, which is involved in self association, a coiled coil domain (helix) that helps in dimerization, a central core domain, and C-terminal multiple WD-40 repeats characteristic of β subunit of trimeric G protein (Deng et al., 1992; Torii et al., 1998). The core domain of COP1 has a bipartite nuclear localization signal located in it, which helps in the nuclear import of COP1 and a cytoplasmic localization signal (CLS) mediates the nuclear exclusion of COP1, which overlaps the helix domain (Stacey et al., 1999). GUS-COP1 fusion protein studies indicated that COP1 acts in the nucleus in the dark to suppress photomorphogenesis and 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). It has been found that at least three photoreceptors, phyA, phyB and cry1, can trigger nuclear depletion of COP1 under their respective light-responsive regimes by regulating the balance between the competing CLS and NLS activities, which further substantiates the notion that COP1 acts downstream of multiple photoreceptors (Osterlund and Deng, 1998; Stacey et al., 1999).

Another regulator which maintains COP1 level in the nucleus is pleiotropic COP/DET/FUS. Except for COP1, COP10 and DET1, all other genes seem to be required for the structural stability of COP9 signalosome, some which exhibits similarity to distinct non ATPase subunits of the 19S regulatory particle of the 26S proteosome. This similarity indicated that the regulation of COP1 nuclear abundance may be mediated by a proteosome protein degradation pathway (Wei et al., 1998; Glickman et al., 1998). In the dark COP9 signalosome protects nuclear COP1 from proteosome degradation, whereas light may abrogate this protection, which results in an accelerated degradation of COP1 in the nucleus.

Suppression of photomorphogenesis in darkness also requires the four-member SUPPRESSOR OF PHYTOCHROME A-105 (SPA) gene family. SPA1 is a phyA specific signaling component that acts as a repressor of photomorphogenesis. Recent reports suggest that SPA1 is also involved in BL and RL signalling pathways in repressing photomorphogenesis (Laubinger et al., 2004). SPA1 belongs to four member gene family: SPA1, SPA2, SPA3 and SPA4 (Laubinger and Hoecker, 2003; Laubinger et al., 2004). Among these four members SPA1, SPA3 and SPA4 are involved in the repression of photomorphogenesis in the light grown seedlings. Seedlings of the spa1 spa2 spa3 spa4 quadruple mutant exhibit constitutive photomorphogenic phenotype that is very similar to that of cop1 mutants, suggesting the functional redundancy among these
SPA proteins in repressing photomorphogenic growth in the dark (Laubinger et al., 2004 and Fittinghoff et al., 2006). All the four SPA proteins have been reported to physically interact with COP1 (Saijo et al., 2003; Laubinger and Hoecker, 2003; Laubinger et al., 2004). Yeast two hybrid and in vitro interaction studies showed that SPA1 strongly and selectively binds to COP1 with the help of coiled-coil domain of both SPA1 and COP1 (Hoecker and Quail, 2001 and Seo et al., 2003). SPA1 probably function in parallel to COP1 in far-red light.

PIF3 is repressor of photomorphogenesis specific to phyB mediated inhibition of hypocotyls elongation, acts as a positive regulator for cotyledon opening and expansion, and known to bind to G-box in LHY and CCA promoters (Kim et al., 2003). It is reported to be stabilized by COP1 and degraded by photoreceptors in a redundant manner (Bauer et al., 2004). PIF1, a bHLH protein, plays major role in negatively regulating light induced seed germination and chlorophyll biosynthesis as well as plays a minor role in light induced suppression of hypocotyl elongation and cotyledon expansion (Haq et al., 2004). PIF1 regulates gibberellic acid metabolic and signaling genes to suppress seed germination (Oh et al., 2006, 2007). This is also involved directly or indirectly in chlorophyll biosynthetic genes to optimise the greening process in Arabidopsis (Moon et al., 2008). It is reported to be phosphorylated, polyubiquitinated and degraded by phytochromes under red and far-ed light conditions (Shen et al., 2008) in an additive manner. Very recently another bHLH family member, phytochrome interacing factor (PIF) 7, has also shown to be involved in red light mediated hypocotyl growth inhibition, and together with PIF3 and PIF4 reported to be involved in the degradation of PHYB protein levels in an additive manner (Leivar et al., 2008).

