

**Supplementary Material**

**Identification and characterisation of blue light photoreceptor gene family and their expression in tomato (*Solanum lycopersicum*) under cold stress**

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## SUPPLEMENT FILES

**Table 1:** Blue light gene specific primers sequences used for quantitative RT- PCR (qRT-PCR) analysis. Primers sequences were designed using Primer Premier software.

Genes	Forward Primer	Revers Primer	Primer efficiency (%)
CRY1	GGCACCAGAGGAAGAAGGGCACT	CATGGTGGTTCTGCAAGTAGC	95.4
CRY2	GGGATCGTTTAATGCAAGCTATAATT	CGAGTTATCAAACACAACCTTCAACAG	96
PHOT1	CACTGATCCTAGGCTTCCCG	GTGGTTAGATCAGTCTCTGGACC	97.6
PHOT2	GAAACTTGGGAAGTTAAATCC	GCATCAAGAAGGGAACAATAAGC	98
PHYA	GAAGCATGGAGGTGCTATGC	TATGCAATACGGTGGTCAAGG	98.5
PHYB	CTCCTCGGCAATGAAGTTGT	CGTGGATGCA TCTTCCTGCC	98
PHYC	ACCGCTGCTGACCGTCGC	TTCGCAAGCATACTGAGA	97

**Table 2:** Blue light photoreceptors with their photosensory domains and proposed modes from X-ray Crystal Structure analyses in tomato

Proteins	Photosensory Domain	PDB ID	Cofactor	Mode of Action
<b>Phototropins</b>	PAS & LOV	2Z6D, 2VOU 2VIA, 2VIB	FMN	Proteins belonging to the LOV domain family form a sub-class of PAS domains that bind to a flavin co-factor. The flavin moiety present in LOV domains could either be flavin mononucleotide (FMN) or flavin dinucleotide (FAD) that are sensitive to blue light (400nm-500 nm) in the visible region of the electromagnetic spectrum. The ZTL family bind to FMN as a chromophore. Photoreception by flavin changes the chemical state of the co-factor that initiates signaling by altering the protein structure
<b>Cryptochromes</b>	PHR/DNA photolyase & MTHF	3FY4, 1QNF	FAD, FMN	
<b>Zeitlupe (FKF1)</b>	Flavin-binding Kelch F-box	N/A	FMN	
<b>Phytochromes</b>	PAS, GAF & PHY	2VEA & 3C2W	Phytochromobilin tetrapyrrol chromophore (P $\phi$ B)	P $\phi$ B bond to a specific Cys in the apoprotein, that occurs autocatalytically with lyase activity within the Phy polypeptide Phy apoproteins are encoded by a small family of genes, with holoproteins derived from each isoform having both distinct and overlapping functions in light perception
<b>UVR8</b>	Seven-bladed $\beta$ -propeller &		No external cofactor and instead uses	Induces hydrogen bonds and hydrophobic interactions between adjacent blades and maintains the core structure. Trp-285 and

	C27. Both interact with COP1		two tryptophan residues, Trp 285 and Trp 233, as the chromophore for ultraviolet-B perception	Trp-233 are the key chromophore components responsible for UV-B sensing
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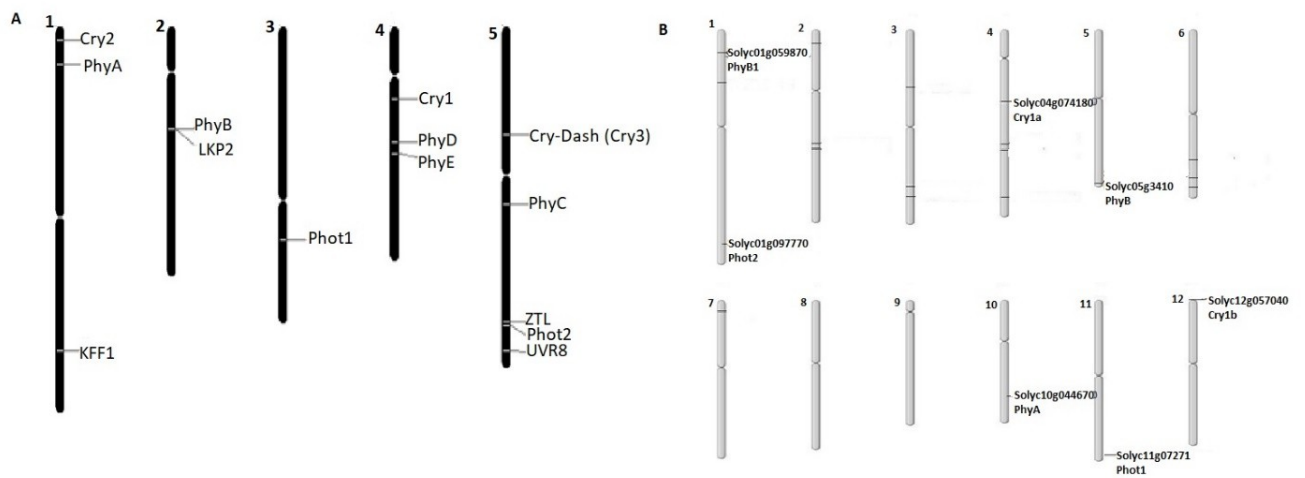
**Table 3:** Blue light photoreceptor gene family and their interaction in abiotic stress response

