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Research Article

Identification of photoperception and light signal transduction pathways in citrus

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Abstract

Studies employing model species have elucidated several aspects of photoperception and light signal transduction that control plant development. However, the information available for economically important crops is scarce. Citrus genome databases of expressed sequence tags (EST) were investigated in order to identify genes coding for functionally characterized proteins responsible for light-regulated developmental control in model plants. Approximately 176,200 EST sequences from 53 libraries were queried and all *bona fide* and putative photoreceptor gene families were found in citrus species. We have identified 53 orthologs for several families of transcriptional regulators and cytoplasmic proteins mediating photoreceptor-induced responses although some important *Arabidopsis* phytochrome- and cryptochrome-signaling components are absent from citrus sequence databases. The main gene families responsible for phototropin-mediated signal transduction were present in citrus transcriptome, including general regulatory factors (14-3-3 proteins), scaffolding elements and auxin-responsive transcription factors and transporters. A working model of light perception, signal transduction and response-eliciting in citrus is proposed based on the identified key components. These results demonstrate the power of comparative genomics between model systems and economically important crop species to elucidate several aspects of plant physiology and metabolism.

Key words: cryptochrome, data mining, light signaling, phototropin, phytochrome.

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Introduction

Plant development is highly plastic, allowing the environment to exert tight control over the transitions between genetic developmental programs in order to maximize growth and reproduction (Meyerowitz, 2002). Light provides spatial and temporal information to regulate plant development throughout its life cycle: from germination and seedling establishment to the onset of the reproductive stage (Schäfer and Nagy, 2006). Plants are able to detect environmental light direction, duration, fluency and wavelength due to a complex system of photoreceptor molecules: the blue (B) and ultraviolet-A (UV-A) 320-500 nm light-sensing cryptochromes (cry) and phototropins (phot) (Banerjee and Batschauer, 2005) and the red (R)/far red (FR) 600-750 nm phytochrome (phy) receptors (Schepens *et al.*, 2004). Recently, a novel family of putative B photoreceptors has been described in *Arabidopsis thaliana*: the ZEITLUPE (ZTL)/ Flavin-binding Kelch repeat F-box protein (FKF1)/LOV Kelch Protein (LKP2) family (Nelson *et al.*, 2000; Schultz *et al.*, 2001; Somers *et al.*, 2000). ZTL/ FKF1/LKP2 are involved in the circadian clock mechanism and photoperiodic flowering response (Imaizumi *et al.*, 2005).

In higher plants, photoreceptor co-action is a common theme and the pathways may function synergistically, antagonistically and additively to control several developmental responses (Schäfer and Nagy, 2006). Moreover, plant photoreceptors operate in concert with numerous other signaling systems; including phytohormones, carbohydrate-mediated, temperature, gravity and the endogenous clock transduction pathways (Halliday and Fankhauser, 2003; Chen *et al.*, 2004; Heggie and Halliday, 2005). The molecular mechanisms involved in light-induced signal transduction include the following: light-regulated sub-cellular localization of the photoreceptors (Guo *et al.*, 1999; Nagy and Schäfer, 2002; Chen *et al.*, 2005; Kong *et al.*, 2006); a large reorganization of the transcriptional program (Casal and Yanovsky, 2005; Franklin *et al.*, 2005) and light-regulated proteolytic degradation of several photoreceptors and signaling components (Höcker, 2005; Huq, 2006).

Studies employing model plant species have demonstrated that photoperception, the signal transduction path-

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ways and the responses elicited by light form a complex interconnected network rather than a linear pathway (Chen *et al.*, 2004; Quecini and Liscum, 2006). The diversity of light responsiveness observed in plants has arisen from the elaboration, combination and re-arrangement of a basic repertoire of mechanisms responsible for light-mediated developmental regulation, allowing adaptation to a wide range of climatic and latitudinal regions. Comparative genomics has provided tools to access the genetic bases of this diversity in non-model species using bioinformatics, which increases the fundamental knowledge of gene interactions and permits analyses of the functional significance of proteins *in silico* (*e.g.* Santelli and Siviero, 2001; Souza *et al.*, 2001; Hecht *et al.*, 2005).

Virtually all information about light-regulated development in woody plants comes from studies with *Populus*, a temperate deciduous perennial (Zhu and Coleman, 2001; Olsen and Juntilla, 2002). Adaptive traits in temperate perennial woody plants involve an integrated physiological response directed at plant survival and nutrient storage over the winter period and are greatly dependent of photoreceptor-mediated perception of seasonal progression (Thomas and Vince-Prue, 1997). Surprisingly, recent evidence has demonstrated that light is also the main factor triggering the transition between vegetative to reproductive developmental stages of trees in Equatorial regions (Borchert *et al.*, 2005). The effects of light on developmental processes in citrus and other neotropical tree species have been described in several situations, although without approaching the molecular aspects of the metabolism (Steppe *et al.*, 2006; Chen L-S *et al.*, 2005; Raveh *et al.*, 2003; Torné *et al.*, 2001). Mutant studies employing transgenically generated plants have demonstrated variable extents of functional conservation in the genes responsible for developmental control between citrus and model species (Pena *et al.*, 2001; Pillitteri *et al.*, 2004). However, the detailed functional characterization of individual genes is a limiting factor in the study of tree species, and new strategies should be devised for the study of gene function (Groover and Robischon, 2006).

Based on evidence of extensive conservation in photoperception and light signal transduction in angiosperms, this work aimed to identify the characterized components of these pathways in citrus. Our results have demonstrated that a large portion of the genes involved in light responses from model species are present in citrus and that they share extensive protein sequence conservation in several regions, including functionally characterized domains. These results demonstrate the potential use of comparative genomic tools to elucidate physiological and metabolic processes in crop species.

Material and Methods

Database searches and alignments

Homologs of *Arabidopsis thaliana* and other model species photoperception and light signal transduction genes were identified in BLAST searches (Altschul *et al.*, 1997) against EST sequences from the citrus index databases at CitEST, consisting of approximately 176,200 ESTs obtained from the sequencing of 53 libraries. Data validation was performed by tBLASTx and tBLASTn searches using BLOSUM80 scoring matrix of the retrieved hits against the databases at NCBI (National Center for Biotechnology Information) built inside the CitEST project. Sequences failing to retrieve the original bait sequence were eliminated from the projects. The resulting alignments were filtered by a threshold e-value of 1e-15 and the validated hits were further analyzed according to functional domain description. Validated sequences were translated and protein (deduced amino acid) alignments were performed using ClustalX (Thompson *et al.*, 1997). When necessary, alignments were manually adjusted using Lasergene MegAlign (DNASTAR, Madison, WI, USA).

Motif analysis and *in silico* characterization

The identified homologs were investigated for the presence and sequence conservation of recognizable functional domains described in several protein analysis and gene function databases (European Bioinformatics Institute-European Molecular Biology Laboratory - EMBL-EBI; Expert Protein Analysis System - ExPaSy from the Swiss Institute of Bioinformatics - SIB; Protein Families database - Pfam).

Phylogenetic analysis

The functionality of citrus genes in comparison to the characterized homologs was assessed by genetic distance and phylogenetic studies. Phylogenetic analyses were performed using distance and parsimony methods in the software PAUP* 4.0b10, using the software default parameters. Re-sampling bootstrap trees containing 1000 random samples were constructed using PSIGNFIT software. Modular functional domains were employed for genetic distance studies for genes previously described as having divergent regions and conserved blocks.

Results and Discussion

The genes characterized as essential players in light-controlled developmental processes in *A. thaliana* and other model species were compiled, including those involved in light sensing, seed germination, seedling establishment, phototropism and shade-avoidance responses. Their protein sequences were used to search citrus EST databases and the retrieved sequences were ranked according to the degree of amino acid sequence conservation and further analyzed. In this way, we have identified 102 EST contigs corresponding to genes involved in light-regulated developmental control in citrus genome (Figure 1, Figure S1). From the total, 27 ESTs and EST contigs share sequence homology with photoreceptor and putative photoreceptor families, 53 represent several phy and cry signal