Other bHLH proteins, PIF4, is involved in the phyB mediated seedling de- etiolation and is selectively binds to Pfr form of phyB through its APB motif (Khanna et al, 2004). Whereas, PIF5 interacts specifically with photoactivated phyB and negatively regulates phyB imposed hypocotyl inhibition in prolonged red light, and also reported to be involved in ethylene signalling cascade (Khanna et al., 2007). OBP3 negatively regulates the cry1 mediated cotyledon expansion. HRB1 acts as a negative regulator of red-blue light mediated inhibition of hypocotyl and petiole elongation (Kang et al., 2005; Ward et al., 2005). EID1 (Empfindlicher Im Dunkelroten Licht) is a F-box protein and acts as negative regulator in phyA mediated light signaling (Buche et al., 2000). EID1 forms SCF\textsubscript{EID} ligase which degrades the positive regulators of phyA signal pathway (Dieterle et al., 2001). Recently it has been shown that MYC2 a bHLH protein, binds to both Z/G-box LREs present in CAB and RBCS minimal promoters and acts as a negative
regulator in blue and far-red light mediated signal transduction whereas, positive regulator of lateral root formation (Yadav et al., 2005). GBF1/ZBF2, a bZIP transcription factor, binds to both Z and G-box LREs present respectively, in CAB and RBCS minimal promoters, acts as a repressor in blue light mediated hypocotyl inhibition and positive regulator for cotyledon expansion and lateral root formation (Mallappa et al., 2006). Also, it differentially regulates CAB and RBCS genes. Very recently, it has been reported that Short Hypocotyl under White light (SHW) 1, acts as a negative regulator of hypocotyl growth in WL and dark, whereas it positively regulates CAB and RBCS expression. It works parallel with COP1 in a non redundant manner in dark. However, WL specific phenotype of shwl mutant is dependent on functional COP1 (Bhatia et al., 2008), in other words cop1 is acing downstream to shwl.

2.3.2. Cryptochrome signaling components

Previous and recent works have identified a number of blue light signaling components, like HY5, HYH, SUB1, AtPP7, HFR1, HRB1, ZBF1/MYC2, SHB1, ZBF2/GBF1 and BIT1 (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; Kang et al., 2006; Mallappa et al., 2006 and Hong et al., 2008). Among these regulatory proteins, HYH, AtPP7, SHB1, ZBF2/GBF1 and ZBF1/MYC2 are specifically involved in blue light mediated photomorphogenic growth (Holm et al., 2002; Moller et al., 2003; Yadav et al., 2005).

HYH is a member of the bZIP family of transcription factors, containing bZIP domain in the C-terminal half and a nuclear localized protein. Overexpression can partially compensate for the loss of HY5 in white light suggesting functional overlap between the two proteins. The accumulation of HYH protein, not the mRNA, is dependent on the presence of HY5. And further HYH and HY5 can, respectively, act as heterodimers and homodimers, thus mediating light-regulated expression of overlapping as well as distinct target genes (Holm et al., 2002). The AtPP7 protein phosphatase acts as a positive regulator in blue light signaling (Moller et al., 2003). Short Hypocotyl under Blue (SHB) 1, encodes a nuclear and cytosolic protein, reported to act in blue light mediated suppression of hypocotyl growth and genetically interact with PIF4 and HFR1 (long hypocotyl in far-red light) (Kang et al., 2006). Recently, ZBF1/MYC2 has been reported to bind to G-box and Z-box LREs. ZBF1/MYC2 acts as a negative regulator of blue light mediated photomorphogenic growth and blue light and far-red light mediated gene expression (Yadav et al., 2005). ZBF2/GBF1 is also reported to be involved in the
blue light signaling. It negatively regulates hypocotyl growth and RBCS1A expression, whereas positively for cotyledon expansion, CAB1 expression and lateral root formation (Mallappa et al., 2006).

2.4. Interaction of COP1 and SPA1 plays critical role in regulation of HY5, LAF1 and HFR1 activity

*Arabidopsis* SPA1 (suppressor of phytochrome A-105), a negative regulator of phyA signaling, is a nuclear localized protein (Hoecker et al., 1999). SPA1 is a member of a small family of four structurally related proteins. SPA proteins contain an N-terminal kinase like domain, followed by a coiled-coil domain and seven WD40 repeats at the carboxyl-terminus. Interestingly, the WD40 domains of the SPA proteins are highly homologous to the COP1 WD40 domain, and all the SPA proteins have been reported to physically interact with COP1 through their mutual coiled-coil domains (Laubinger and Hoecker, 2003; Saijo et al., 2003; Laubinger et al., 2004). While the quadruple *spa* mutants exhibit strong *cop* phenotypes almost indistinguishable from *cop1* mutants, single, double and triple *spa* mutants show no or weak photomorphogenic phenotypes, suggesting that the SPA proteins modulate COP1 activity in a redundant and overlapping manner (Laubinger and Hoecker, 2003; Laubinger et al., 2004).

