

**SÃO PAULO STATE UNIVERSITY – UNESP
JABOTICABAL CAMPUS**

**THE ROLE OF CRYPTOCHROME 1A IN LONG-DISTANCE
SIGNALING OF WATER DEFICIT IN TOMATO**

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SIGNALING OF WATER DEFICIT IN TOMATO**

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TÍTULO DA TESE: THE ROLE OF CRYPTOCHROME 1A IN LONG-DISTANCE SIGNALING OF WATER DEFICIT IN TOMATO

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“Education is the key to unlock the golden door
of freedom.”

George Washington Carver

DEDICATION

I dedicate this work to my family, especially my parents Maria Ângela Dalalana D'Amico and Valdeci Aparecido Damião, for all the care and support during my walk.

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SUMMARY

	Page
ABSTRACT	iv
CHAPTER 1 – General considerations: Cryptochrome-related abiotic stress responses in plants	1
1. INTRODUCTION	1
2. LITERATURE REVIEW	6
2.1. Osmotic stress acclimation: How do cryptochromes regulate this response? ...	6
2.1.1. Drought stress.....	6
2.1.2. Salt stress	7
2.2. Blue light mediates light stress responses through cryptochromes	8
2.3. What can cryptochromes reveal about other plant stresses?	10
2.3.1. Temperature stress	11
2.4. Epilogue	11
3. REFERENCES	14
CHAPTER 2 – Cryptochrome 1a influences source-sink partitioning during diferente stages of growth in tomato	22
ABSTRACT	22
1. INTRODUCTION	23
2. MATERIALS AND METHODS.....	25
2.1. Plant material and growth conditions	25
2.2. Determination of vegetative parameters	25
2.2.1. Growth analysis	25
2.2.2. Pigment content.....	26
2.3. Morphometric analysis of fruit parameters	26
2.4. Data analysis.....	26
3. RESULTS.....	27
3.1. Vegetative parameters	27
3.1.1. Growth analysis	27
3.1.2. Pigment content.....	27
3.2. Morphometric analysis of fruit parameters	29
4. DISCUSSION	30
5. CONCLUSIONS	35

6. REFERENCES.....	36
CHAPTER 3 – Cryptochrome 1a depends on blue light fluence rate to mediate osmotic stress responses in tomato	39
ABSTRACT	39
1. INTRODUCTION.....	40
2. MATERIALS AND METHODS.....	44
2.1. Plant material, growth conditions and light sources	44
2.2. Growth analysis.....	45
2.3. Cotyledon and hypocotyl pigment analyses	45
2.4. Lipid peroxidation and H ₂ O ₂ formation	46
2.5. Compatible osmolyte contents	47
2.6. Statistical analyses.....	47
3. RESULTS.....	49
3.1. The tomato <i>cry1a</i> mutant inhibits growth under osmotic stress conditions	49
3.2. Cotyledon pigmentation is altered in <i>cry1a</i> seedlings under osmotic stress ...	51
3.3. Biochemical responses to osmotic stress are differentially changed in <i>cry1a</i> seedlings.....	52
3.4. The growth of <i>cry1a</i> seedlings under osmotic stress is affected in response to blue light fluence rate	55
3.5. Pigmentation of <i>cry1a</i> seedlings under osmotic stress are dependent on the blue light fluence rate	57
3.6. Blue light-dependent biochemical changes in response to osmotic stress are altered in <i>cry1a</i> mutant.....	60
4. DISCUSSION	62
4.1. <i>cry1a</i> depends on blue light fluence to modulate tomato photomorphogenesis	62
4.2. Blue light fluence signals <i>cry1a</i> to alleviate osmotic stress in tomato	64
5. CONCLUSIONS	68
6. REFERENCES.....	70
CHAPTER 4 – Cryptochrome 1a of tomato mediates long-distance signaling of soil water deficit	78
ABSTRACT	78
1. INTRODUCTION.....	79
2. MATERIALS AND METHODS.....	82

2.1. Plant material, growth condition and grafting technique	82
2.2. Water deficit treatment	82
2.3. Growth analysis.....	83
2.4. Leaf pigments content.....	83
2.5. Lipid peroxidation and H ₂ O ₂ content	83
2.6. Leaf relative water content	84
2.7. Stomatal measurements	84
2.8. Leaf gas exchange	84
2.9. Data analyses	85
3. RESULTS	86
3.1. Response of own-rooted tomato plants to water deficit.....	86
3.2. Responses of reciprocally grafted plants to water deficit	92
3.3. Correlations between plant variables	99
4. DISCUSSION	100
4.1. cry1a photoreceptor positively regulates tomato growth	100
4.2. Importance of tomato cry1a in regulating long-distance-signaling of water deficit.....	103
5. CONCLUSIONS	106
6. REFERENCES	108
APPENDICES	113
Appendix A. Supplementary material for chapter 3	114
Appendix B. Supplementary material for chapter 4	121

THE ROLE OF CRYPTOCHROME 1A IN LONG-DISTANCE SIGNALING OF WATER DEFICIT IN TOMATO

ABSTRACT – It is well known that light is a crucial environmental factor that has a fundamental role in plant growth and development from seed germination to fruiting. For this process, plants contain versatile and multifaceted photoreceptor systems to sense variations in the light spectrum and to acclimate to a range of ambient conditions. Five main groups of photoreceptors have been found in higher plants, cryptochromes, phototropins, UVR8, zeaxanthins and phytochromes, but the last one red/far red wavelengths photoreceptor is the most characterized. Among the many responses modulated by phytochromes, these molecules play an important role in biotic and abiotic stress responses, which is one of the most active research topics in plant biology, especially their effect on agronomic traits. However, regarding the light spectrum, it is not surprising to consider that other photoreceptors are also part of the stress response modulated by light. In fact, it has become increasingly evident that cryptochromes, which mainly absorb in the blue light region, also act as key regulators of a range of plant stress responses, such as drought, salinity, heat and high radiation. However, this information is rarely evidenced in photomorphogenetic studies. Therefore, the scope of the chapter 1 is (i) to compile and discuss the evidence on the abiotic stress responses in plants that are modulated by cryptochromes. In addition, chapters 2, 3 and 4 refer to the respective manuscripts: (ii) physiological characterization of the tomato cry1a mutant; (iii) characterization of tomato cry1a mutant seedlings under osmotic stress and different rates of blue light fluencies; and (iv) the study of plant water stress responses using the photomorphogenetic mutant cry1a and grafting to understand the role of this photoreceptor in long-distance signaling.

Keywords: blue-light fluence, cryptochrome 1a, osmotic stress, root-shoot signaling, *Solanum lycopersicum* L., water deficit

CHAPTER 1 – General considerations: Cryptochrome-related abiotic stress responses in plants

1. INTRODUCTION

Sunlight is the most essential environmental signal for plant growth and development. Interestingly, its influence is not restricted to the photosynthesis process because light also plays a key role in transferring essential information, such as quality, quantity, periodicity and direction, that ensures plant survival under environmental fluctuations (Kami et al., 2010; Demarsy et al., 2018). For this reason, photomorphogenesis has been well elucidated by modulating a plethora of plant processes from seed germination to fruiting (Kendrick and Kronenberg, 1994; Kami et al., 2010). In plants, several of these photomorphogenic responses are largely governed by the visible light spectrum (400–700 nm). In fact, variations in light are sensed by photoreceptors, which are photoreversible proteins with a prosthetic group/cofactor or an intrinsic amino acid (tryptophan) as a chromophore. Currently, five photoreceptor systems have been identified in higher plants: phytochromes (phys), which absorb wavelengths of red (600–700 nm) and far-red light (700–750 nm); blue light (BL)/ultraviolet (UV)-A photoreceptors (315–500 nm), which are represented by cryptochromes (crys), phototropins (phot) and zeitlupes (ZTL, FKF1 and LKP2); and UV Resistance Locus 8 (UVR8) photoreceptor, which operate through UV-B light (280–315 nm) (Chen and Chory, 2011; Christie et al., 2012; Ito et al., 2012; Christie et al., 2015; Jenkins, 2014; Mishra and Khurana, 2017).

Although there are several classes of plant photoreceptors, phytochromes were the first type of photoreceptor discovered and are the most characterized. These molecules are dimeric apoproteins (~ 130 KDa) covalently linked to phytochromobilin, which is a linear tetrapyrrole that functions as a chromophore. The interconvertible forms of phytochromes allow their activation via the absorption of red light, as well as their inactivation via the absorption of far-red light (Chen and Chory, 2011). Thus, this sensor triggers the light-dependent signal transduction cascade to regulate the expression of numerous genes that result in specific physiological responses (Viczián

et al., 2017). Furthermore, various reports have shown that the plant signaling pathways involved in the responses to abiotic and biotic stresses, including insect herbivory, salinity, drought, hot or cold temperatures, and UV-B radiation, are modulated by phytochromes (Donohue et al., 2008; Ballaré, 2009; Carvalho et al., 2011; D'Amico-Damião et al., 2015; Gavassi et al., 2017). However, these responses remain unclear due to the complex light signaling pathways that are operated by other photoreceptors along the light spectrum (Kami et al., 2010; Fiorucci and Fankhauser, 2017; Demarsy et al., 2018). Thus, regarding the intricate photomorphogenic organization, it is important to remember that, in addition to phytochromes, other molecules, such as crys, have also increasingly received attention with regard to light signaling.

Increasingly, crys have been shown to modulate plant responses from germination to fruiting, especially through wavelengths of blue, green and UV-A light (Cashmore et al., 1999; Chaves et al., 2011). Crys are present from bacteria to humans, in plants, these photoreceptors are basically an apoprotein with two prosthetic groups (Figure 1A), the N-terminal photolyase homology-related (PHR) domain and a cryptochrome C-terminal extension (CCE) domain. The PHR domain contains the noncovalent binding site for 5, 10-methenyl tetrahydrofolate (MTHF) and flavin adenine dinucleotide (FAD), the two chromophores. FAD is a chromophore for BL that triggers photomorphogenesis, and the MTHF chromophore is a derivative of pterine that acts in the UV-A region, transferring excitation energy to FAD, which is a catalytic cofactor (Mishra and Khurana, 2017). In the model plant *Arabidopsis thaliana*, three crys were identified (cry1, cry2 and cry3). cry1 and cry2 are located predominantly in the nucleus (Cashmore et al., 1999; Kleiner et al., 1999) and play a multifaceted role in various aspects of plant growth and development. For instance, cry1 primarily regulates photomorphogenic responses related to the inhibition of hypocotyl elongation, anthocyanin accumulation and cotyledon expansion, while cry2 plays a role in the hypocotyl inhibition, circadian clock and photoperiod-dependent flowering (Yu et al., 2010). However, cry3 is a DASH protein located in chloroplasts and mitochondria (Kleine et al., 2003), which works to repair UV-damaged DNA in a light-dependent manner (Mishra and Khurana, 2017). Overall, cryptochrome-dependent signaling

pathways remain unclear because these physiological responses change with BL intensity (Kami et al., 2010) as well as with the plant species in question (Yang et al., 2017).