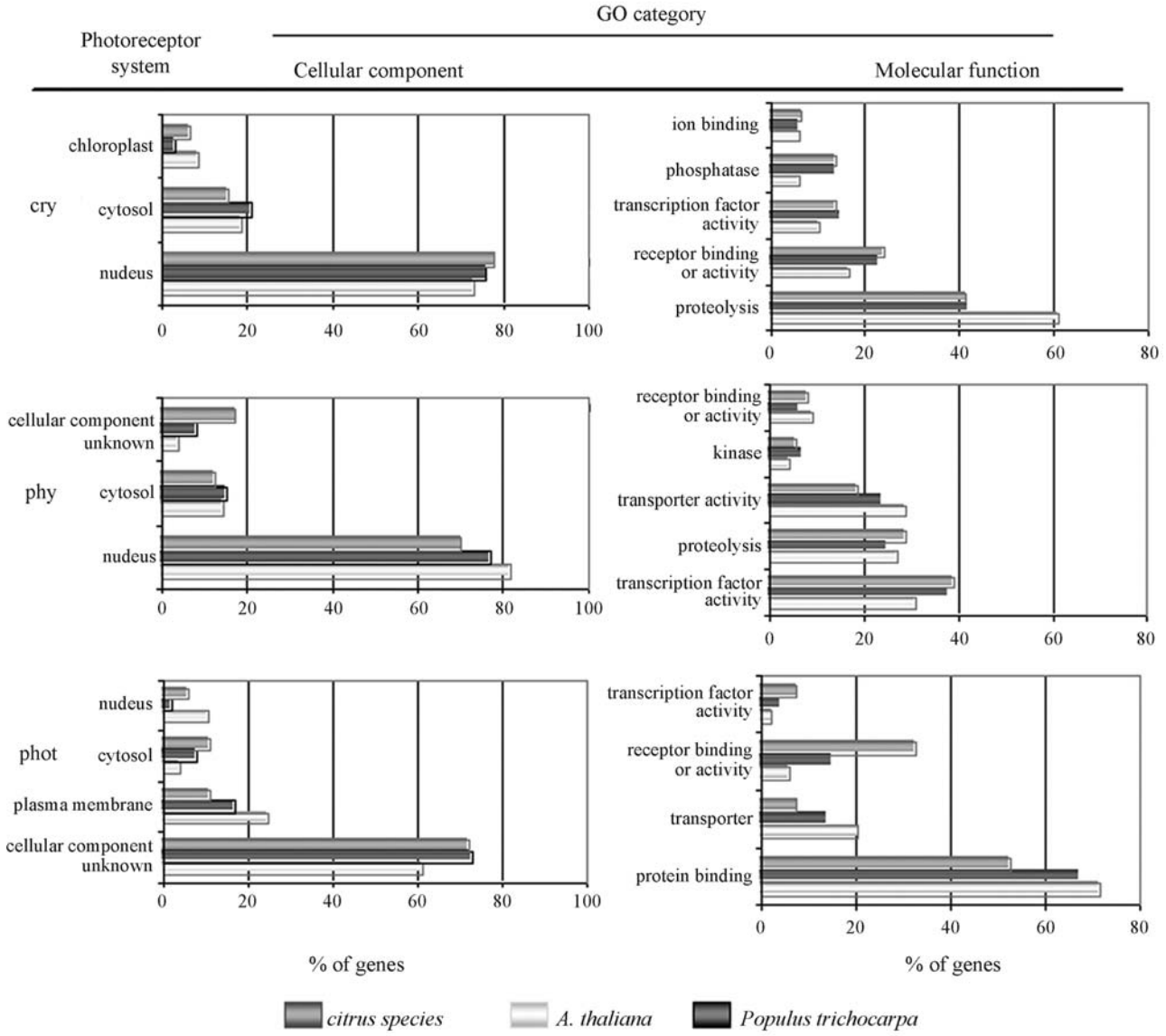

Figure 1 - Functional classification of citrus transcripts associated to photoperception and light signal transduction based on gene ontology (GO) categories in comparison to *Populus trichocarpa* transcriptome and *Arabidopsis thaliana* proteome. Photoreceptor systems are indicated by the columns: cry; cryptochrome, phy; phytochrome and phot; phototropin. Assignments are based on the data available at the TIGR *Arabidopsis thaliana* Gene Index version 13.0.

transduction components and light-regulated transcription factors, whereas the remaining 22 are similar to phot signaling partners (Figure 1). Homologs from the transcriptional regulators PAT1 (PHYTOCHROME A SIGNAL TRANSDUCTION 1, AT5G48150) (Bolle *et al.*, 2000), HRB1 (HYPERSENSITIVE TO RED AND BLUE 1, AT1G02340) (Kang *et al.*, 2005), OBP3 (OBF4-BINDING PROTEIN 3, AT3G55370) (Ward *et al.*, 2005) and from the novel signaling components FHL (FHY1-LIKE, AT5G02200) (Zhou *et al.*, 2005) and SRR1 (SENSITIVITY TO RED LIGHT REDUCED 1, AT5G59560) (Staiger *et al.*, 2003) were absent from CitEST databases. However, the existence of citrus orthologs cannot be ruled out at this point due to the restricted coverage of the transcriptome analysis, the expression levels and patterns of these genes and the post-translational modifications required to generate functional components. In *Arabidopsis* and rice, several genes involved in light signaling have been identified by biochemical, forward and reverse genetic assays (Zhou *et al.*, 2005; Kevei *et al.*, 2006)

Photoreceptor-related genes

Citrus EST database contains homologs of members of all families of plant photoreceptors: namely, the phytochrome, cryptochrome and phototropin families (Table S1, Figure 2, Figure 3, Figure 4). In higher plants, phy are responsible for the control of major developmental processes, such as seed, germination, de-etiolation, shade avoidance,

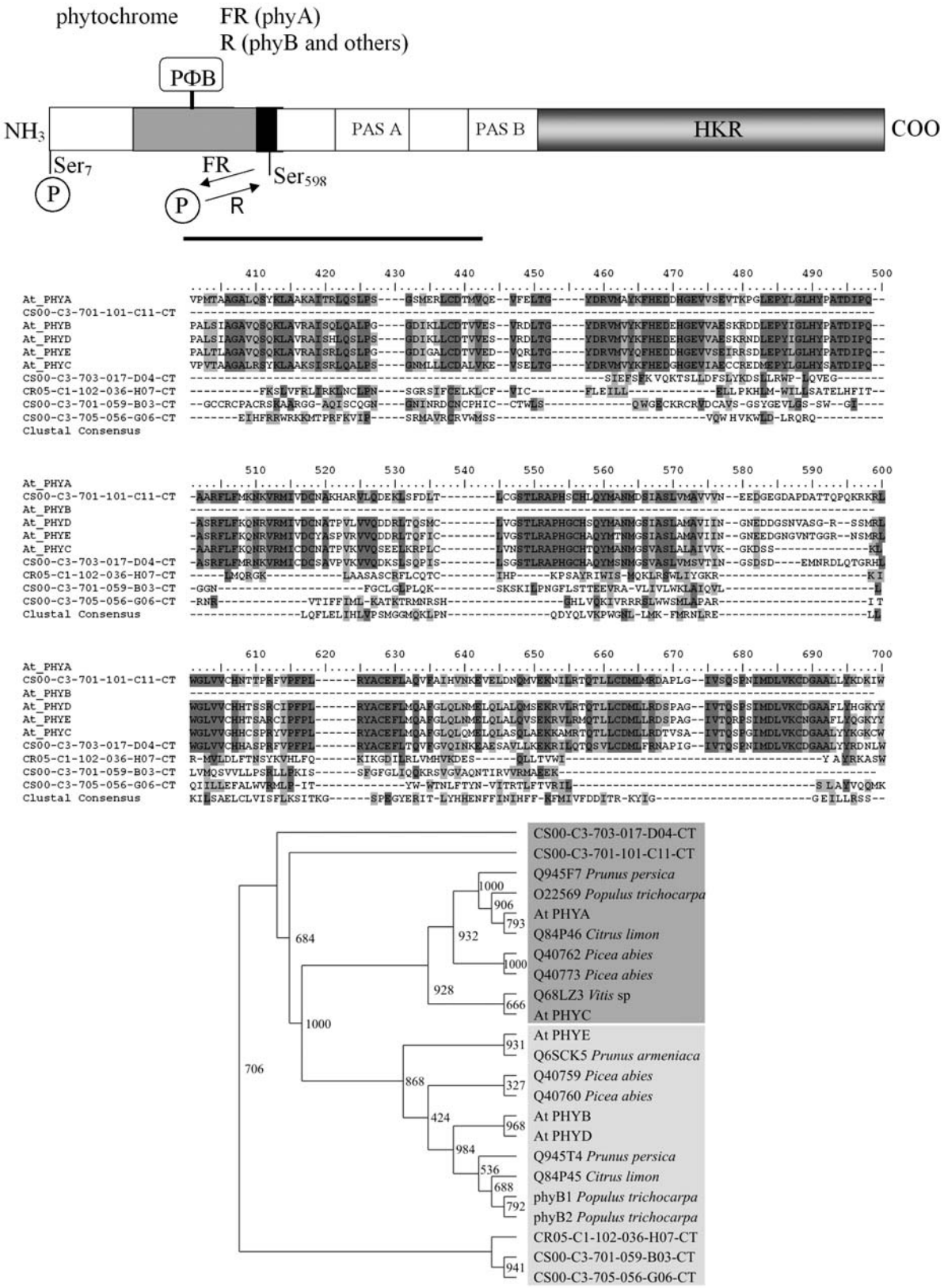

Figure 2 - Domain structure, phylogenetic analyses and alignment of the predicted amino acid sequence of phytochrome family in citrus. Neighbor-joining trees for citrus and tree species deduced amino acid and *Arabidopsis* full length sequences aligned with ClustalX are shown. Bootstrap values are indicated above each branch. Dark and light gray shading indicate sequence identity and similarity, respectively. At, *Arabidopsis thaliana*; C Number, contig number; Cit, citrus; CS, *Citrus sinensis*; CR, *Citrus reticulata*; FAD, flavin adenosine diphosphate; FR, far-red light; HKRD, histidine kinase-related domain; PAS, Per-ARNT, Sym domain; PΦB, phytochrome bilin; R, red light; ser, serine residue.