Type of Stress	Photoreceptors Genes	Abiotic Stress Response	Reference
Drought	Cry1 & Cry2	<p>a) Cry1 &amp; Cry2 are photosensory receptors that regulate growth and development and circadian clock in plant by mediating important agronomic traits in crop species (entrainment of the circadian clock, guard cell development, stomatal opening, root growth, plant height, high-light stress response, osmotic stress response, shade avoidance).</p> <p>b) Constitutively photomorphogenic 1 (COP1), which represses light signaling, is a core photomorphogenic regulator</p> <p>c) As an E3 ubiquitin ligase COP1 contains a RING-finger motif, a coiled-coil domain, and WD40 repeats; the RING-finger motif interacts with ubiquitin-conjugating enzyme E2, the coiled-coil domain is involved in the formation of a complex between COP1 and SUPPRESSOR OF PHYA-105 (SPA), and WD40 repeats interact with target proteins in the regulation of stomatal movements in response to dehydration</p>	(Fantini et al., 2019)
	PHYs	<p>a) Phytochromes regulate the transcription of light-responsive genes by modulating the activity of several transcription factors.</p> <p>b) PhyA, PhyB and PhyE are involved in suppressing drought tolerance. These results imply a possible function of phytochrome C (PhyC) in mediating</p>	(Wang <i>et al.</i> , 2016)

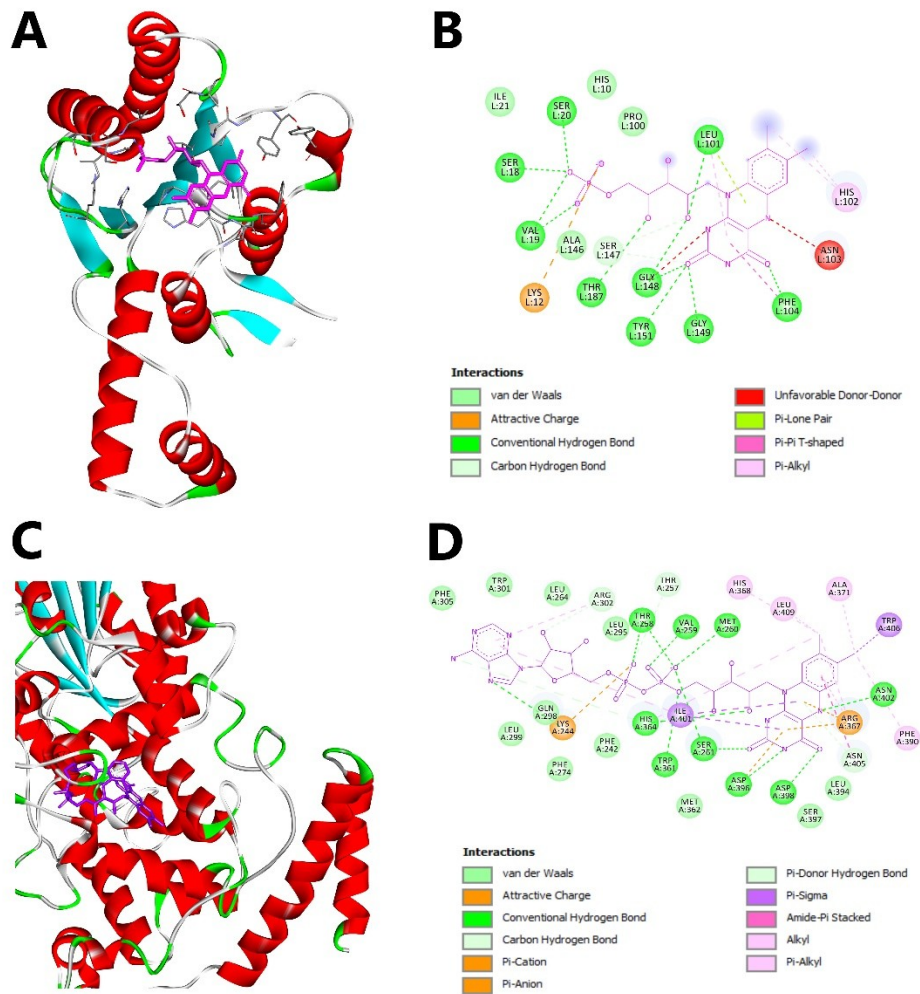
		<p>osmotic stress</p> <p>c) In high R/FR condition, PhyA is degraded and PhyB play critical role in regulating promoting stomatal opening and many other adaptive reactions</p>	
Drought & Salinity	Cry1 & Cry2	Cryptochrome-induced altered expression of stress/ABA-responsive genes	(Zhou <i>et al.</i> , 2018)
Salinity	Cry1 ZTL group	Overexpression of Cry1a confers oversensitive to salt stress	(D'Amico-Damião & Carvalho, 2018)
Heat	Cry1 ZTL	<p>a) PIF4 interact with Cry1 controlling hypocotyl elongation under heat stress</p> <p>b) ZTL and its interacting molecular chaperone, HSP90, establish a heat stress-inducible protein quality control system, which contributes to maintaining thermostable growth and circadian behaviors at high temperatures.</p> <p>c) In response to heat stress, ZTL and HSP90 are localized to insoluble protein aggregates and direct their degradation via the ubiquitin–proteasome pathways</p> <p>d) Under heat stress, HSP90 and other HSP members, including HSP70, recognize insoluble protein aggregates, to which ZTL is recruited through the interactions with the HSP family members.</p> <p>e) HSP family members and their interacting E3 ubiquitin ligase C-terminus of HSP70 Interacting Protein (CHIP) are known to mediate thermo-tolerance in plants further supporting</p>	(Gil & Park, 2019; Ma <i>et al.</i> , 2016)