In addition to the variety of mechanisms orchestrated by crys, the modes of action by which these extraordinary photoreceptors work are very dynamic. For example, recent studies have revealed the role of crys in BL-dependent biosynthesis of reactive oxygen species (ROS) (Consentino et al., 2015; Jourdan et al., 2015; El-Esawi et al., 2017), which is a key signaling pathway for genes, such as those related to biotic and abiotic stresses, that have ROS-dependent transcription (Suzuki and Katano, 2018). In fact, these new findings contribute to the already established knowledge regarding the acclimation responses to biotic and abiotic stress mediated by crys, such as drought, salinity, heat, freezing, high radiation, UV-B light and pathogen attack (Mao et al., 2005; Catalá et al., 2011; Ma et al., 2016; Zhou et al., 2017; Demarsy et al., 2018; Zhou et al., 2018). However, although this information is mixed throughout few research studies already performed in the literature, here we discuss the evidence on cryptochrome-controlled plant acclimation responses under stressful environments and how these responses may occur (Table 1). Furthermore, the present review has compiled the most classic and current advances inherent to cry defense mechanisms against abiotic stress conditions.

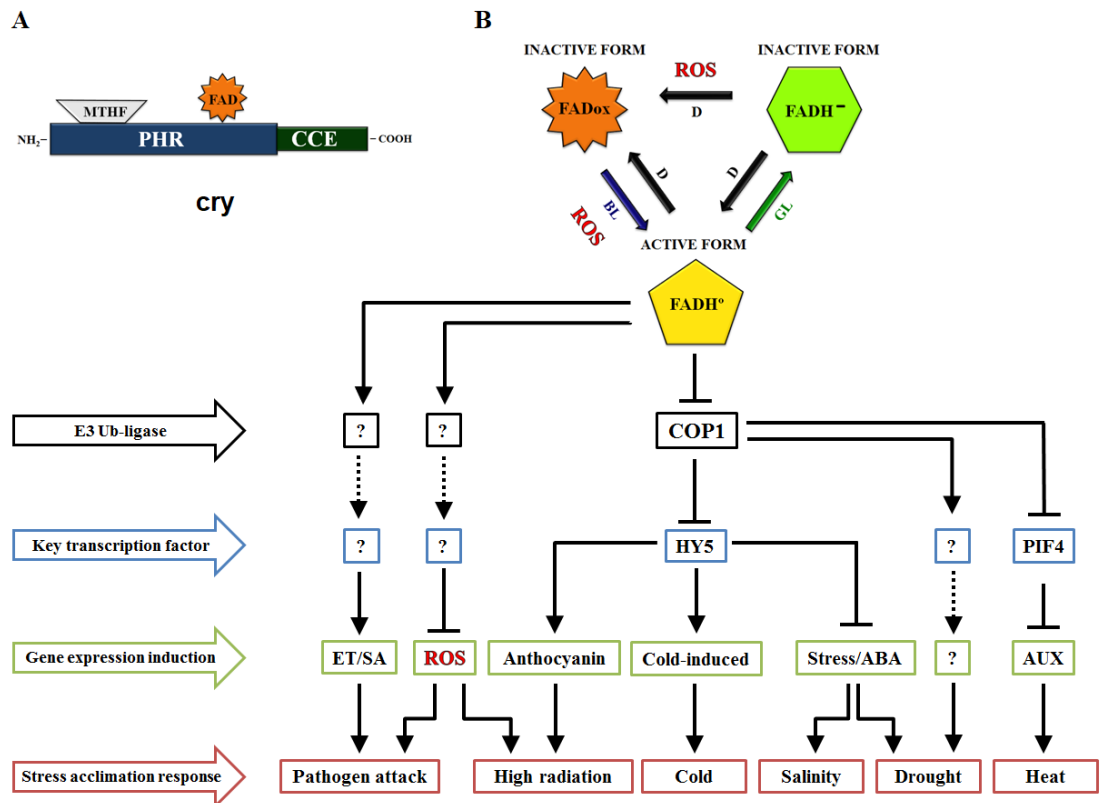


Figure 1. Schematic view of cryptochromes (cry) role in stress acclimation responses. (A) Domain structure of cry consists of Photolyase-Homologous Region (PHR) domain and CRY C-terminal extension (CCE) domain. The cry chromophores methenyltetrahydrofolate (MTHF) and FAD are binding to PHR, a light perception domain. (B) FAD chromophore redox states of cry. FAD is oxidized (FADox) in the darkness (D), with the absorption peak in blue light (BL). On light exposure, FADox changes to the neutral semireduced state (FADH⁰), which allows the biological activity of cry in the plant cell. With the absorption peak in green light (GL), FADH⁰ is induced to change to the totally reduced form (FAD⁻). An unclear subject around the reoxidation of FAD is the production of reactive oxygen species (ROS) during the process. Stress signaling pathway regulated by cryptochromes. From the blue light signaling, cry coordinate the negative regulation of COP1-dependent degradation of HY5 and PIF4 transcription factors, triggering changes in stress response target genes expression. Note that these are key pathways in the induction of genes related to signaling and/or biosynthesis of hormones, ROS and stress components. Although the complex signaling of cry in stress acclimation still involves some unknown components, certainly this photoreceptor play a crucial role in BL-dependent stress responses. Arrows means upregulation, T-bars indicate downregulation and dotted lines indicate unknown (?) signaling routes. E3 ubiquitin-ligase; COP1, constitutive photomorphogenic 1; HY5, long hypocotyl 5; PIF4, phytochrome interacting factor 4; ET, ethylene; SA, salicylic acid; ABA, abscisic acid; AUX, auxin.

Table 1. General description of stress responses controlled by cryptochromes in plants.

Species	Type of stress	Cryptochrome	Stress response	Reference
<i>Arabidopsis thaliana</i>	Drought	cry1, cry2	COP1, a repressor of stomatal opening is downregulated by crys	Mao et al., 2005
	Drought and Salinity	cry1, cry2	Cryptochromes-induced altered expression of stress/ABA-responsive genes	Xu et al., 2009; Xu and Ma, 2009
	Salinity	cry1	Overexpression of <i>SbCRY1a</i> confers oversensitive to salt stress	Zhou et al., 2018
	High-light	cry1, cry2	Cryptochromes in affects chloroplast light-harvesting complex and redox equilibrium of photosynthetic apparatus	Walters et al., 1999; Weston et al., 2000
			Cryptochromes promotes the transcription of ROS-responsive genes	Chen et al., 2013
		cry1	Singlet oxygen-related programmed cell death is coordinated by cry1	Danon et al., 2006
		cry1, cry2	SIG5 modulates chloroplast transcripts in a cryptochromes-dependent manner	Belbin et al., 2017
		cry1	Cry1 promote the photoprotective genes expression under excessive radiation	Kleine et al., 2007; Shaikhali et al., 2012
	Heat	cry1	PIF4 interact with cry1 controlling hypocotyl elongation under high temperature	Ma et al., 2016
	Cold	-	Cryptochromes share transcription factors with cold acclimatization mechanisms	Catalá et al., 2011
	Biotic	cry1	Cry1 play a role in plant defense against pathogen attack	Zhou et al., 2017
<i>Brassica napus</i>	Drought and Salinity	cry1	Several osmotic stress responsive genes are downregulated by cry1	Sharma et al., 2014
<i>Solanum lycopersicum</i>	High-light	cry1, cry2	Cryptochromes modulates a classic high-light stress response, the anthocyanin biosynthesis	Giliberto et al., 2005; Liu et al., 2018b

2. LITERATURE REVIEW

2.1. Osmotic stress acclimation: How do cryptochromes regulate this response?

2.1.1. Drought stress

Drought is one of the most critical factors of plant production in agricultural systems (Lesk et al., 2016; González-Villagra et al., 2017; Sseremba et al., 2018). Given the evidence regarding the mechanisms that control plant responses to drought stress (Bechtold, 2018; Hussain et al., 2018; Meng, 2018), we would not be surprised that crys are an important part of these responses (Mishra and Khurana, 2017). Previous studies have demonstrated that crys play an interesting role in Arabidopsis drought stress tolerance (Mao et al., 2005). The authors found that Arabidopsis double mutant *cry1cry2* plants were clearly more drought-tolerant than the wild type (WT) after irrigation was suspended for 1 week. However, transgenic *CRY1-ovx* plants, which overexpress the CRY1 protein, exhibited excessive water loss, which was a response mainly associated with the enhanced stomatal aperture of the transgenic plants. This response seems to be strongly related to crys, particularly through their interaction with the downstream signaling protein CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (Kang et al., 2009); COP1 represses stomatal opening (Delgado et al., 2012). However, the mechanisms by which crys modulate water loss under drought stress are still very unclear because this event is dependent on many factors, including hormones, mainly abscisic acid (ABA) (Miller et al., 2010; Saradadevi et al., 2017), and interactions with other BL photoreceptors, such as phototropins (Mao et al., 2005).

Regarding ABA, some evidence shows that this hormone is part of cry signaling during water stress. For example, transgenic lines of Arabidopsis overexpressing *TaCRY1a* and *TaCRY2* from monocot wheat (*Triticum aestivum*) exhibited a lower tolerance than did WT lines to osmotic stress (300 mM mannitol) and an ABA exogenous treatment (0.3 or 10 μ M ABA) throughout germination and postgermination development (Xu et al., 2009). Furthermore, these authors also showed the differential expression of *responsive to desiccation 29A* (*RD29A*) and *alcohol dehydrogenase 1*

(*ADH1*), two ABA/stress-responsive genes, in the transgenic lines. In fact, the plants overexpressing *TaCRY1a* showed a strong reduction in *RD29A* expression and a lower induction of *ADH1* under osmotic stress in comparison to plants overexpressing *TaCRY2*; the plants overexpressing *CRY1a* were also more sensitive to osmotic stress. In addition, *NAD(P)-binding Rossmann-fold superfamily protein (ABA2)*, which is a gene involved in adjusting ABA biosynthesis, was downregulated in *TaCRY1a-GFP* transgenic lines under ABA treatment and was downregulated in either *TaCRY1a-GFP* or *TaCRY2-GFP* transgenic lines under mannitol treatment, showing how crys coordinate molecular responses under drought via interactions with this stress hormone (Xu and Ma, 2009).