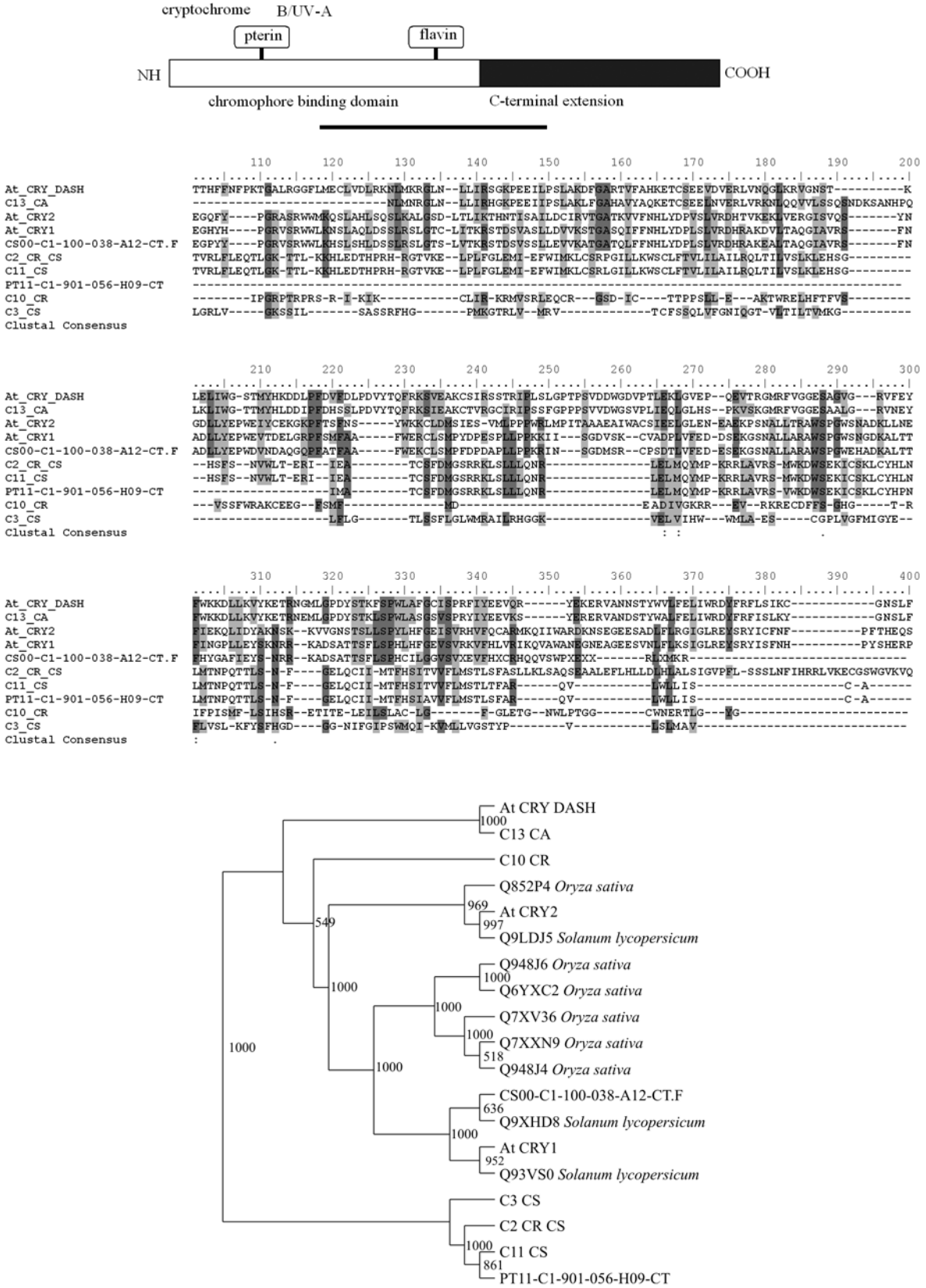

Figure 3 - Domain structure, phylogenetic analyses and alignment of the predicted amino acid sequence of the cryptochrome family in citrus. Neighbor-joining trees for citrus deduced amino acid and *Arabidopsis* full length sequences aligned with ClustalX are shown. Bootstrap values are indicated above each branch. Dark and light gray shading indicate sequence identity and similarity, respectively. At, *Arabidopsis thaliana*; B, blue light; C Number, contig number; CA, *Citrus aurantium*; Cit, citrus; CS, *Citrus sinensis*; CR, *Citrus reticulata*; FAD, flavin adenosine diphosphate.

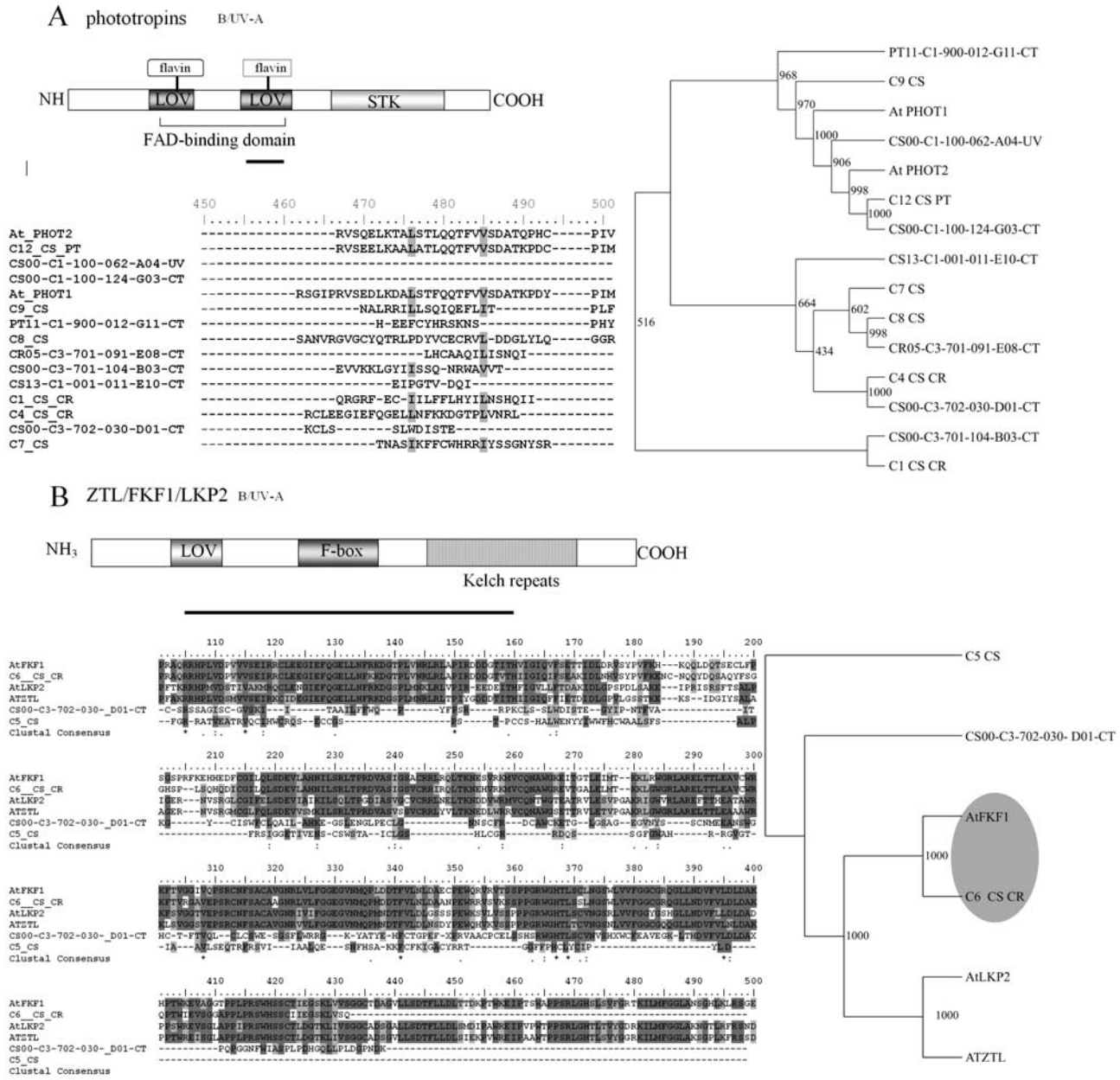

Figure 4 - Domain structure, phylogenetic analyses and alignment of the predicted amino acid sequence of phototropin and zeitlupe in citrus. **A.** phototropin family. **B.** zeitlupe family. Neighbor-joining trees for citrus deduced amino acid and *Arabidopsis* full length sequences aligned with ClustalX are shown. Bootstrap values are indicated above each branch. Dark and light gray shading indicate sequence identity and similarity, respectively. At, *Arabidopsis thaliana*; B, blue light; C Number, contig number; CA, *Citrus aurantium;* Cit, citrus; CS, *Citrus sinensis*; CR, *Citrus reticulata*; FAD, flavin adenosine diphosphate; LOV, light, oxygen, voltage subtype of PAS; PT, *Poncirus trifoliata*; R, red light; STKD, serine/threonine kinase domain; UV-A, ultraviolet A light.

floral induction and entrainment of the circadian clock (Chen *et al.*, 2004). Four EST singlets sharing sequence similarities to phy family genes were identified; three in *C. sinensis* and one in *C. reticulata* genome (Table S1). Two of *C. sinensis* ESTs show higher levels of sequence similarity to *PHYA* genes, whereas the remaining ones are related to *PHYB*-type of sequences. Interestingly, the *PHYB* homologs in citrus appear to be more distantly related from the *Populus PHYB1* and *PHYB2* sequences than from the *A. thaliana PHYE* gene (Figure 2). The branching in the *PHYB* genes is a relatively recent event and is absent from many plant species, including *Arabidopsis* (Mathews, 2006). The partial nature of the sequences prevent us from speculating whether citrus genome has a single *PHYB* gene or two, like the current model woody plant *Populus*.