How exactly SPA proteins regulate COP1 activity is still not clearly understood. However, evidences from Saijo et al., 2003, suggests that, COP1 and SPA1 interact physically and they form a complex together, also, double mutants of *cop1* and *spa1* accumulate higher HY5 protein level compared to both the single mutants. It has been shown that SPA1 alters the ubiquitination status of the COP1 on HY5 and LAF1 (Saijo et al., 2003; Seo et al., 2003), also, SPA1 interacts with HY5 through WD40 domain (Saijo et al., 2003), suggesting that the SPA proteins might be helping COP1 for the degradation of positive regulators. Alternately, the SPA proteins might be essential for the stability of COP1 complexes, since COP1 and SPA1 co-fractionate in large complexes in *Arabidopsis* seedlings (Saijo et al., 2003). SPA1 may function in concert with COP1 to target transcription factors such as HY5, LAF1 and HFR1 for degradation (Saijo et al., 2003; Seo et al., 2003; Yang et al., 2005b). LAF1, a myb transcription activator participates in the transmission of phyA signals to downstream responses (Ballesteros et al., 2001). LAF1 is localized in nuclear bodies along with COP1 and SPA1. LAF1 is ubiquitinated by COP1 and this ubiquitination is dependent on RING motif but not WD40 repeat domain of COP1. Interestingly, SPA1 coiled-coil domain stimulates LAF1
ubiquitination by COPI when latter is present at low concentrations (Seo et al., 2003). HY5, a bZIP transcription factor acts as a positive regulator of photomorphogenesis in red light, far-red light and blue light signaling pathways (Oyama et al., 1997). The abundance of HY5 protein correlates with the extent of photomorphogenesis and is primarily regulated at the level of protein degradation via the proteasome pathway (Osterlund et al., 2000). COPI is known to directly interact with HY5 and subject it for proteasome mediated degradation (Osterlund et al., 2000).

Saijo et al., 2003, by the genetic analyses of double mutants reported the synergistic enhancement of the cop1 and spa1 mutations. HY5 was found to accumulate to high levels in spa1-3 mutants in FR conditions and synergistic effect of the cop1-6 and spa1-3 mutations was found on HY5 abundance. Further SPA1 interacts with HY5 through WD40 domain and with COP1 through coiled coil domain. SPA1 acts as a component of the COP1 complex and stimulate the degradation of HY5 (Saijo et al., 2003). HFR1, a photomorphogenesis promoting bHLH protein, is required for a number of exaggerated photoresponses of the spa1 mutants including hypocotyl elongation and light responsive gene expression under specific light conditions (Fairchild et al., 2000; Duek and Fankhauser, 2003; Yang et al., 2005a). HFR1 undergoes proteasome mediated degradation through COPI (Jang et al., 2005; Yang et al., 2005a; Duek et al., 2004). SPA1 physically interacts with HFR1 through coiled-coil domain, and degradation of HFR1 is defective in spa1 mutants under all light conditions. Thus, it has been proposed that SPA1 represses light signaling by modulating COPI-dependent degradation of HFR1 at a post translational level (Yang et al., 2005b).

2.5. Cross-talk between light and hormone signaling pathways

Plant growth and development are regulated through coordinated interactions between light and phytohormones, and in many cases the signals from plant hormones and those derived from light interacts either agonistic or antagonistic manner. Additionally, it is also possible that light itself plays important role in the regulation of certain hormonal pathways either directly or indirectly. For example, exogenous application of either auxins 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, it regulates biosynthesis and/or signaling of gibberellins (Garcia-Martinez and Gil, 2001). GA is also known to inhibit the expression of CAB2 and RBCS genes whereas, with the addition of paclobutazol, a GA biosynthesis inhibitor cancels the effect
of GA suggesting its involvement in the etiolation (Alabadi et al., 2004). More conclusive evidences for Light and GA cross-talk at the molecular level came from very recent work (Feng et al., 2008; de Lucas et al., 2008), wherein, 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 block PIF3 and PIF4 transcriptional activity by binding to the DNA recognition domain of PIFs. This effect abrogated by the addition of GA. In the presence of GA, DELLAs will be targeted for degradation and thereby allowing PIF3 and 4 to exert their effect by directly binding to the promoters of genes mediating cell elongation. Recent reports (Oh et al., 2006) suggest the involvement of PIF1 in gibberellin (GA)-mediated seed germination by repressing GA biosynthetic genes and by activating GA catabolic genes. PIF1 controls GA sensitivity by directly activating the expression of repressors of GA signalling such as, GAI and RGA.

Brassinosteroid biosynthesis genes are generally down regulated by light (Ma et al., 2001). It has been reported that photomorphogenesis is modulated by inactivators of brassinosteroids, phyB activation-tagged suppressor (BAS) 1 and suppressor of phyB-4 7 (SOB7) (Turk et al., 2003, 2005). Also, BR deficient mutants show partial or complete de-etiolation in the dark. Several auxin resistance, aux/iaa mutants suggest their role in etiolation (Neff et al., 2000), auxins are influenced by light in their transport and/or distribution, at the level of signal transduction, and in their homeostasis (Morelli and Ruberti, 2000; Tian et al., 2002; Hoecker et al., 2004; Vandenbussche et al., 2005). A recent work from Dohmann et al., 2008, indicates the partial impairment of auxin responses in the csn (COP9 signalosome) mutants.