Although crys have been widely investigated in the *Arabidopsis* plant model (Liu et al., 2018a), recent molecular evidence in *Brassica napus*, an agriculturally important crop, has shown an interaction between cry1 and drought stress-related genes (Sharma et al., 2014). Moreover, *Brassica napus* cry1 overexpression (*OE-BnCRY1*) resulted in plants that were very sensitive to stress induced by a low osmotic potential (mannitol) when compared to WT, whereas the plants that were transformed with the antisense for cry1 (*AS-BnCRY1*) were more tolerant. Interestingly, cry1 overexpression reduced the transcript levels of genes related to the responses induced by water deficits (e.g., *late embryogenesis abundant protein 4-1*, *LEA4-1*; *dehydrin family protein*, *RAB18*; *nitrate reductase 1*, *NIA1*; *ascorbate peroxidase 1*, *APX1*; and *NAC domain containing protein 60*, *NAC060*). Indeed, these observations show us the involvement of crys in the drought stress acclimation responses. However, several questions, such as those related to cry stress response cascades and crosstalk mechanisms in many plant species under water deficit conditions, illuminate a new path for future research. Thus, molecular manipulation of plant crys or their signaling components, through the photoreceptor engineering, could potentially play a key role in improving drought tolerance traits.

2.1.2. Salt stress

In addition to the fact that drought and salt stress can share a common signaling pathway mainly due to their osmotic effects (Chaves et al., 2009), it was also shown that crys are part of the salt stress response via an ionic effect. Xu et al. (2009) observed that transgenic lines of *Arabidopsis* overexpressing *TaCRY1a* and *TaCRY2* (from *Triticum aestivum*) were more highly sensitive to high salt stress (120 mM NaCl) when compared with the watered control. Although salt stress impaired WT germination, this effect was more harmful to the transgenic lines than to the WT. However, the plants overexpressing *TaCRY1a* were more salt-sensitive than the plants overexpressing *TaCRY2*. In addition, real-time PCR revealed a remarkable increase in *TaCRY2* transcripts in *Arabidopsis* roots treated with salt stress (250 mM NaCl) after 12 h of stress induction for a 28 h period. Interestingly, the authors found that transcription of *TaCRY1a* was induced by salt stress after 24 h of exposure to the stressor agent.

An additional molecular event that supports the role of crys in salt stress responses comes from the overexpression of the *SbCRY1a* gene of sweet sorghum (*Sorghum bicolor*) in *Arabidopsis* under salinity conditions, in which a wide range of stress-responsive genes were modified (Zhou et al., 2018). Notably, the *cry1* mutant had a greater germination and seedling survival rate than the WT plants in response to ABA and salt conditions, showing more tolerance to salinity. On the other hand, the transgenic line *SbCRY1a* was oversensitive to salt stress. These mechanisms are likely regulated through the “LONG HYPOCOTYL 5-ABA INSENSITIVE 5” (HY5-ABI5) regulon, which triggers the differentiated expression of ABA/stress-responsive transcripts (*RD29A* and *RD22*), as speculated by the authors. Indeed, *cry1* has been shown to control salt stress responses by ABA-dependent signaling pathways (Sharma et al., 2014). Obviously, these results allow researchers to provide more evidence on how cry signal transduction affects plant acclimation in salinity environments.

2.2. Blue light mediates light stress responses through cryptochromes

The metabolic adjustment of plants in stressful environments of excess or limited light depends on complex mechanisms that are already well established

(Demmig-Adams and Adams III, 1992; Demarsy et al., 2018). For instance, responsive molecules have important roles in plant photoprotection against high radiation, including anthocyanin accumulation (Petrussa et al., 2013). Notably, BL modulation of anthocyanin accumulation in *Arabidopsis* seedlings is cry-dependent because *cry1* C-terminal mutant alleles are known to have altered anthocyanin levels (El-Esawi et al., 2017). In addition, leaf anthocyanin accumulation increased with the overexpression of *CRY1a* in tomato (*Solanum lycopersicum* L.) (Liu et al., 2018b). These authors showed that anthocyanin accumulation was related to variations in the mRNA and protein levels of the LONG HYPOCOTYL 5 (HY5) transcription factor, which interacts with the promoters of genes for anthocyanin biosynthesis (*dihydroflavonol 4-reductase*, *chalcone synthase 1* and *2*) (Figure 1B). Similarly, it was also reported that *CRY2* overexpression (*OE-LeCRY2*) in seedlings and leaves of tomato resulted in plants with an abundant accumulation of anthocyanins (Giliberto et al., 2005), indicating the pivotal role of crys on anthocyanin accumulation, even though light stress was not directly associated with the pigments in the above works.

Nevertheless, numerous studies have highlighted the participation of crys in other high light acclimation responses, such as the regulation of redox equilibrium of photosynthetic electron transport chain under high light stress (Walters et al., 1999). Moreover, Weston et al. (2000) observed that crys modulate key photoprotective components, even the chlorophyll a/b ratio and light-harvesting complex of photosystem II (LHCB), in high white light. WT *Arabidopsis* and its background *cry1* and *cry2* mutants and *cry1cry2* double mutant were submitted to continuous white light under a high ($600 \mu\text{mol m}^{-2} \text{s}^{-1}$) or low ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) intensity. In this context, the double mutant *cry1cry2* showed a strong decrease in LHCB accumulation and in the chlorophyll a/b ratio. Currently, strong evidence shows that crys can be part of the response to a light-induced redox imbalance through their interaction with the oxidative stress system (Danon et al., 2006) and specifically through their function in ROS production (Consentino et al., 2015), including the modifications of transcript levels of *ascorbate peroxidase 2* (*APX2*), *salt tolerance zinc finger* (*ZAT10*), sigma factor binding protein 1 (*SIB1*), *ethylene responsive element binding factor 4* (*ERF4*), and *NAD(P)H dehydrogenase B2* (*NDB2*) in *Arabidopsis* (Chen et al., 2013) (Figure 1B).

More recently, it was shown that the circadian regulation of sigma factor SIG5 transcription, which is a part of the chloroplast signaling pathway in response to light stress adaptation, is predominantly dependent on BL and crys (Belbin et al., 2017). Certainly, nuclear gene expression is severely damaged when light intensities are greater than the maximum potential of the chloroplast electron capacity (Demarsy et al., 2018; Foyer, 2018). In relation to this evidence, Shaikhali et al. (2012) identified two zinc finger GATA-type transcription factors as essential regulators of the photoprotective mechanisms orchestrated by crys. Through cry1 coordination, “ZINC FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM LIKE1” (ZML1) and “ZML2” play a fundamental role in ROS scavenger responses to excess light. In another way, some discussions have been considered about ROS biosynthesis during crys inactivation (Consentino et al., 2015 Jourdan et al., 2015; El-Esawi et al., 2017). However, the link between the ROS produced under light and dark conditions merits further investigation (Figure 1B).

Regarding specific short-wavelength light signaling, such as UV-B, it has also been speculated that crys are part of these responses (Fortunato et al., 2015). In general, it is well known that UV-B light causes photodamage to DNA polymers and affects the electron balance in photosystem II (Czégény et al., 2016; Dotto and Casati, 2017), and it has been shown that cry-DASH homologues participate in restoring the photosynthetic efficiency of the photosystem II reaction center complexes in photosynthetic organisms (Vass et al., 2014). However, it is still hard to understand the mechanisms in which crys are part of high light stress response because a large system of photoprotective mechanisms is triggered under this condition, besides the fact that BL induces UV-B stress tolerance (Adamse et al., 1994; Hoffmann et al., 2015), and numerous high light-responsive genes are modulated by cry1 in *Arabidopsis* (Kleine et al., 2007). Therefore, there are potential opportunities for future studies about how cry signal transduction is involved in light stress responses to ambient fluctuations.

2.3. What can cryptochromes reveal about other plant stresses?

2.3.1. Temperature stress

Temperature stress triggers a large amount of metabolic damage in plants with well-known effects on protein stability and enzymatic reactions (Szymańska et al., 2017). In addition, the changes between the regulatory mechanisms of ROS and multiple pathways under heat stress have been recently reviewed, showing that downstream heat shock protein (HSP) is largely associated with these responses (Chen et al., 2018; Suzuki and Katano, 2018). In view of this, the HSP transcription profile is strongly regulated by crys (Yang et al., 2008; Facella et al., 2008), allowing a new perspectives on this BL photoreceptor in high temperature responses. A previous study demonstrated that cry1 repressed auxin biosynthesis to acclimate to heat stress, resulting in morphological changes in *Arabidopsis* seedlings (Ma et al., 2016). In addition, cry1 interacted with promoters such as *flavin-binding monooxygenase family protein* (YUC8), *indole-3-acetic acid inducible 19* (IAA19), and *indole-3-acetic acid inducible 29* (IAA29) under high temperature in a PHYTOCHROME-INTERACTING FACTOR 4 (PIF4)-dependent manner, which is an important part of BL-dependent signal transduction. On the other hand, the mechanisms of plant acclimation to cold stress in *Arabidopsis* include HY5, COP1 and Z-box, which are involved in these responses (Catalá et al., 2011); Z-box is a regulatory cis-element located in the promoter of responsive genes, such as HY5 transcription factor (Yadav et al., 2005). Coincidentally, these key regulators of light signaling are known to be mediated by crys through various interactions with other photoreceptors and signaling molecules (Mishra and Khurana, 2017), causing speculation that these BL photoreceptors have a role in a part of low temperature tolerance. However, as can be observed in this topic, temperature response modulated by crys in plants is still uncommon, but intensive research on this subject should be considered.