The cryptochrome family is represented in the citrus genome by two ESTs corresponding to *Arabidopsis CRY1* and five sequences similar to CRY-DASH (Table S1, Figure 3). Although, all the identified sequences share similarities to higher plant cry sequences, for the majority of them

(five), the deduced amino acid sequence identity is restricted to the photolyase-like domain (Figure 3), indicating that these genes may function as photolyases rather than *bona fide* B photoreceptors. The C-terminal extension, essential for cry1 function in *Arabidopsis*, is conserved in *C. sinensis* EST and in *C. reticulata* EST contig (Table S1, Figure 3). Cryptochromes are mainly responsible for de-etiolation under blue light in *Arabidopsis*, including control of transcriptional regulation, inhibition of hypocotyls growth, promotion of cotyledons expansion, and synthesis of several non-photoreceptor pigments, such as chlorophyll and anthocyanins (Li and Yang, 2006). In addition, this class of photoreceptor acts in coordination with phy to reset the circadian clock and to control the transition to flowering (Yanovsky and Kay, 2002). At least two of the identified sequences (CS00-C1-100-038-A12.CT and C13-CA) are likely to code for functional cry family members in citrus.

In *Arabidopsis*, the phototropin photoreceptor family consists of two closely related members that share almost 60% protein identity. In the genome of citrus species, an EST contig whose deduced amino acid sequence shows 71% identity to PHOT2 and four cDNAs with sequences approximately 50% identical to PHOT1 and PHOT2, were identified (Figure 4A, Table S1). The overall sequence conservation between citrus and other species PHOT proteins is high, including at the N-terminal LOV domains, essential for chromofore binding and protein function in *Arabidopsis,* reviewed in Quecini and Liscum (2006). The remaining identified ESTs and EST contigs present high levels of sequence identity restricted to the C-terminal serine/threonine kinase and thus, may not perform photoperception-related functions. In *Arabidopsis* and rice, phot family controls a specific sub-set of physiological processes, including phototropic stem curvature, stomata opening control and chloroplast relocation (Quecini and Liscum, 2006). Only recently, phots have been demonstrated to be involved in B-mediated seedling de-etiolation (Folta *et al.*, 2003; Takemyia *et al.*, 2005). In *Arabidopsis*, phot1 and phot2 have specialized and overlapping roles, phot1 being the most important photoreceptor sensing directional B under low fluence rates and phot2 responsible for high light responses (Briggs and Christie, 2002). The presence of multiple PHOT-like sequences in citrus genome suggests that such a fluence rate-specific role might occur.

Recently, a three-member family of putative photoreceptors has been characterized in *Arabidopsis*; the ZTL/FKF1/LKP2 family (Somers, 2001). It is represented by three distinct sequences in citrus genome databases: one highly similar to FKF1 with lower homology to ZTL and two sharing sequence similarity to FKF1 and LKP2 (Table S1, Figure 4B). *Arabidopsis* ZTL/FKF1/LKP2 proteins are characterized by the presence of a flavin-binding LOV domain at the protein N-terminal, an F-box domain and a stretch of Kelch repeats, providing a direct link between light perception and ubiquitin-mediated protein degradation. The family has been functionally associated to the endogenous time-keeping mechanism and the control of photoperiodic flowering time (Imaizumi *et al.*, 2003; Imaizumi *et al.*, 2005). In *Arabidopsis*, ZTL and FKF1 are thought to be components of an Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase complex (Vierstra, 2003). ZTL has been implicated in the ubiquitin-mediated proteolytic degradation of the clock component TOC1 (Más *et al.*, 2003) and in the regulation of developmental responses to R, possibly through its interaction with phyB (Kevei *et al.*, 2006), while FKF1 has been demonstrated to control the levels of the photoperiod-sensing *CONSTANS* (*CO*) gene via degradation of its transcriptional repressor, a DOF type transcription factor, CDF1 (Imaizumi *et al.*, 2003; Imaizumi *et al.*, 2005). Thus, ZTL post-translationally regulates TOC1 levels and FKF1 controls daily *CO* expression in part by degrading CDF1. Citrus *FKF1*/*ZTL*- and *LKP2*-like EST contigs are highly conserved at the F-box and Kelch repeats domains (Figure 4B), suggesting a function in the protelolytic degradation of circadian-clock associated factors.

Phy and cry signal transduction components

Phytochrome responses are associated with changes in gene expression (Casal and Yanovsky, 2005) and members of several transcription factor families are required for phy signaling or are early targets of phy-mediated responses. In citrus, 22 EST contigs corresponding to the *Arabidopsis* transcriptional regulators and nuclear factors involved in phy and cry light signaling were identified, along with 17 transcripts associated to light-mediated proteolysis and seven transcripts similar to several signaling events of light signaling, such as Ca^{2+}-binding and post-translational protein modification (Figure 1, Table S2).

In *Arabidopsis*, the PIF (PHYTOCHROME-INTERACTING FACTOR) and PIL (PIF-LIKE) family of bHLH (basic Helix-Loop-Helix) transcriptional regulators, which includes HFR1/REP1/RSF1 (LONG HYPOCOTYL IN FAR RED 1/REDUCED PHYTOCHROME SIGNALING 1/REDUCED SENSITIVITY TO FAR-RED LIGHT 1), is one of the best-characterized phy signaling partners (Quail, 2002). PIF3 interacts with the R-activated form of phyA and phyB in a conformation-specific manner (Ni *et al.*, 1999) and binds specifically to a *cis*-acting regulatory element (G-box) in the promoters of several phytochrome-responsive genes. Simultaneous binding of PIF3 to promoters of light-responsive genes and to the Pfr form of phyB (Martinéz-Garcia *et al.*, 2000) indicates that PIF3 recruits phyB to the promoters of actively transcribed genes (Figure 5A). Moreover, PIF3 can heterodimerize with other bHLH proteins, such as HFR1 (Fairchild *et al.*, 2000) and PIF4 (Huq and Quail, 2002). Thus, the current hypothesis for PIF/PIL function in *Arabidopsis* is that: (i) phytochromes, mainly phyA, act through PIF3 and other yet un-

identified factors to regulate transcription of a master set of regulators, such as *CCA1* (Wang and Tobin, 1998), *LHY1* (Schaffer *et al.*, 1998), *TOC1* and *CO* (Harmer *et al.*, 2000; Tepperman *et al.*, 2001); and (ii) these regulators then control the transcription of genes encoding functions necessary for the terminal steps of the signaling cascade. Interestingly, in citrus genome databases, the *PIF*/*PIL*/*HFR* family is represented by a single EST contig from *C. aurantifolia* and *C. latifolia*, displaying higher identity to PIF4 and HFR1 protein (Table S2, Figure 5). Another two highly similar EST contigs (55.5% deduced amino acid sequence identity) showed moderate (15.0 to 17.5%) and low (7.5 to 2.9%) identity to HFR1 and PIF/PIL gene products, respectively. The functional significance of these transcripts as PIF/PIL/HFR-like light-induced transcriptional regulators remains unclear. The absence of PIF-like transcripts in *C. sinensis* and *C. reticulata* transcriptomes, which together correspond to approximately 72% of CitEST database, is noteworthy given their importance in light-mediated responses in *Arabidopsis*.

Several basic domain/zinc finger (Zn finger) and MYB-type factors function as downstream convergent targets of phy and cry signaling in *Arabidopsis*, independently of G-box photoreceptor binding (Oyama *et al.*, 1997; Chattopadhyay *et al.*, 1998; Ballesteros *et al.*, 2001). In citrus species transcriptome, six cDNAs corresponding to this class of transcriptional regulators were present: namely, one HY5 (LONG HYPOCOTYL 5), one HYH (HY5-HOMOLOGOUS) and four LAF1 (LONG AFTER FAR RED 1) homologs (Table S2, Figure 6A). Down-regulation of these signaling pathways occurs when phyA and the transcription factors, HY5 and LAF1, are degraded in a light-dependent fashion by the proteasome in a mechanism that involves COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) (Saijo *et al.*, 2003; Seo *et al.*, 2004; Jang *et al.*, 2005). The COP9 signalosome (CSN) is also involved in HY5 degradation (Peng *et al.*, 2001). Moreover, SPA1 (SUPRESSOR OF PHYA-105 MUTATION) and the other members of the SPA family regulate the ubiquitin-ligase activity of COP1 (Saijo *et al.*, 2003; Seo *et al.*, 2003). In citrus EST databases, COP1, other components of the CSN and a small group of SPA-like transcripts were identified, suggesting the existence of a similar mechanism of phy-mediated signal desensitization route (Table S2, Figure 6B).