		the physiological relevance of the ZTL-HSP module in thermostabilizing plant growth and the clock function.	
High-light	Cry1 & Cry2	<ol style="list-style-type: none"> <li>1. Cryptochromes affects chloroplast light-harvesting complex and redox equilibrium of photosynthetic apparatus.</li> <li>2. They also promote the transcription of ROS-responsive genes</li> </ol>	(El-Esawi <i>et al.</i> , 2017)
	Phot1 & Phot2	<ol style="list-style-type: none"> <li>a) Induce the opening stomatal pore to regulate CO<sub>2</sub> in leaf and stem epidermis.</li> <li>b) Induce chloroplast accumulation movement to the upper cell surface that promote light capture for photosynthesis.</li> <li>c) Promote cotyledon and leaf expansion and stimulate increases in cytosolic Ca<sup>2+</sup>.</li> <li>d) Regulate hypocotyl phototropism in response to high intensities of unilateral blue light.</li> </ol>	(Christie, 2007)
Cold & Drought	Cry ZTL LKP2 FKF1	<ol style="list-style-type: none"> <li>a) Cryptochromes share transcription factor with cold acclimatization mechanisms.</li> <li>b) GIGANTEA (GI) was originally identified based on its roles in photoperiodic flowering and circadian clock regulation. GI interacts with F-box protein ZEITLUPE (ZTL) through the amino-terminal flavin-binding LIGHT, OXYGEN or VOLTAGE domain of ZTL, which is necessary to sustain a normal circadian period by regulating the proteasome-dependent degradation of the central circadian oscillator, TIMING OF CAB EXPRESSION 1.</li> <li>c) Overexpression of LKP2 enhances drought tolerance.</li> <li>d) Overexpression of LOV KELCH</li> </ol>	(Greenham & McClung, 2015; Franklin <i>et al.</i> , 2014)

		<p>PROTEIN 2 (LKP2), a homolog of ZEITLUPE (ZTL) and FKF1, confers drought stress tolerance due to activation of DREB1 genes.</p> <p>e) LKP2 degrades PRR5 protein redundantly with ZTL and FKF1 to confer drought stress tolerance through degradation of Pseudo-Response Regulator 5 (PRR5).</p>	
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**Figure 1:** Distribution of the blue light photoreceptor gene family on Arabidopsis and tomato chromosomes. The chromosomal location image was generated by the Chromosome Map Tool (<https://www.arabidopsis.org/jsp/ChromosomeMap/tool.jsp>) and MapInspect tool



**Figure 2:** 3D structure of DNA Binding Domain (DBD) identified in tomato blue light photoreceptor genes. (A): PAS/LOV1 domain in interaction with its cofactor FMN. (B): Intermolecular (protein-ligand) interactions of PAS/LOV1 domain with FMN. (C): 3D structure of PHR/DNA Photolyase in complex with its cofactor FAD. (D): Intermolecular (protein-ligand) interactions of PHR/DNA Photolyase with FAD.