2.4. Epilogue

The role of crys in several events related to plant growth and development has been well established, including biotic stress response (Jeong et al., 2010; Wu and

Yang, 2010; Zhou et al., 2017). However, few papers consider the participation of these photoreceptors in one of the most scientifically explored topics, abiotic stress in plants. Although much still needs to be revealed and understood about the subject, this review aimed to address and discuss different insights in the key function of BL in the acclimation to abiotic stresses via cry regulation. Thus, in this paper, we focus on compiling the responses to abiotic stress mediated by crys, although biotic stress is an interesting matter that may need to be intensively addressed (Table 1). In fact, since large changes in agronomically desirable traits have been shown in mutants and transgenic lines of important crops (Mawphlang and Kharshiing, 2017), regarding stress tolerance, potential future perspectives could be generated for agriculture on the manipulation of this multifaceted photoreceptor. However, we are aware that the subject is complex because it involves the interaction of crys with other photoreceptors as well as with the signaling of other important stress-modulating molecules (Figure 1B), such as plant hormones (Casal, 2000; Warpeha and Montgomery, 2016; Xu et al., 2018). Therefore, we encourage new investigations that clarify the underlying mechanisms of cry-related stress responses in different plant species. Certainly, these efforts will contribute to revealing other crys functions in the acclimation to stressful environmental conditions. Overall, exploring the molecular mechanisms by which crys mediate abiotic stress responses are issues that deserve further investigation.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

VD and RC designed this review, wrote the manuscript and approved it for publication.

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3. REFERENCES

- Adamse P, Britz SJ, Caldwell CR (1994) Amelioration of UV-B damage under high irradiance. II: role of blue light photoreceptors. **Photochemistry and Photobiology** 60:110–115. doi: 10.1111/j.1751-1097.1994.tb05075.x
- Ballaré CL (2009) Illuminated behaviour: phytochrome as a key regulator of light foraging and plant anti-herbivore defence. **Plant, Cell & Environment** 32:713–725. doi: 10.1111/j.1365-3040.2009.01958.x
- Bechtold U (2018) Plant life in extreme environments: how do you improve drought tolerance? **Frontiers in Plant Science** 9:543. doi: 10.3389/fpls.2018.00543
- Belbin FE, Noordally ZB, Wetherill SJ, Atkins KA, Franklin KA, Dodd AN (2017) Integration of light and circadian signals that regulate chloroplast transcription by a nuclear-encoded sigma factor. **New Phytologist** 213:727–738. doi: 10.1111/nph.14176
- Carvalho RF, Campos ML, Azevedo RA (2011) The role of phytochromes in stress tolerance. In: Ahmad P, Azooz MM, Prasad MNV (Eds.) **Salt Stress in Plants**. New York: Springer, p. 283–299. doi: 10.1007/978-1-4614-6108-1_12
- Casal JJ (2000) Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. **Photochemistry and Photobiology** 71:1–11. doi: 10.1562/0031-8655(2000)0710001PCPPII2.0.CO2
- Cashmore AR, Jarillo JA, Wu YJ, Liu D (1999) Cryptochromes: blue light receptors for plants and animals. **Science** 284:760–765. doi: 10.1126/science.284.5415.760
- Catalá R, Medina J, Salinas J (2011) Integration of low temperature and light signaling during cold acclimation response in *Arabidopsis*. **Proceedings of the National Academy of Sciences, USA** 108:16475–16480. doi: 10.1073/pnas.1107161108
- Chaves I, Pokorny R, Byrdin M, Hoang N, Ritz T, Brettel K, Essen LO, van der Horst GTJ, Batschauer A, Ahmad M (2011) The cryptochromes: blue light photoreceptors in plants and animals. **Annual Review of Plant Biology** 62:335–364. doi: 10.1146/annurev-arplant-042110-103759
- Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. **Annals of Botany** 103:551–560. doi: 10.1093/aob/mcn125

- Chen B, Feder ME, Kang L (2018) Evolution of heat-shock protein expression underlying adaptive responses to environmental stress. **Molecular Ecology** 27:3040–3054. doi: 10.1111/mec.14769
- Chen D, Xu G, Tang W, Jing Y, Ji Q, Fei Z, Lin R (2013) Antagonistic basic helix-loop-helix/bZIP transcription factors form transcriptional modules that integrate light and reactive oxygen species signaling in *Arabidopsis*. **The Plant Cell** 25:1657–1673. doi: 10.1105/tpc.112.104869
- Chen M, Chory J (2011) Phytochrome signaling mechanisms and the control of plant development. **Trends in Cell Biology** 21:664–671. doi: 10.1016/j.tcb.2011.07.002
- Christie JM, Arvai AS, Baxter KJ, Heilmann M, Pratt AJ, O'Hara A, Kelly SM, Hothorn M, Smith BO, Hitomi K, Jenkins GI, Getzoff ED (2012) Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges. **Science** 335:1492–1496. doi: 10.1126/science.1218091
- Christie JM, Blackwood L, Petersen J, Sullivan S (2015) Plant flavoprotein photoreceptors. **Plant and Cell Physiology** 56:401–413. doi: 10.1093/pcp/pcu196
- Consentino L, Lambert S, Martino C, Jourdan N, Bouchet PE, Witczak J, Castello P, El-Esawi M, Corbineau F, d'Harlingue A, Ahmad M (2015) Blue-light dependent reactive oxygen species formation by *Arabidopsis* cryptochrome may define a novel evolutionarily conserved signaling mechanism. **New Phytologist** 206:1450–1462. doi: 10.1111/nph.13341
- Czégény G, Máta A, Hideg É (2016) UV-B effects on leaves—Oxidative stress and acclimation in controlled environments. **Plant Science** 248:57–63. doi: 10.1016/j.plantsci.2016.04.013
- D'Amico-Damião V, Cruz FJR, Gavassi MA, Santos DMM, Melo HC, Carvalho RF (2015) Photomorphogenic modulation of water stress in tomato (*Solanum lycopersicum* L.): the role of phytochromes A, B1, and B2. **The Journal of Horticultural Science and Biotechnology** 90:25–30. doi: 10.1080/14620316.2015.11513149
- Danon A, Coll NS, Apel K (2006) Cryptochrome-1-dependent execution of programmed cell death induced by singlet oxygen in *Arabidopsis thaliana*. **Proceedings of the National Academy of Sciences, USA** 103:17036–17041. doi: 10.1073/pnas.0608139103

Delgado D, Ballesteros I, Torres-Contreras J, Mena M, Fenoll C (2012) Dynamic analysis of epidermal cell divisions identifies specific roles for COP10 in *Arabidopsis* stomatal lineage development. **Planta** 236:447–461. doi: 10.1007/s00425-012-1617-y

Demarsy E, Goldschmidt-Clermont M, Ulm R (2018) Coping with ‘dark sides of the sun’ through photoreceptor signaling. **Trends in Plant Science** 23:260–271. doi: 10.1016/j.tplants.2017.11.007

Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress. **Annual Review of Plant Biology** 43:599–626. doi: 10.1146/annurev.pp.43.060192.003123

Donohue K, Heschel MS, Butler CM, Barua D, Sharrock RA, Whitelam GC, Chiang GC (2008) Diversification of phytochrome contributions to germination as a function of seed-maturation environment. **New Phytologist** 177:367–379. doi: 10.1111/j.1469-8137.2007.02281.x

Dotto M, Casati P (2017) Developmental reprogramming by UV-B radiation in plants. **Plant Science** 264:96–101. doi: 10.1016/j.plantsci.2017.09.006

El-Esawi M, Arthaut LD, Jourdan N, d’Harlingue A, Link J, Martino CF, Ahmad M (2017) Blue-light induced biosynthesis of ROS contributes to the signaling mechanism of *Arabidopsis* cryptochrome. **Scientific Reports** 7:13875. doi: 10.1038/s41598-017-13832-z

Facella P, Lopez L, Carbone F, Galbraith DW, Giuliano G, Perrotta G (2008) Diurnal and circadian rhythms in the tomato transcriptome and their modulation by cryptochrome photoreceptors. **PLoS ONE** 3:e2798. doi: 10.1371/journal.pone.0002798

Fiorucci AS, Fankhauser C (2017) Plant strategies for enhancing access to sunlight. **Current biology** 27:R931–R940. doi: 10.1016/j.cub.2017.05.085

Fortunato AE, Annunziata R, Jaubert M, Bouly JP, Falciatore A (2015) Dealing with light: the widespread and multitasking cryptochrome/photolyase family in photosynthetic organisms. **Journal of Plant Physiology** 172:42–54. doi: 10.1016/j.jplph.2014.06.011

Foyer CH (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. **Environmental and Experimental Botany** 154:134–142. doi: 10.1016/j.envexpbot.2018.05.003

Gavassi MA, Monteiro CC, Campos ML, Melo HC, Carvalho RF (2017) Phytochromes are key regulators of abiotic stress responses in tomato. **Scientia Horticulturae** 222:126–135. doi: 10.1016/j.scienta.2017.04.035

Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, Tavazza M, Giuliano G (2005) Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. **Plant Physiology** 137:199–208. doi: 10.1104/pp.104.051987

González-Villagra J, Kurepin LV, Reyes-Díaz MM (2017) Evaluating the involvement and interaction of abscisic acid and miRNA156 in the induction of anthocyanin biosynthesis in drought-stressed plants. **Planta** 246:299–312. doi: 10.1007/s00425-017-2711-y

Hoffmann AM, Noga G, Hunsche M (2015) High blue light improves acclimation and photosynthetic recovery of pepper plants exposed to UV stress. **Environmental and Experimental Botany** 109:254–263. doi: 10.1016/j.envexpbot.2014.06.017

Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L (2018) Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. **Frontiers in Plant Science** 9:393. doi: 10.3389/fpls.2018.00393

Ito S, Song YH, Imaizumi T (2012) LOV domain-containing f-box proteins: light-dependent protein degradation modules in Arabidopsis. **Molecular Plant** 5:47–56. doi: 10.1093/mp/sss013

Jenkins GI (2014) The UV-B photoreceptor UVR8: from structure to physiology. **The Plant Cell** 26:21–37. doi: 10.1105/tpc.113.119446

Jeong RD, Chandra-Shekara AC, Barman SR, Navarre D, Klessig DF, Kachroo A, Kachroo P (2010) Cryptochrome 2 and phototropin 2 regulate resistance protein-mediated viral defense by negatively regulating an E3 ubiquitin ligase. **Proceedings of the National Academy of Sciences, USA** 107:13538–13543. doi: 10.1073/pnas.1004529107

Jourdan N, Martino C, El-Esawi M, Witczak J, Bouchet PE, d'Harlingue A, Ahmad M (2015) Blue-light dependent ROS formation by *Arabidopsis* cryptochrome-2 may contribute toward its signaling role. **Plant Signaling & Behavior** 10:e1042647. doi: 10.1080/15592324.2015.1042647