Photoreceptor-initiated signaling pathways also consist of cytosolic components in *Arabidopsis* and other model species. General transduction pathways, such as G-protein, Ca^{+2}-calmodulin and protein phosphorylation cascades have been demonstrated to take part in light-triggered signaling (Bowler *et al.*, 1994). Homologs of several Ca^{+2}-binding and protein phosphorylation factors in phy- and cry-initiated signal transduction were identified in citrus transcriptome (Table S2). Phosphorylation may be responsible for the fine tuning of light signal transduction at several checkpoints, including the degradation of active phyA mediated by the ubiquitin/26S proteasome pathway; the interaction of light-signaling positive factors with COP1, an E3 ubiquitin-protein ligase functioning as a negative regulator of photomorphogenesis (Seo *et al.*, 2004), reducing the affinity of phosphorylated phyA to its signaling partners (Kim *et al.*, 2004) and controlling the phosphorylation level and, consequently, the signaling activity, of phy (Ryu *et al.*, 2005). Extensive functional conservation in angiosperms phosphorylation and post-translational protein modification suggests that the transcripts identified in citrus are involved in the proteolytic degradation of light-signaling components.

Phot signal transduction components

Phototropin-mediated signaling involves several families of transducing partner proteins. *NPH3* and *RPT2* are members of the 32-gene *NRL* (*NPH 3* and *RPT2*-LIKE) family in *Arabidopsis* (Quecini and Liscum, 2006). Nine citrus EST contigs whose deduced amino acid sequences share high homology to NPH3 and RPT2 were identified (Table S3). Moreover, 12 cDNAs with the conserved N-terminal signature BTB/POZ (BRIC-À-BRAC, TRAMTRACK AND BROAD COMPLEX/POXVIRUS AND ZINC FINGER) domain and C-terminal coiled-coil, respectively, were found. The differential growth of plant organs relative to a B-light stimulus is triggered by phot directional light sensing and hypothesized to be brought about by differential auxin concentration or activity (review in Quecini and Liscum, 2006). The relocation of PIN family auxin transporters has been associated to phot-mediated stem curvature response (Friml *et al.*, 2002; Friml, 2003). The ARF transcriptional activator, NPH4, represent a connection between auxin-mediated asymmetric growth and phototropism, indicating that phototropism requires an auxin-regulated transcriptional response (Harper *et al.*, 2000; Tatematsu *et al.*, 2004). Recently, NPH4/ARF7 has also been implicated in leaf expansion and auxin-induced lateral root formation in *Arabidopsis* (Wilmoth *et al.*, 2005). The citrus genome databases have two EST contigs and one singlet corresponding to *Arabidopsis* PIN1 and PIN3 and two, highly similar to NPH4/ARF7 (Table S3), that are likely to be functionally equivalent to their *Arabidopsis* counterparts due to extensive identity. Phot-mediated B light perception involves H^+-ATPase phosphorylation and association with 14-3-3 proteins in stomatal guard cells (Kinoshita and Shimazaki, 2002, Fuglsang *et al.*, 2003). At this point, the function of the 14-3-3 family proteins remain elusive, although they have been shown to be required for H^+-ATPase activity induced by B light (Fuglsang *et al.*, 2003). Four citrus EST contigs showing more than 50% deduced amino acid identity to 14-3-3 proteins were identified; however, their association to phot-mediated signal transduction remains to be determined.

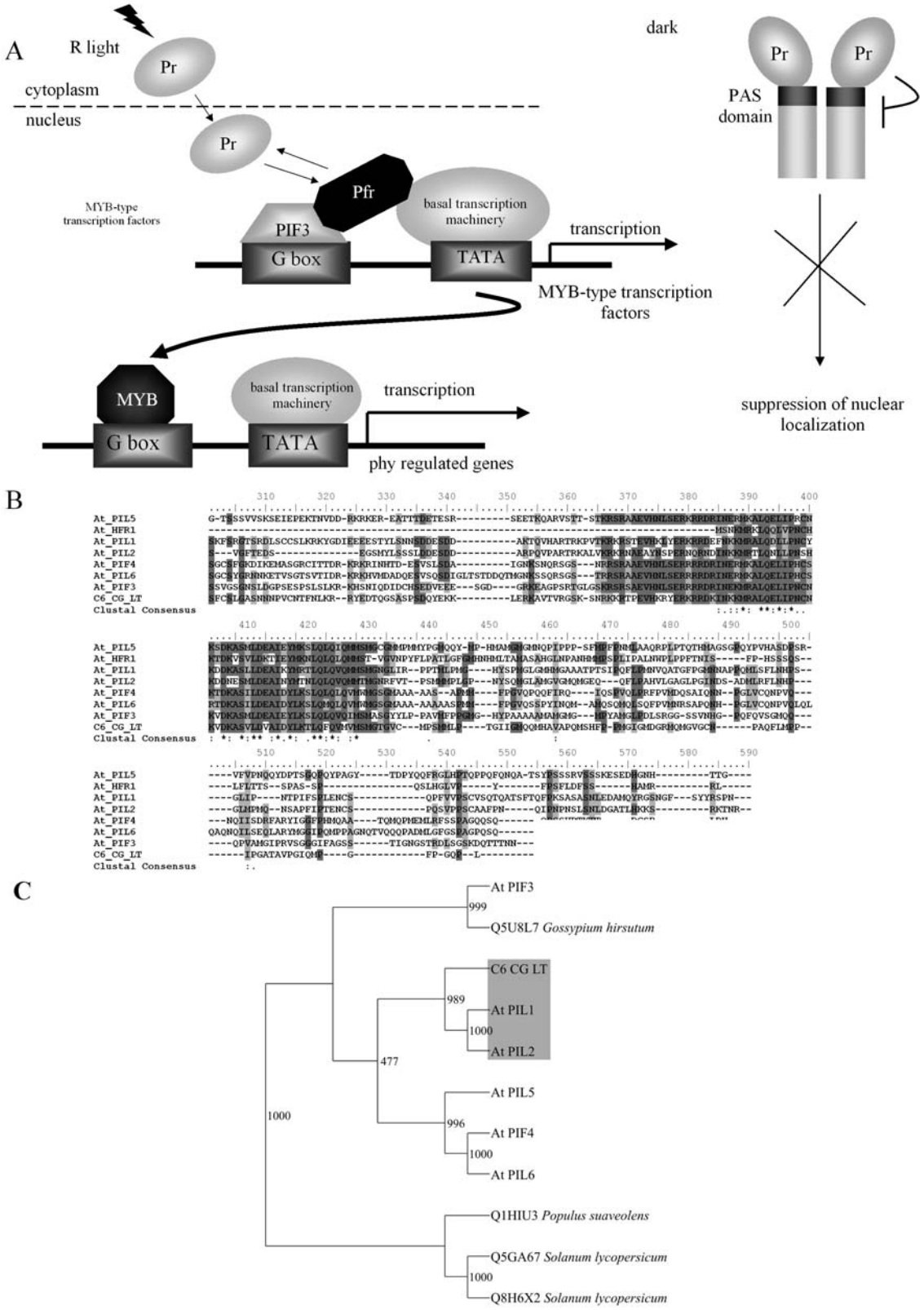

Figure 5 - Light-signaling associated bHLH transcriptional regulator family in citrus. **A.** schematic representation of phytochrome-regulated PIF3 transcriptional activation. **B.** alignment of the bHLH DNA-binding domainPIF/PIL family in *Arabidopsis* and citrus. C. phylogenetic analysis of plant PIF/PIL genes. Neighbor-joining trees for a concatenation of the DNA-binding amino acid domains aligned with ClustalX is shown. Bootstrap values are indicated above each branch. Dark and light gray shading indicate sequence identity and similarity, respectively. The sequences from species other than *A. thaliana* and citrus are indicated by their ExPaSy entry code. At, *Arabidopsis thaliana*; bHLH, basic loop-helix-loop DNA binding domain; C Number, contig number; CG, *Citrus aurantifolia;* LT, *Citrus latifolia*.