On the contrary, cytokinins mimic the effects of light in dark grown plants (Chory et al., 1994; Deikman and Hammer, 1995). Dark grown etiolated seedlings treated with exogenous cytokinins have the short hypocotyl and expanded cotyledon of light-grown plants, and even show true leaf formation. Moreover in tissue culture, exogenous cytokinins enhance greening and shoot formation in calli. In this respect, exogenous cytokinins produce a similar phenotype in seedlings as mutations in COP1 and other subunits of either COP1 or COP9 signalosomes. Phytochrome B and a cytokinin related two component signaling pathways have been suggested to converge (Sweere et al., 2001; Heyl and Schmulling, 2003; Salome et al., 2006) through the demonstration of direct interaction of phytochrome B (phyB) with the Arabidopsis response regulator 4 protein (ARR4) (Sweere et al., 2001). It has been suggested that phyB, ARR4 and the circadian oscillator may function as signaling intermediates to integrate light and cytokinin
pathways (Zheng et al., 2006). ARR4 has been shown to directly interact with the phosphotransfer protein Arabidopsis histidine phosphotransfer protein 1 (AHP1) (Urao et al., 2000), which is translocated to the nucleus in the presence of cytokinin (Hwang and Sheen, 2001).

The connection between cytokinin and cryptochrome signaling pathways came recently with the work of Vandenbussche et al., 2007, wherein they demonstrated that cytokinin and cryptochrome act additively and independently on hypocotyl growth inhibition. Cytokinin regulation of anthocyanin accumulation occurs via a mechanism that requires light and act downstream of the photoreceptors. HY5 is involved in the transcriptional regulation of anthocyanin biosynthetic enzymes by both blue light and cytokinins, and response to cytokinins in blue light requires prior signal by cry1. Levels of HY5 protein are directly regulated by both light and cytokinins. HY5 is proposed to be a common intermediate in both the light and cytokinin signaling pathways (Vandenbussche et al., 2007). HY5 can also bind to promoters of AXR2 in vitro, and the negative regulators of auxin signalling such as AUXIN RESISTANCE 2 (AXR2/IAA7 (INDOLE ACETIC ACID 7) and IAA14/SLR (SOLITARY ROOT) are underexpressed in hy5 mutants. Increased expression of AXR2 in hy5 mutant background partially rescues the elongated hypocotyl phenotype (Cluis et al., 2004). Report from Sibout et al., 2006, using hy5 hyh double mutants indicates that, HY5 and HYH are important negative regulators of auxin signaling in embryogenesis and seed development.

Interaction of light and ABA in the regulation of light inducible gene expression in Lemna gibba was demonstrated by Weatherwax et al., 1996. A report by Yadav et al., 2005, demonstrated that ZBF1/MYC2, a bHLH transcription factor, acts as a negative regulator of photomorphogenesis in cryptochrome signaling pathway, and acts as a point of cross-talk between light, ABA, JA (jasmonic acid) and ethylene signaling pathways (Boter et al., 2004; Lorenzo et al., 2004; Yadav et al., 2005; Dombrecht et al, 2007). Very recently, it has been reported that light and ABA signalling cascades are integrated through HY5 (Chen et al., 2008), wherein they have shown that HY5 also mediates ABA response in seed germination, early seedling growth and root development in Arabidopsis. HY5 binds to the promoter of the transcription factor gene ABI5 with high affinity and is required for the expression of ABI5 and ABI5 targeted late embryogenesis abundant genes in seeds. Recently mutation in CULLIN 1 has been shown to have altered response to hormone and light signals in Arabidopsis. The CUL1 mutants show reduced sensitivity to hormones such as auxin and ethylene in red and blue light specific manner (Moon et al., 2007).
Very recently, Dombrecht et al., 2007, have reported that 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 has been reported to modulate JA responses via differential regulation of an intermediate spectrum of TFs with activating or repressing roles in JA signaling. Moreover, MYC2 induces the JAZ protein (jasmonate ZIM-domain) in a JA dependent manner, and these JAZ proteins further negatively regulates the MYC2 expression, suggesting involvement of a feedback loop between JAZ and MYC2 for pulsed response to JA (Chini et al., 2007). It is known that JA positively regulates MYC2 expression, however, mitogen-activated protein kinase (MAPK) cascade, a JA activated MAPK KINASE 3 (MKK3)–MAP KINASE 6 (MPK6), has been reported to negatively regulate MYC2. Hence, both positive and negative regulation may be used for the fine tuning of MYC2 expression in JA ignaling (Takahashi et al., 2007).