Kami C, Lorrain S, Hornitschek P, Fankhauser C (2010) Light-regulated plant growth and development. In: Timmermans MCP (Ed.) **Current Topics in Developmental Biology**. San Diego: Elsevier Academic Press Inc, p. 29-66. doi: 10.1016/S0070-2153(10)91002-8

Kang CY, Lian HL, Wang FF, Huang JR, Yang HQ (2009) Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in *Arabidopsis*. **The Plant Cell** 21:2624–2641. doi: 10.1105/tpc.109.069765

Kendrick RE, Kronenberg GHM (Eds.) (1994) Photomorphogenesis in Plants. Dordrecht: Kluwer Academic Publishers, 864p. doi: 10.1007/978-94-011-1884-2

Kleine T, Kindgren P, Benedict C, Hendrickson L, Strand Å (2007) Genome-wide gene expression analysis reveals a critical role for CRYPTOCHROME1 in the response of *Arabidopsis* to high irradiance. **Plant Physiology** 144:1391–1406. doi: 10.1104/pp.107.098293

Kleine T, Lockhart P, Batschauer A (2003) An *Arabidopsis* protein closely related to *Synechocystis* cryptochrome is targeted to organelles. **The Plant Journal** 35:93–103. doi: 10.1046/j.1365-313X.2003.01787.x

Kleiner O, Kircher S, Harter K, Batschauer A (1999) Nuclear localization of the *Arabidopsis* blue light receptor cryptochrome 2. **The Plant Journal** 19:289–296. doi: 10.1046/j.1365-313X.1999.00535.x

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. **Nature** 529:84–87. doi: 10.1038/nature16467

Liu CC, Ahammed GJ, Wang GT, Xu CJ, Chen KS, Zhou YH, Yu JQ (2018a) Tomato CRY1a plays a critical role in the regulation of phytohormone homeostasis, plant development, and carotenoid metabolism in fruits. **Plant, Cell & Environment** 41:354–366. doi: 10.1111/pce.13092

Liu CC, Chi C, Jin LJ, Zhu J, Yu JQ, Zhou YH (2018b) The bZip transcription factor HY5 mediates CRY1a-induced anthocyanin biosynthesis in tomato. **Plant, Cell & Environment** 41:1762–1775. doi: 10.1111/pce.13171

Ma D, Li X, Guo Y, Chu J, Fang S, Yan C, Noel JP, Liu H (2016) Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. **Proceedings of the National Academy of Sciences, USA** 113:224–229. doi: 10.1073/pnas.1511437113

Mao J, Zhang YC, Sang Y, Li QH, Yang HQ (2005) A role for *Arabidopsis* cryptochromes and COP1 in the regulation of stomatal opening. **Proceedings of the National Academy of Sciences, USA** 102:12270–12275. doi: 10.1073/pnas.0501011102

Mawphlang OI, Kharshiing EV (2017) Photoreceptor mediated plant growth responses: implications for photoreceptor engineering toward improved performance in crops. **Frontiers in Plant Science** 8:1181. doi: 10.3389/fpls.2017.01181

Meng LS (2018) Compound synthesis or growth and development of roots/stomata regulate plant drought tolerance or water use efficiency/water uptake efficiency. **Journal of Agricultural and Food Chemistry** 66:3595–3604. doi: 10.1021/acs.jafc.7b05990

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. **Plant, Cell & Environment** 33:453–467. doi: 10.1111/j.1365-3040.2009.02041.x

Mishra S, Khurana JP (2017) Emerging roles and new paradigms in signaling mechanisms of plant cryptochromes. **Critical Reviews in Plant Sciences** 36:89–115. doi: 10.1080/07352689.2017.1348725

Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S, Vianello A (2013) Plant flavonoids—biosynthesis, transport and involvement in stress responses. **International Journal of Molecular Sciences** 14:14950–14973. doi: 10.3390/ijms140714950

Saradadevi R, Palta JA, Siddique KH (2017) ABA-mediated stomatal response in regulating water use during the development of terminal drought in wheat. **Frontiers in Plant Science** 8:1251. doi: 10.3389/fpls.2017.01251

Shaikhali J, Barajas-López J, Östväos K, Kremnev D, Sánchez Garcia A, Srivastava V, Wingsle G, Bako L, Strand Å (2012) The CRYPTOCHROME1-dependent response to excess light is mediated through the transcriptional activators ZINC FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM LIKE1 and ZML2 in *Arabidopsis*. **The Plant Cell** 24:3009–3025. doi: 10.1105/tpc.112.100099

Sharma P, Chatterjee M, Burman N, Khurana JP (2014) Cryptochrome 1 regulates growth and development in *Brassica* through alteration in the expression of genes involved in light, phytohormone and stress signalling. **Plant, Cell & Environment** 37:961–977. doi: 10.1111/pce.12212

Sseremba G, Tongoona P, Eleblu J, Danquah EY, Kizito EB (2018) Heritability of drought resistance in *Solanum aethiopicum* Shum group and combining ability of genotypes for drought tolerance and recovery. **Scientia Horticulturae** 240:213–220. doi: 10.1016/j.scienta.2018.06.028

Suzuki N, Katano K (2018) Coordination between ROS regulatory systems and other pathways under heat stress and pathogen attack. **Frontiers in Plant Science** 9:490. doi: 10.3389/fpls.2018.00490

Szymańska R, Ślesak I, Orzechowska A, Kruk J (2017) Physiological and biochemical responses to high light and temperature stress in plants. **Environmental and Experimental Botany** 139:165–177. doi: 10.1016/j.envexpbot.2017.05.002

Vass IZ, Kós PB, Knoppová J, Komenda J, Vass I (2014) The cry-DASH cryptochrome encoded by the *sll1629* gene in the cyanobacterium *Synechocystis* PCC 6803 is required for Photosystem II repair. **Journal of Photochemistry and Photobiology B: Biology** 130:318–326. doi: 10.1016/j.jphotobiol.2013.12.006

Viczián A, Klose C, Ádám É, Nagy F (2017) New insights of red light-induced development. **Plant, Cell & Environment** 40:2457–2468. doi: 10.1111/pce.12880

Walters RG, Rogers JJ, Shephard F, Horton P (1999) Acclimation of *Arabidopsis thaliana* to the light environment: the role of photoreceptors. **Planta** 209:517–527. doi: 10.1007/s004250050756

Warpeha KM, Montgomery BL (2016) Light and hormone interactions in the seed-to-seedling transition. **Environmental and Experimental Botany** 121:56–65. doi: 10.1016/j.envexpbot.2015.05.004

Weston E, Thorogood K, Vinti G, López-Juez E (2000) Light quantity controls leaf-cell and chloroplast development in *Arabidopsis thaliana* wild type and blue-light-perception mutants. **Planta** 211:807–815. doi: 10.1007/s004250000392

Wu L, Yang HQ (2010) CRYPTOCHROME 1 is implicated in promoting R protein-mediated plant resistance to *Pseudomonas syringae* in *Arabidopsis*. **Molecular Plant** 3:539–548. doi: 10.1093/mp/ssp107

Xu F, He S, Zhang J, Mao Z, Wang W, Li T, Hua J, Du S, Xu P, Li L, Lian H, Yang HQ (2018) Photoactivated CRY1 and phyB interact directly with AUX/IAA proteins to inhibit auxin signaling in *Arabidopsis*. **Molecular Plant** 11:523–541. doi: 10.1016/j.molp.2017.12.003

Xu P, Ma Z (2009) Plant cryptochromes employ complicated mechanisms for subcellular localization and are involved in pathways apart from photomorphogenesis. **Plant Signaling & Behavior** 4:200–201. doi: 10.4161/psb.4.3.7756

Xu P, Xiang Y, Zhu H, Xu H, Zhang Z, Zhang C, Zhang L, Ma Z (2009) Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. **Plant Physiology** 149:760–774. doi: 10.1104/pp.108.132217

Yang Y, Li Y, Li X, Guo X, Xiao X, Tang D, Liu X (2008) Comparative proteomics analysis of light responses in cryptochrome1-304 and Columbia wild-type 4 of *Arabidopsis thaliana*. **Acta Biochimica et Biophysica Sinica** 40:27–37. doi: 10.1111/j.1745-7270.2008.00367.x

Yadav V, Mallappa C, Gangappa SN, Bhatia S, Chattopadhyay S (2005) A basic helix-loop-helix transcription factor in Arabidopsis, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth. **The Plant Cell** 17:1953–1966. doi: 10.1105/tpc.105.032060

Yang Z, Liu B, Su J, Liao J, Lin C, Oka Y (2017) Cryptochromes orchestrate transcription regulation of diverse blue light responses in plants. **Photochemistry and Photobiology** 93:112–127. doi: 10.1111/php.12663

Yu X, Liu H, Klejnot J, Lin C (2010) The cryptochrome blue light receptors. **The Arabidopsis Book** 8:e0135. doi: 10.1199/tab.0135

Zhou T, Meng L, Ma Y, Liu Q, Zhang Y, Yang Z, Yang D, Bian M (2018) Overexpression of sweet sorghum cryptochrome 1a confers hypersensitivity to blue light, abscisic acid and salinity in Arabidopsis. **Plant Cell Reports** 37:251–264. doi: 10.1007/s00299-017-2227-8

Zhou X, Zhu T, Zhu LS, Luo SS, Deng XG, Lin HH, Xi DH (2017) The role of photoreceptors in response to cucumber mosaic virus in *Arabidopsis thaliana*. **Journal of Plant Growth Regulation** 36:257–270. doi: 10.1007/s00344-016-9635-9

CHAPTER 2 – Cryptochrome 1a influences source-sink partitioning during diferente stages of growth in tomato

ABSTRACT – Cryptochromes are photoreceptors that coordinate multiple processes during the plant life cycle through UV-A/blue light. In the present study, we used tomato *cry1a* mutant to understand how *LeCRY1a* influences the vegetative and reproductive development during different stages of growth. When compared to the wild type of tomato, the *cry1a* mutant showed greater plant height, number of leaves and leaf area during the life cycle. However, leaf pigment biosynthesis in *cry1a* was impaired compared with wild-type plants. Moreover, *cry1a* influenced the partitioning of photoassimilates between the shoot and root, mainly by positively influencing the initial root growth. Although the wild-type plants exhibited lower shoot and root biomass accumulation at the final stage of plant growth than did the mutant, the wild type exhibited higher yield, as evidenced by its greater number of ripe fruits per plant. The results reveal a complex role of *cry1a* throughout shoot and root growth and fruit development in tomato, causing this photoreceptor to stand out as target molecules for plant breeding programs.