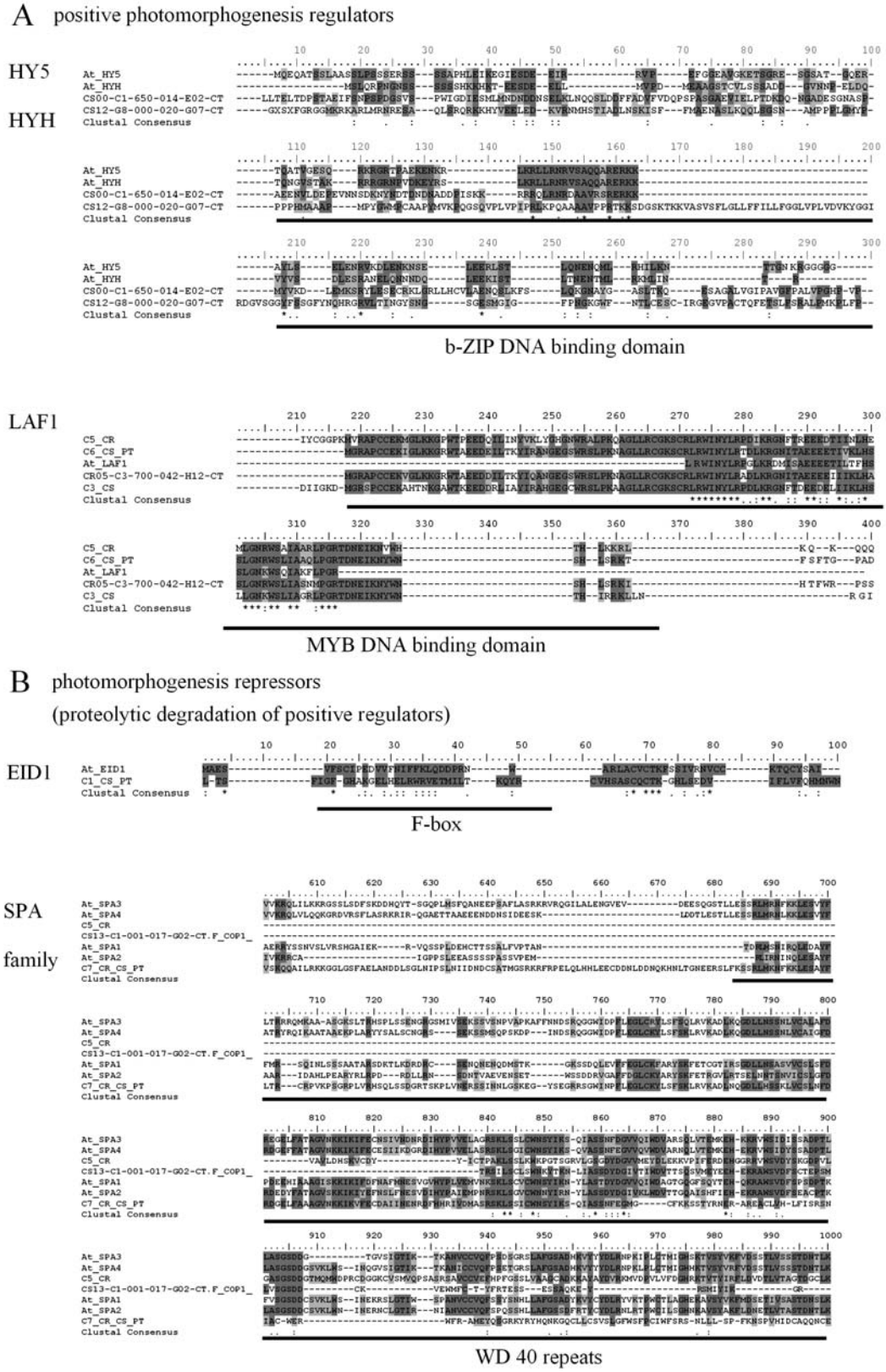

Figure 6 - Proteolysis-mediated photomorphogenesis control pathway in citrus. **A.** alignment of positive photomorphogenesis regulator families HY5/HYH and LAF1. **B.** alignment of negative photormorphogenesis regulator families EID1 and SPA1. Dark and light gray shading indicate sequence identity and similarity, respectively. At, *Arabidopsis thaliana*; bZIP, basic leucine zipper; C, contig; CS, *Citrus sinensis*; CR, *Citrus reticulata*; PT, *Poncirus trifoliata*. Underlined sequence represents functional domains. Dark and light gray shading indicate sequence identity and similarity, respectively.

Concluding Remarks

This preliminary survey of citrus photoperception-associated genes has provided useful information for further studies of light developmental control in these species. It has allowed the identification of conserved members of light-triggered signaling in a non-model species and the elaboration of a work model frame for light perception and signaling in citrus (Figure 7). These prospects are particularly attractive considering the range of economically important physiological processes of citrus that are regulated by light, including secondary metabolism regulation and shading responses. An immediate goal of plant genomics is to transfer knowledge between model and crop species, allowing a better understanding of the mechanisms underlying several aspects of plant physiology. Thus, genomic and functional information can be integrated into the accumulated knowledge of citrus genetics and physiology to advance basic and applied research. These studies will help to elucidate the molecular basis of developmental plasticity and to understand how environmental factors modulate plant development and the expression of phenotypic characters. The results obtained provide a new perspective on several aspects of light-regulated physiological processes in citrus, such as de-etiolation, seedling establishment and shade-avoidance response.

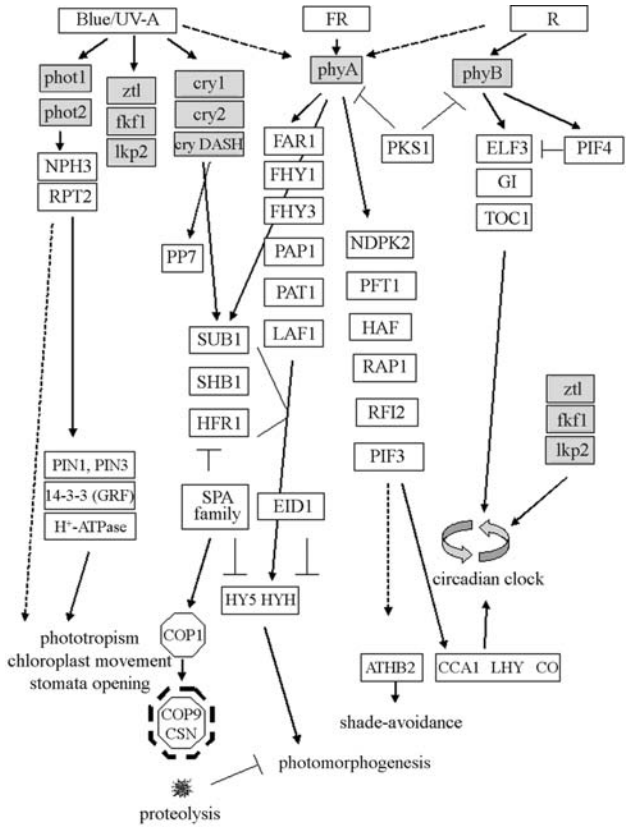

Figure 7 - Schematic overview of the photosensory signaling pathways in citrus based on comparative genomic analysis to *A. thaliana*. Shaded boxes represent photoreceptors. Positive and negative interactions are represented by arrowheads and square ends, respectively. Dashed arrows indicate mechanistically undefined processes.

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Internet Resources

Citrus Biotechnology Laboratory, http://citest.centrodecitricultura.br (September 13, 2006)

Cluster v.2.11 Software, http://rana.lbl.gov/EisenSoftware.htm.

DNASTAR Lasergene Software, http://www.dnastar.com/web/index.php.

European Bioinformatics Institute-European Molecular Biology Laboratory (EMBL-EBI), www.ebi.ac.uk/interpro/ (September 04, 2006).

Expert Protein Analysis System (ExPaSy), http://www.expasy.org/prosite/ and http://www.us.expasy.org/sprot/ (October 05, 2006).

Gene Ontology (GO), http://www.godatabase.org/cgi-bin/amigo/go.cgi (October 23, 2006).

PAUP* 4.0b10 Software, http://paup.csit.fsu.edu/.

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Tree View v.1.6 Software, http://rana.lbl.gov/EisenSoftware.htm.

Supplementary Material

The following online material is available for this article:

Table S1
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Supplemental References
Figure S1

This material is available as part of the online article from http://www.scielo.br/gmb.

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Table S1. Citrus ESTs with homology to photoreceptor and putative photoreceptor gene families of *Arabidopsis thaliana*.

Arabidopsis thaliana		CitEST			Protein motifs[d] and	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**References**
CRY1	AT4G08920	C10-CR (2)	23.3	3e-91	FAD binding domain, DNA photolyase,	Cashmore *et al.*, 1999
		CS00-C1-100-038-A12-CT	74.6	1e-106	B photoreceptor, photomorphogenesis, circadian clock entrainment, photoperiodic responses	
CRY-DASH	AT5G24850	C2-CR/CS (13)	23.5	1e-93	Cryptochrome family, putative B	Brudler *et al.*, 2003,
		C3-CS (4)	22.2	1e-89	photoreceptor, transcriptional regulator in	Kleine *et al.*, 2003
		C11-CS (3)	21.2	2e-87	*Synechocystis*	
		C13-CA (2)	64.1	1e-106		
		PT11-C1-901-056-H09-CT	12.7	1e-62		
PHOT1	AT3G45780	C8- CS (5)	19.8	1e-56	LOV1 and LOV2 domain, serine-	Huala *et al.*, 1997
		CS00-C3-701-104-B03-CT	50.0	4e-84	threonine kinase domain, B	
		CS00-C3-702-030-D01-CT	50.0	7e-54	photoreceptor, phototropism, chloroplast	
		CR05-C3-701-091-E08-CT	18.0	6e-53	movement, stomata opening control	
		CS00-C1-100-062-A04-UV	37.8	1e-53		
		PT11-C1-900-012-G11-CT	17.0	2e-45		
PHOT2		C12-CS/PT (17)	71.1	7e-94	phototropin family	Jarillo *et al.*, 2001
		C7-CS (7)	33.4	5e-69		
		C9-CS (2)	38.1	3e-63		
		C1-CR/CS (2)	27.4	4e-59		
		C4-CR/CS (2)	17.5	3e-38		
		CS00-C3-701-104-B03-CT	47.5	4e-84		
		CS00-C1-100-124-G03-CT	42.1	6e-74		
PHYA	AT1G09570	CS00-C3-701-101-C11-CT	58.3	6e-86	PAS1, PAS2, chromophore binding	Sharrock and Quail,
		CS00-C3-705-056-G06-CT	34.7	4e-81	domain, HKL domain, R/FR photoreceptor	1989
PHYB	AT2G18790	CR05-C1-102-036-H07-CT	15.3	3e-75	phytochrome family	Reed *et al.*, 1993
		CS12-G8-000-003-D03-CT	8.1	3e-55		
FKF1	AT1G68050	C5-CS (2)	76.3	1e-142	Kelch repeats, F-box domain, LOV	Nelson *et al.*, 2000
		C6-CR/CS (3)	19.3	1e-46	domain, putative photoreceptor, photoperiodic flowering control, circadian clock	
LKP2	AT2G18915	CS00-C3-702-030-D01-CT	22.0	1e-76	putative photoreceptor, circadian clock	Schultz *et al.*, 2001
ZTL	AT5G57360	C5-CS	29.7	8e-71	putative photoreceptor, circadian clock	Somers *et al.*, 2000

[a]Gene name abbreviations: CRY: cryptochrome; DASH: *Drosophila*, *Arabidopsis*, *Synechocistis*, human; PHOT: phototropin; PHY: phytochrome, FKF1: F-box, Kelch repeat, Flavin-binding protein1; LKP2: LOV domain, Kelch repeat protein2; ZTL: zeitlupe.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Functional domains abbreviations: FAD: flavina adenosine dinucleotide; LOV: light, oxygen, voltage subtype PAS domain; PAS: Per, ARNT, Sym domain; HKL: histidine kinase-like.

Table S2. Citrus ESTs with homology to genes involved in phytochrome- and cryptochrome-mediated responses in *Arabidopsis thaliana*.

Arabidopsis thaliana		CitEST			Protein motifs[d] and	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**Reference**
ATHB2	AT4G16780	C6-CS/PT (23)	40.1	3e-51	homeobox leucine zipper,	Carabelli *et al.*, 1996
		CS00-C2-003-056-F03-CT	27.5	2e-34	shade avoidance response	
COP1	AT2G32950	C5-CS/CG (5)	81.9	1e-89	E3 ubiquitin ligase, Zn finger and RING	Osterlund *et al.*, 2000,
		C2-CS (2)	65.1	3e-78	finger domains, proteolysis	Seo *et al.*, 2004
		CA26-C1-002-001-E08-CT	40.4	9e-61		
COP8/FUS4	AT5G42970	CS00-C3-702-101-D09-CT		2e-75	subunit 4 of COP9 signalosome complex,	Serino *et al.*, 1999
/FUS8		CR05-C1-102-033-G01-CT		2e-72	subunit of the 19S regulatory particle of the	
		CA26-C1-002-037-H04-CT		7e-69	26S proteasome	
COP9/FUS7	AT4G14110	C1-CS (3)	21.9	3e-66	COP9 signalosome subunit, identical to cDNA	Dohmann *et al.*, 2005
		LT33-C1-003-023-F05-CT	28.0	2e-56	CSN complex subunit 8 (CSN8)	
COP10/FUS9	AT3G13550	C2-CR/CS (6)	64.8	5e-69	ubiquitin-conjugating enzyme (COP10),	Yanagawa *et al.*, 2004
		C3-CS/PT (23)	39.0	5e-41	proteolysis	
		C4-CR/CS (8)	39.0	1e-40		
		CR05-C1-102-060-B12-CT	38.5	1e-39		
COP11/FUS6	AT3G61140	C1-CS/PT(2)	16.9	2e-86	COP9 signalosome complex subunit 1 / CSN complex subunit 1 (CSN1) / COP11 protein (COP11) / FUSCA protein (FUS6)	Kang *et al.*, 2000
EID1	AT4G02440	C1-CS/PT (5)	22.0	7e-51	Cyclin-like F-box protein, protein degradation, photomorphogenesis	Marroco *et al.*, 2006
FAR1	AT4G15090	C9-PT (2)	37.3	1e-29	FAR1 family, transposase-like domain	Hudson *et al.*, 1999
		CS00-C1-100-058-F05-CT	32.7	5e-35		
FHY3	AT3G22170	C9-PT (2)	34.2	1e-29	FAR1 family, transpose-like domain	Wang and Deng, 2002,
		CS00-C1-100-058-F05-CT	24.6	1e-33		Lin and Wang, 2004
FHY1	AT2G37680	CR05-C1-100-082-A02-CT	68.3	2e-71	no recognizable domain, phyA-mediated	Shen *et al.*, 2005a
		CS00-C1-100-124-B05-CT	58.7	2e-71	photomorphogenesis	
		CR05-C3-700-106-G10-CT	58.3	2e-71		
		CS00-C1-102-029-H04-CT	18.3	2e-70		
HAF2	AT3G19040	C3-CA/CR (2)	47.5	2e-56	TATA-binding protein-associated factor TAF1 (TAFII250), bromodomain, ubiquitin domain, histone acetyltransferase activity	Bertrand *et al.*, 2005
HFR1	AT1G02340	C6-CG/LT (2)	29.4	4e-32	transcriptional regulator, bHLH domain, de-etiolation	Duek and Fankhauser, 2003
HY5	AT5G11260	CS00-C1-650-014-E02-CT	17.9	1e-35	transcription regulator, bZIP DNA binding domain, photomorphogenesis	Chattopadhyay *et al.*, 1998
HYH	AT3G17609	CS12-G8-000-020-G07-CT	16.1	2e-42	transcriptional regulator, bZIP DNA binding motif	Holm *et al.*, 2002
LAF1	AT4G25560	C6-CS/PT (2)	73.9	2e-78	MYB transcription factor, R2R3 group,	Ballesteros *et al.*, 2001
		C3-CS (2)	67.4	1e-68	de-etiolation	
		C5-CR (2)	63.0	6e-62		

		CR05-C3-700-042-H12-CT	67.4	2e-60		
NDPK2	AT5G63310	C7-LT (2)	53.5	3e-53	nucleoside diphosphate kinase, ATP binding	Shen *et al.*, 2005b
		C2-CS (2	45.3	1e-46	protein, histidine kinase, phy- and auxin-mediated signal transduction	Choi et al, 2005
PAP1	AT1G56650	C3-CS (2)	28.6	5e-56	transcriptional regulator, auxin responsive, anthocyanin biosynthesis	Teng *et al.*, 2005
		CR05-C3-700-042-H12-CT	28.2	2e-58		
		CR05-C3-700-004-E04-EU	25.4	1e-55		
		PT11-C1-900-084-F06-CT	24.6	1e-49		
PFT1	AT1G25540	C1-CG/CS (2)	28.4	1e-97	von Willebrand factor type A (VWF-A), glutamine-rich C-terminal, flowering time	Cerdán and Chory, 2003
		C2-CS/CR (6)	65.4	1e-148		
PIF3	AT1G09530	C6-CG/LT (2)	25.5	4e-27	transcriptional regulator, bHLH domain, photomorphogenesis	Ni *et al.*, 1998, Ni *et al.*, 1999
PIF4	AT2G43010	C6-CG/LT (2)	24.5	1e-22	PIF family, transcriptional regulator, bHLH domain, de-etiolation (cell expansion)	Huq and Quail, 2002
PKS1	AT2G02950	C1-CR/CS (2)	20.2	1e-18	no recognizable domain, phytochrome kinase substrate,	Lariguet *et al.*, 2003
PP7	AT5G63870	C3-CS/CR/PT (3)	29.6	2e-55	metallo-phosphoesterase motif, serine/threonine specific protein phosphatases signature, de-etiolation	Møller *et al.*, 2003
		C12-CS (11)	23.5	7e-45		
		CS13-C1-001-008-C12-CT	25.3	9e-46		
		PT11-C9-005-041-C05-CT	23.9	1e-42		
RAP1 / ATMYC2	AT1G32640	C2-CR/CS (2)	25.8	1e-39	MYC-related transcriptional activator, bHLH leucine zipper motif, photomorphogenesis.	Heim *et al.*, 2003
		CR05-C3-701-055-H07-CT	39.5	4e-42		
RFI2	AT2G47700	C1-CS (3)	34.5	4e-39	zinc finger (C3HC4-type RING finger) family protein, photomorphogenesis	Chen and Ni, 2006
SPA1	AT2G46340	C7-CR (3)	32.2	1e-114	proteolysis targeting, WD-repeat domain, light-regulated proteolysis	Höcker *et al.*, 1999, Laubinger *et al.*, 2004
		C5-CR/CS/PT (3)	25.6	2e-31		
		CS13-C1-001-017-G02-CT	26.8	4e-31		
SHB1	AT4G25350	C1-LT/CS (3)	27.8	2e-53	EXS domain, SPX domain, photomorphogenesis under B	Kang and Ni, 2006
		PT11-C1-900-042-H02-CT	17.1	2e-25		
SUB1	AT4G08810	C3-CG/CS/PT (11)	37.0	2e-64	Ca^{+2}-binding protein, de-etiolation	Guo *et al.*, 2001
		PT11-C1-901-057-B05-CT	22.4	5e-44		