Keywords: agronomic traits, blue-light photoreceptor, *cry1a* mutant, root-to-shoot development, *Solanum lycopersicum*

1. INTRODUCTION

Light regulates plant growth and development from seed germination to fruiting through a process known as photomorphogenesis. This phenomenon is strongly controlled by the quantity and quality of light reaching the plant (Kami et al., 2010; Mawphlang and Kharshiing, 2017). For this reason, higher plants developed a complex of light-absorbing photoreceptor proteins capable of perceiving variations in the quality and quantity of incident light. For example, for the red light spectrum, phytochromes are widely investigated photoreceptors that modulate several morphophysiological responses via their photoreversibility between red (600–700 nm) and far-red (700–750 nm) light absorption (Vicizian et al., 2017). During the plant life cycle, the phytochromes may control seed germination, seedling establishment, shade avoidance, leaf expansion and development, flowering, carbon assimilation and sugar metabolism (Eckstein et al., 2016; Kharshiing and Sinha, 2016; Yang et al., 2016; Mawphlang and Kharshiing, 2017). It has been shown in crops that phytochromes are important for agricultural traits (Gupta et al., 2014; Kharshiing and Sinha, 2015). The use of genetic tools involving phytochromes, such as mutant and transgenic plants, allows high planting densities, early flowering and increased biomass accumulation (Yang et al., 2016; Ballare and Pierik, 2017; Mawphlang and Kharshiing, 2017) as well as tolerance to biotic and abiotic disturbances (Carvalho et al., 2011; Gavassi et al., 2017), drawing attention to the importance of the manipulation of these photoreceptors for plant breeding.

However, beyond the phytochrome system, the complex photomorphogenic control of plant growth is modulated by different wavelengths and perceived by several classes of photoreceptors that operate at low or high light intensity (Kami et al., 2010). For example, for the ultraviolet (UV)-A/blue light spectrum (315–500 nm), three important photoreceptors strongly modulate plant growth: phototropins, zeitlupe and cryptochromes (Christie et al., 2015); the last type is clearly involved in a wide variety of plant developmental processes, such as seed germination, leaf senescence, plant height and stress responses, which are important agronomic characteristics (Mishra and Khurana, 2017; D'Amico-Damião and Carvalho, 2018). However, for the most part,

these approaches are restricted to species of low agronomic interest, such as the model plant *Arabidopsis thaliana* (Kami et al., 2010). On the other hand, in other crops such as tomato, one of the most important vegetables in the world, transgenic lines overexpressing *CRY1a* or *CRY2* showed alterations in plant height, shoot dry weight, leaf area, pigment content, and flavonoid and lycopene contents in the fruits (Giliberto et al., 2005; Lopez et al., 2012; Liu et al., 2018). However, mechanisms by which cryptochromes control plant growth seem to be complex because the *cry1a* (loss of-function) mutant of tomato showed some enhanced shoot and fruit traits (Liu et al., 2018), and in our current work, we showed that the *cry1a* mutant alters its growth based on an over time orchestrated modulation of roots, shoots, and especially fruits, revealing new roles of this photoreceptor in tomato growth.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Seeds of the tomato (*Solanum lycopersicum* L.) *cry1a* mutant (Weller et al., 2001) and its near isogenic line, the wild type (WT) of cv Moneymaker, were sown in trays containing a 1:1 (v/v) mixture of a commercial substrate (BioPlant, Brazil) and vermiculite. Fifteen days after sowing, seedlings were transferred to 7 L pots filled with eutrophic oxisol supplemented with 1 g L⁻¹ of a 30:20:15 (m/m/m) mix of NPK fertilizer and 0.7 g L⁻¹ lime (MgCO₃ plus CaCO₃) (Malavolta, 1980). The plants were, then, maintained in a greenhouse with an average temperature of 20.1°C (SD ± 1.64°C) and a relative humidity of 66% (SD ± 8.05%) under a 12 h photoperiod at a photosynthetic photon flux density (PPFD) of 450–700 µmol m⁻² s⁻¹. All plants were watered daily throughout the experimental period (120 days) to maintain the maximum water holding capacity of the substrate. Growth evaluations were performed at 30, 60, 90 and 120 days after sowing (DAS). After these periods, plants were harvested for morphometric analysis of fruit parameters.

2.2. Determination of vegetative parameters

2.2.1. Growth analysis

Plant height and stem diameter were measured with a graduated ruler and digital caliper, respectively. The number of leaves was counted, whereas the leaf area was evaluated using an image analysis system (Delta-T Devices, Cambridge, UK). Roots of each genotype were digitized using a Hewlett Packard 125C scanner, and the images were analyzed by Delta-T Scan software to determine total root length, diameter, area and density. Subsequently, both the shoot and root were oven dried at 60°C for 72 h, and biomass accumulation was quantified using an analytical scale (AA-200, Denver Instrument Company).

2.2.2. Pigment content

Leaf discs were obtained from the third fully expanded leaf (top to bottom) for pigment extraction, and the determination of pigment content was performed by spectrophotometry at 661.6 nm (chlorophyll a), 644.8 nm (chlorophyll b) and 470 nm (carotenoids). The total chlorophyll content (a + b) and carotenoid content were estimated by the Lichtenthaler method (Lichtenthaler, 1987).

2.3. Morphometric analysis of fruit parameters

After the experimental period (120 days), plants were harvested for morphometric analysis of fruit. The first fruit insertion length was measured with a graduated ruler. The numbers of ripe fruits per plant and seeds per fruit were determined by direct counting. Fruit diameter was evaluated with a digital caliper, and the total soluble solids (°Brix) in fruits was determined using a portable handheld refractometer (model ATCS/Mill-E). Afterwards, the ripe fruits were oven dried at 60°C for 72 h, and dry mass was measured using an analytical scale (AA-200, Denver Instrument Company). Finally, the ratio of fruit to shoot/root dry weight was calculated using all fruits produced per plant.

2.4. Data analysis

The experimental design was completely randomized, with three replicates for growth analysis and four replicates for pigment content and morphometric analysis of fruit, and the treatments were represented by the two genotypes (WT and *cry1a*). The morphometric data of fruit parameters were subjected to analysis of variance (ANOVA), and mean values were compared using Tukey's test (significance at $p \leq 0.05$) in AgroEstat software (www.agroestat.com). In figures, all mean values are accompanied by standard errors (SE).

3. RESULTS

3.1. Vegetative parameters

3.1.1. Growth analysis

Throughout the life cycle, *cry1a* mutant showed greater shoot growth than the WT, as evidenced by increased plant height at 30 (46%), 60 (16%), 90 (25%) and 120 (21%) DAS (Figure 1A). Although the stem diameter of the mutants did not change compared to that of the WT (Figure 1B), we observed in the mutants a greater number of leaves at 30 (25%) and 120 (30%) DAS (Figure 1C) and a greater leaf area at 90 (35%) and 120 (41%) DAS (Figure 1D). However, the WT plants showed greater shoot biomass accumulation at 30 (12%) and 90 (38%) DAS (Figure 1E) when compared with the *cry1a* mutants. In contrast, WT plants exhibited a reduction in shoot biomass (- 24%) only at 120 DAS.

There was larger total root length (60%; Figure 1F), root area (72%; Figure 1G) and root density (60%; Figure 1H) in the WT in relation to *cry1a* at 30 DAS. However, positive changes were observed in the roots of *cry1a*, which exhibited larger diameter at 120 DAS (44%; Figure 1I) and greater biomass accumulation at 90 and 120 DAS (both 28%; Figure 1J) when compared to the roots of WT plants. The source-sink relationships resulted in a significant increase in the ratio of shoot to root biomass accumulation in WT plants at 90 (85%) DAS (Figure 1J, inset).

3.1.2. Pigment content

The leaf pigments per area differed among genotypes (Figure 2). Interestingly, at 30, 60 and 90 DAS, WT plants exhibited a high total chlorophyll content (Figure 2A) (32%, 6% and 33% at 30, 60 and 90 DAS, respectively) and carotenoid content (Figure 2B) (19%, 15% and 27% at 30, 60 and 90 DAS, respectively); the biosynthesis of these pigments was downregulated in the mutant.