[a]Gene name abbreviations: *ATHB: Arabidopsis thaliana* homeobox; *ATMYC*: *Arabidopsis thaliana* MYC-type; *COP*: constitutively photomorphogenic ; *EID:* Eimpfindlicher Im Dunkelroten Licht*; FAR:* far-red impaired response; *FHY :* far-red elongated hypocotyl ; *FUS*: Fusca; *HAF*: histone acetylation factor; *HFR* : long hypocotyl in FR light ; *HY:* long hypocotyl; *HYH :* HY5-homologue; *LAF:* long after far red; *NDPK:* nucleotide diphosphate protein kinase*; PAP:* production of anthocyanin pigment; *PAT*: phytochrome A-signal transduction ; *PFT:* phytochrome and flowering time*; PIF:* phytochrome-interacting factor*; PP:* protein phosphatase *; RAP:*ethylene response factor subfamily B-4 of ERF/AP2 transcription factor family*; RFI:*red and far red insensitive ; *SPA:* suppressor of *phytochrome A-105; SHB:* short hypocotyl under blue; *SUB:* short under blue.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level;

[d]Functional domains abbreviations: ATP: adenosine triphosphate; bHLH: basic helix-loop-helix; bZIP: basic Zipper; FAD: flavina adenosine dinucleotide; HKL: histidine kinase-like; LOV: light, oxygen, voltage subtype PAS domain; PAS: Per, ARNT, Sym domain; TAF: transcription ancillary factor.

Table S3. Citrus ESTs with homology to genes involved in phototropin-mediated responses in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Functional information and**	
Name	**Gene**	**EST**	**%**	**e value**	**biological process**	**Reference**
ARF7/	AT5G20730	C4-CR[d]	55.1	1e-89	B3 DNA binding domain,	Harper *et al.*,
NPH4		C1-CS/CR[e]	41.5	3e-62	AUX/IAA family, auxin-regulated	2000
		CS00-C3-705-071-D01-CT	47.8	5e-93	transcription	
GRF1 and	AT4G09000	C2-CS[f]	71.1	1e-113	regulatory factor1-G-box factor	Ferl, 2004
GRF family		C4-CS[g]	64.3	1e-104	14-3-3 homolog isoform family,	
		C9-CA/PT[h]	51.6	4e-99	signal transduction (scaffolding)	
		C10-CA/CS[i]	52.2	1e-102		
		CS00-C1-101-018-E05-CT	39.5	1e-101		
		CS00-C3-700-041-F04-CT	29.9	2e-97		
NPH3 and	AT5G64330	C5-CS[j]	47.1	9e-71	plant-specific NPH3 domain,	Motchoulski and
NRL family		C6-CR[k]	21.6	3e-59	BTB/POZ domain, signal	Liscum, 1999,
		C8-CR/CS[l]	19.3	4e-59	transduction (scaffolding)	Haga *et al.*, 2005
		CS00-C3-701-013-G03-CT	25.2	6e-82		
		CS00-C3-701-060-E12-CT	35.4	5e-96		
		PT11-C1-900-042-G09-CT	39.3	1e-78		
		PT11-C1-900-043-E10-CT	33.3	4e-67		
PIN1 and	AT1G73590	C3-CG/CS[m]	23.4	2e-75	auxin efflux carrier, tropic	Blakeslee *et al.*,
PIN family		C11-CA/CS[n]	24.6	3e-89	responses	2004
		CS00-C3-700-106-C03-CT	38.7	4e-97		
RPT2	AT2G30520	C1-CS[o]	27.6	2e-65	plant-specific NPH3 domain,	Inada *et al.*, 2004
		C7-CR[p]	70.8	1e-132	BTB/POZ domain, signal	
		CS00-C3-702-027-G06-CT	18.3	4e-42	transduction (scaffolding)	

[a]Gene name abbreviations: *ARF:* auxin-responsive factor *; GRF:*general regulatory factor (14-3-3 protein) ; *NPH:* non-phototropic hypocotyl *; PIN:* pin-formed *; RPT:* root phototropism.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Functional domains abbreviations: BTB: bric-à-brac, tramtrack, broad complex; POZ: poxvirus and zinc finger.

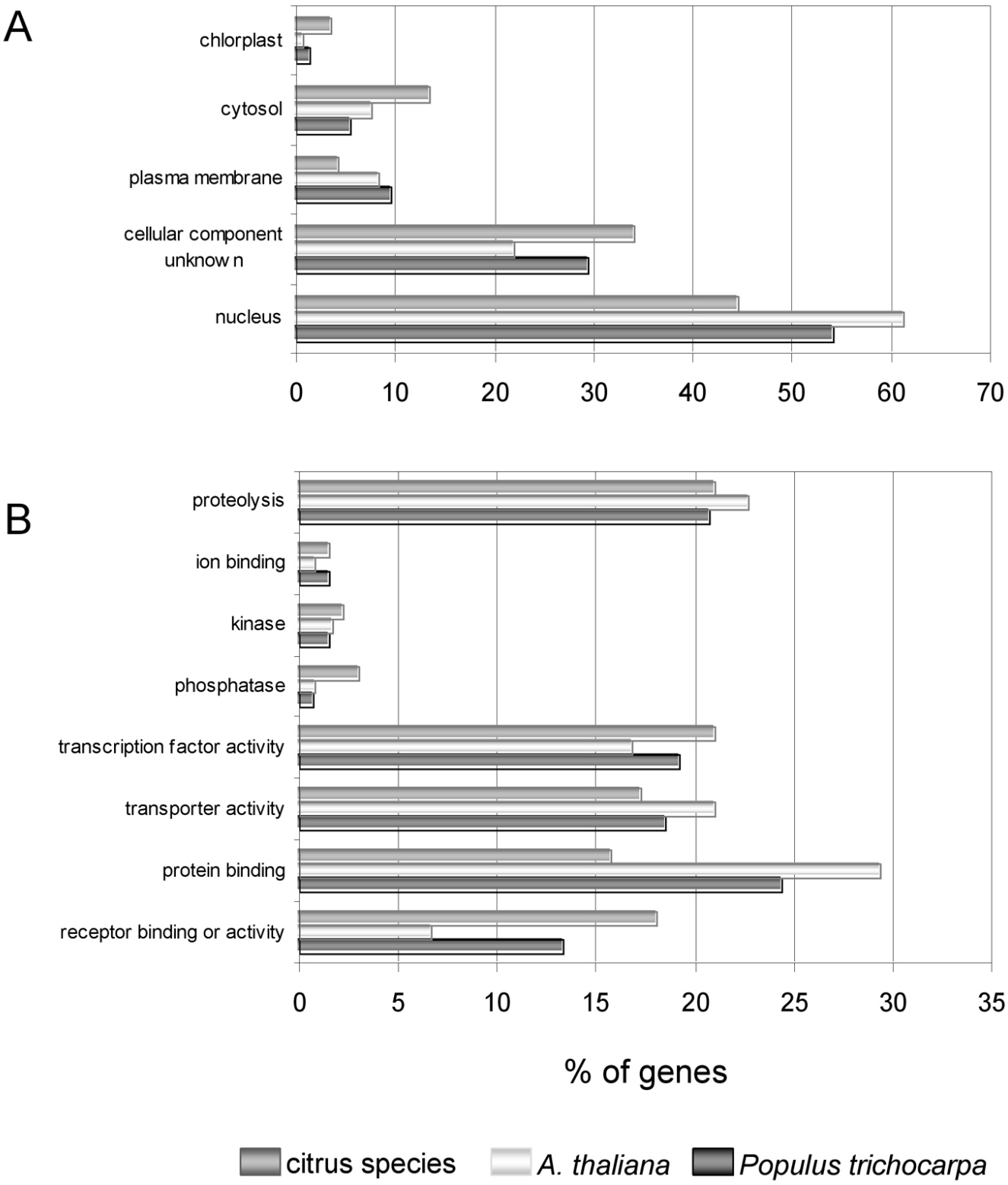

Figure S1 – Functional classification of citrus transcripts associated to photoperception and light signal transduction according to gene ontology (GO) categories in comparison to *Populus trichocarpa* transcriptome and *Arabidopsis thaliana* proteome. **A.** cellular component. **B.** molecular function. Assignments are based on the data available at the TIGR *Arabidopsis thaliana* Gene Index version 13.0.