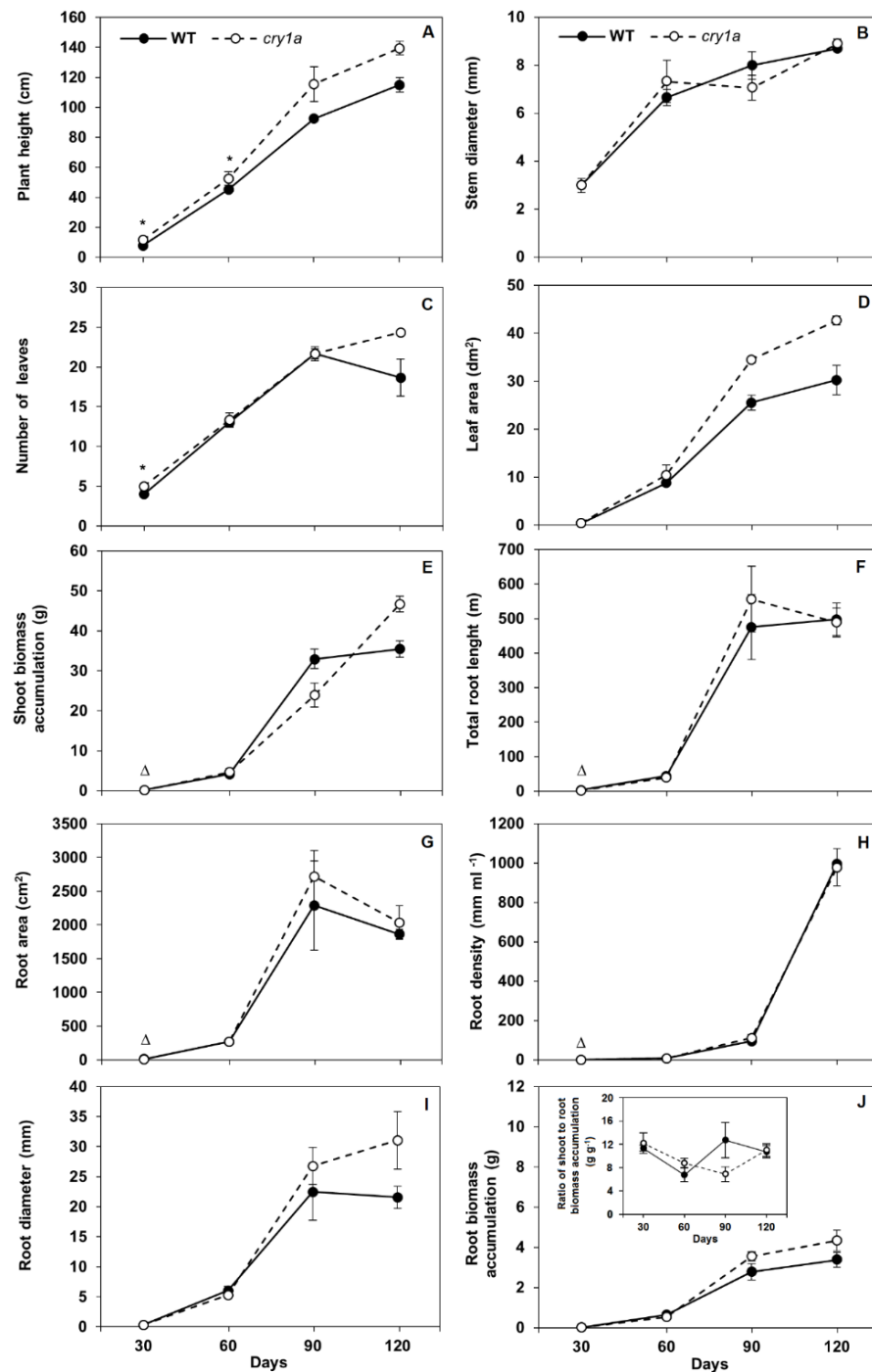


Figure 1. Plant height (A), stem diameter (B), number of leaves (C), leaf area (D), shoot biomass accumulation (E), total root length (F), root area (G), root density (H), root diameter (I) and root biomass accumulation (J) of *cry1a* and WT plants at 30, 60, 90 and 120 days after sowing. Inset (J): ratio of shoot to root biomass accumulation of *cry1a* and WT plants at 30, 60, 90 and 120 days after sowing. Nonvisible differences are represented by triangles for the WT and asterisks for *cry1a*, indicating a significant genotype effect. Data are means $\pm$ SEs ($n = 3$).

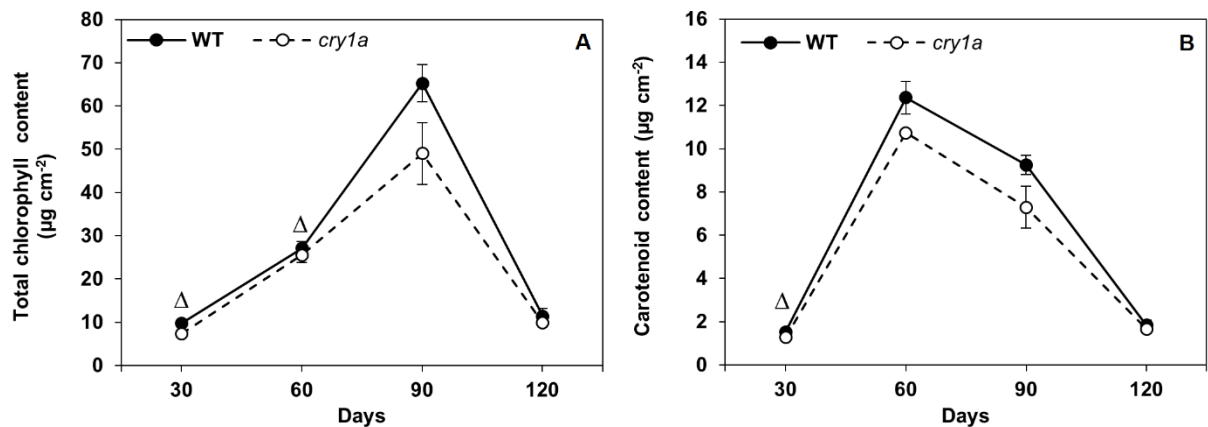


Figure 2. Total chlorophyll content (A) and carotenoid content (B) of *cry1a* and WT plants at 30, 60, 90 and 120 days after sowing. Nonvisible differences are represented by triangles for the WT and asterisks for *cry1a*. Data are means $\pm$ SEs ($n = 3$).

3.2. Morphometric analysis of fruit parameters

Surprisingly, the WT had different fruit parameters than *cry1a*, namely, a lower first fruit insertion length (- 40%) (Figure 3A) and fruit dry weight (- 34%) (Figure 3B). In contrast, in WT a strong increase of 98% in the ratio of fruit to shoot/root dry weight (Figure 3B, inset) and an increase in the number of ripe fruits per plant (62%) was observed (Figure 3C) when compared to *cry1a*. Although fruit diameter (Figure 3D) and total soluble solids (Figure 3E) showed no difference between genotypes, the WT plants produced more seeds per fruit (+ 107%) than *cry1a* (Figure 3F).

4. DISCUSSION

The photomorphogenic control mediated by cryptochromes is well elucidated in the model plant *Arabidopsis* (Kami et al., 2010). However, this species does not produce commercial fruits, and in most cases, this knowledge is obtained from the characterization of seedlings or young plants. Recently, the role of cryptochromes in growth has been shown in crops, such as tomato (Liu et al., 2018), one of the most important vegetable plants in the world. However, the *cry1a* mutant was used in the present study in order to provide a broad overview of how cryptochrome 1a influences shoot/root growth and fruit development throughout the life cycle of tomato. For this, we used a tomato mutant with the loss of CRY1a function and its near isogenic line (WT) to gain more information about blue light signaling through the *cry1a* photoreceptor.

In comparison with the vegetative parameters of WT tomato plants, *cry1a* plants were taller throughout the life cycle (Figure 1A). Because the inhibition of hypocotyl elongation by blue light is well characterized, e.g., by the long hypocotyl phenotype in *cry1a* under blue light (Weller et al., 2001), we expected this phenomenon to occur in our study. However, we observed no changes in stem diameter (Figure 1B). This result suggests that the increased plant height was due to greater stem biomass accumulation; according to a previous report, *cry1a* presented a lower ratio of leaf to stem dry weight (Liu et al., 2018). In contrast, etiolated plants can present a strongly reduced stem diameter (Wu et al., 2017). Though these results indicate the relocation of photoassimilate to the stem in the mutant, in the present study, shoot biomass accumulation was measured by the sum of the stem and leaf biomass (Figure 1E). Furthermore, the number of leaves (Figure 1C) and leaf area (Figures 1D and 4) in the WT were lower than those in *cry1a*, showing an interesting role of cryptochromes in leaf expansion to better perform light harvesting. Actually, the WT with full blue light perception greatly altered its growth relative to that of the *cry1a* mutant (Figure 1), which exhibited exaggerated growth due to disruption in the encoding of *CRY1a* (Weller et al., 2001).

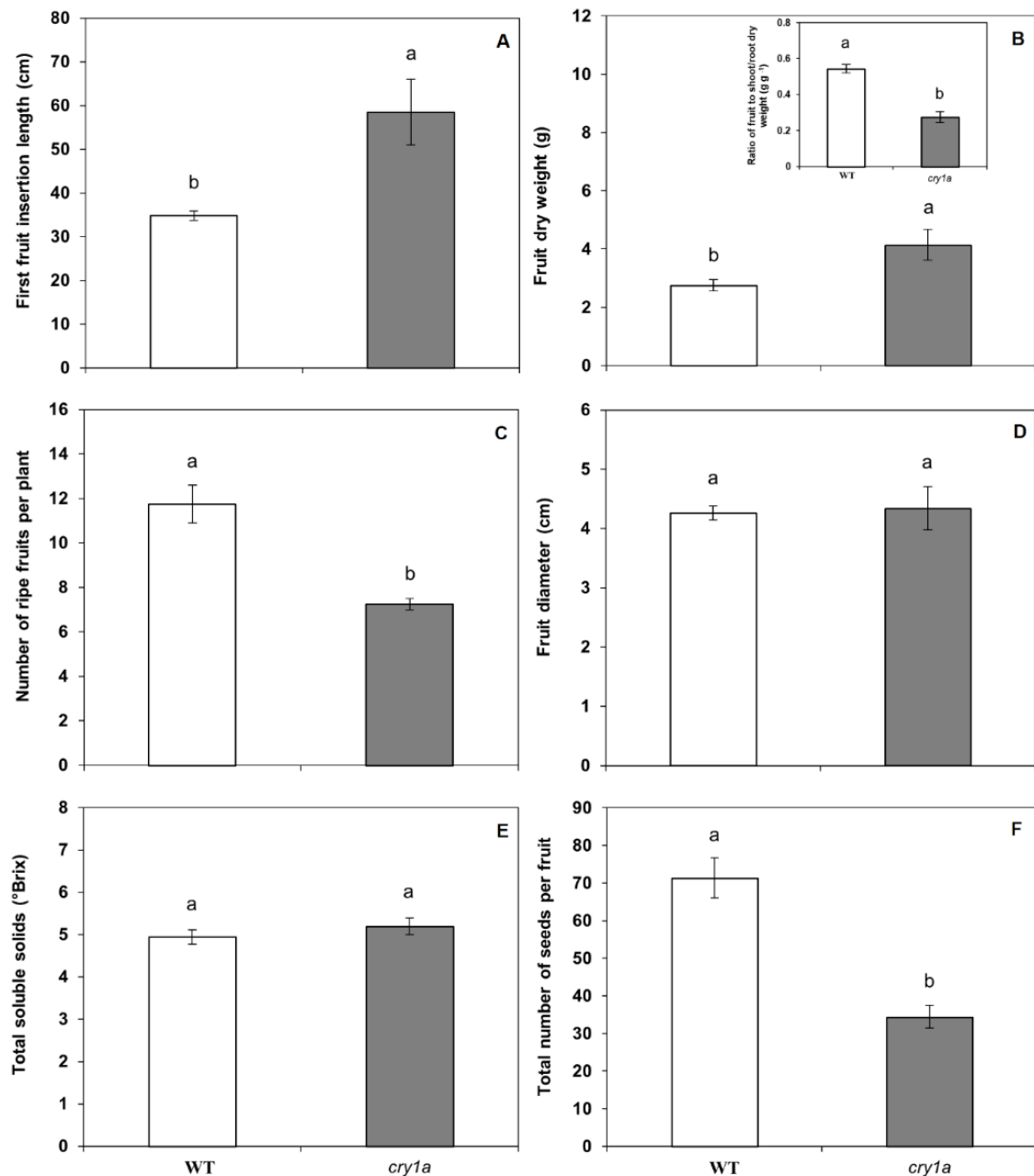


Figure 3. First fruit insertion length (A), fruit dry weight (B), number of ripe fruits per plant (C), fruit diameter (D), total soluble solids (E) and total number of seeds per fruit (F) of *cry1a* and WT plants. Inset (B): ratio of fruit to shoot/root dry weight of *cry1a* and WT plants. Data are means $\pm$ SEs ($n = 4$). Letters above the bars indicate significant differences according to Tukey's test ($p < 0.05$).

In contrast, WT plants exhibited greater shoot biomass accumulation than the mutants at 30 and 90 DAS (Figure 1E). However, at the final stage of tomato development (120 DAS), the WT accumulated 76% of *cry1a* shoot biomass. This fact

can be explained by the greater number of leaves in the mutant at 120 DAS (Figure 1C) and the smaller leaf mass per area in *cry1a*, which, despite showing a larger leaf area at 42 DAS, still produced thinner leaves and lower ratio of leaf to stem dry weight than did the WT (Liu et al., 2018). These data reflect the higher total chlorophyll (Figure 2A) and carotenoid (Figure 2B) contents per area in WT leaves, although increased biosynthesis of these leaf pigments per unit fresh weight was demonstrated in *cry1a* (Liu et al., 2018). Therefore, here we suggest a dilution of leaf pigments in *cry1a* due to the greater leaf area in this mutant (Figure 1D).

Our data also show that cryptochrome 1a influences partitioning of shoot and root photoassimilates, based on the exaggerated ratio of shoot to root biomass accumulation in WT plants relative to that in the *cry1a* mutant at 90 DAS (Figure 1J, inset). Although the lower root growth of *cry1a* was expected, due to the greater investment of photoassimilates in shoot growth, we found greater total root length (Figure 1F), root area (Figure 1G) and root density (Figure 1H) at 30 DAS in the WT when compared to *cry1a*, which exhibited greater root diameter at 120 DAS (Figures 1I and 4) and root biomass at 90 and 120 DAS (Figure 1J). These patterns suggest that the mechanisms involved in tomato root growth and development are influenced by *cry1a*. These results also indicate that cryptochrome 1a positively influences initial tomato root growth, which is in contrast to the inhibitory effects of blue light on hypocotyl elongation mediated by *cry1a*, as reported previously (Mishra and Khurana, 2017).

Given the morphophysiological changes mediated by cryptochrome 1 in shoots and roots, it is not surprising that the fruit growth is influenced by cryptochromes. In tomato, a previous study reported that the fruit yield per plant was approximately 30% higher in *cry1a* than in the wild type, although no difference was observed in the number of fruits per plant (Liu et al., 2018).

In the present study, we elucidated the fruit characteristics of tomato WT and *cry1a* mutant, revealing changes in the first fruit insertion length (Figure 3A), fruit dry weight (Figure 3B), number of ripe fruits per plant (Figure 3C) and number of seeds per fruit (Figure 3F). The first fruit insertion length was lower in the WT than in *cry1a*, probably due to the greater growth of the mutant during plant development (Figure 1A).

On the other hand, WT plants showed a greater number of ripe fruits per plant than *cry1a* (Figure 3C), although the mutant fruits exhibited a greater mass (Figure 3B). Indeed, the largest shoot biomass accumulation in the WT (30 and 90 DAS) was reduced at the end of the cycle (120 DAS), showing strong carbon translocation to the fruits in this genotype during this time (Figure 1E). Actually, WT photosynthetic products seemed to be exponentially invested in fruit production, which resulted in a higher ratio of fruit to shoot/root dry weight in the WT than in the *cry1a* mutant (Figure 3B, inset). Fruit diameter (Figures 3D and 4) and total soluble solids (Figure 3E) showed no difference. Although the fruit diameter did not change, the high fruit dry weight in *cry1a* may explain the similarity in soluble solids. On the other hand, other researchers observed greater total soluble solids content in WT fruits than in *cry1a* fruits (Liu et al., 2018). Actually, the morphometric analysis of fruit parameters in tomato are exponentially influenced by cryptochrome 1a, as demonstrated in *cry1a* mutant, which showed greater fruit dry weight (Figure 3B) but fewer ripe fruits per plant (Figure 3C). Hence, these results probably favored the propagative attributes in WT, which produced more seeds per fruit (Figure 3F).



Figure 4. Aspects of the fruit, leaf and root which are 120, 60, and 120 days old, respectively, of WT and *cry1a* mutant. Although the fruit diameter does not differ between genotypes, the mutant fruit accumulates more biomass than WT (Figure 3B).

5. CONCLUSIONS

In the present work, using tomato *cry1a* mutant, we demonstrated the intricate role of cryptochrome 1a in contributing towards shoot/root growth and fruit development throughout the life cycle of tomato plants. Actually, the important vegetative and fruit characteristics in tomato plants appear to be modulated by *cry1a*, making this photoreceptor a target molecule plant breeding programs. However, clarifying how and which biochemical routes these responses signal are necessary.

Acknowledgements

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6. REFERENCES

- Ballare CL, Pierik R (2017) The shade-avoidance syndrome: multiple signals and ecological consequences. **Plant, Cell & Environment** 40:2530–2543. doi: 10.1111/pce.12914
- Carvalho RF, Campos ML, Azevedo RA (2011) The role of phytochromes in stress tolerance. In.: Ahmad P, Azooz MM, Prasad MNV (Eds.) **Salt Stress in Plants**. New York: Springer, p. 283–299. doi: 10.1007/978-1-4614-6108-1_12
- Christie JM, Blackwood L, Petersen J, Sullivan S (2015) Plant flavoprotein photoreceptors. **Plant and Cell Physiology** 56:401–413. doi: 10.1093/pcp/pcu196
- D'Amico-Damião V, Carvalho RF (2018) Cryptochrome-related abiotic stress responses in plants. **Frontiers in Plant Science** 9:1897. doi: 10.3389/fpls.2018.01897
- Eckstein A, Jagiełło-Flasińska D, Lewandowska A, Hermanowicz P, Appenroth KJ, Gabryś H (2016) Mobilization of storage materials during light-induced germination of tomato (*Solanum lycopersicum*) seeds. **Plant Physiology and Biochemistry** 105:271–281. doi: 10.1016/j.plaphy.2016.05.008
- Gavassi MA, Monteiro CC, Campos ML, Melo HC, Carvalho RF (2017) Phytochromes are key regulators of abiotic stress responses in tomato. **Scientia Horticulturae** 222:126–135. doi: 10.1016/j.scienta.2017.04.035
- Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, Tavazza M, Giuliano G (2005) Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. **Plant Physiology** 137:199–208. doi: 10.1104/pp.104.051987
- Gupta S K, Sharma S, Santisree P, Kilambi HV, Appenroth K, Sreelakshmi Y, Sharma R (2014) Complex and shifting interactions of phytochromes regulate fruit development in tomato. **Plant, Cell & Environment** 37:1688–1702. doi: 10.1111/pce.12279
- Kami C, Lorrain S, Hornitschek P, Fankhauser C (2010) Light-regulated plant growth and development. In.: Timmermans MCP (Ed.) **Current Topics in Developmental Biology**. San Diego: Elsevier Academic Press Inc, p. 29–66. doi: 10.1016/S0070-2153(10)91002-8
- Kharshiing E, Sinha SP (2015) Plant productivity: can photoreceptors light the way?. **Journal of Plant Growth Regulation** 34:206–214. doi: 10.1007/s00344-014-9454-9

Kharshiing E, Sinha SP (2016) Deficiency in phytochrome A alters photosynthetic activity, leaf starch metabolism and shoot biomass production in tomato. **Journal of Photochemistry & Photobiology B-Biology** 165:157–162. doi: 10.1016/j.jphotobiol.2016.10.026

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In.: Colowick SP, Kaplan NO (Eds.) **Methods in Enzymology**. New York: Academic Press, p. 350–382. doi: 10.1016/0076-6879(87)48036-1

Liu CC, Ahammed GJ, Wang GT, Xu CJ, Chen KS, Zhou YH, Yu JQ (2018) Tomato CRY1a plays a critical role in the regulation of phytohormone homeostasis, plant development, and carotenoid metabolism in fruits. **Plant, Cell & Environment** 41:354–366. doi: 10.1111/pce.13092

Lopez L, Carbone F, Bianco L, Giuliano G, Facella P, Perrotta G (2012) Tomato plants overexpressing cryptochrome 2 reveal altered expression of energy and stress-related gene products in response to diurnal cues. **Plant, Cell & Environment** 35:994–1012. doi: 10.1111/j.1365-3040.2011.02467.x

Malavolta E (Ed.) (1980) Elementos de nutrição mineral de plantas. São Paulo: Agronômica Ceres, 252p.

Mawphlang OIL, Kharshiing EV (2017) Photoreceptor mediated plant growth responses: Implications for photoreceptor engineering toward improved performance in crops. **Frontiers in Plant Science** 8:1181. doi: 10.3389/fpls.2017.01181

Mishra S, Khurana JP (2017) Emerging roles and new paradigms in signaling mechanisms of plant cryptochromes. **Critical Reviews in Plant Science** 36:89–115. doi: 10.1080/07352689.2017.1348725

Viczian A, Klose C, Adam E, Nagy F (2017) New insights of red light-induced development. **Plant, Cell & Environment** 40:2457–2468. doi: 10.1111/pce.12880

Weller JL, Perrotta G, Schreuder MEL, van Tuinen A, Koornneef M, Giuliano G, Kendrick RE (2001) Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. **The Plant Journal** 25:427–440. doi: 10.1046/j.1365-313x.2001.00978.x

Wu Y, Gong W, Yang W (2017) Shade inhibits leaf size by controlling cell proliferation and enlargement in soybean. **Scientific Reports** 7:9259. doi: 10.1038/s41598-017-10026-5

Yang DY, Seaton DD, Krahmer J, Halliday KJ (2016) Photoreceptor effects on plant biomass, resource allocation, and metabolic state. **Proceedings of the National Academy of Sciences, USA** 113:7667–7672. doi: 10.1073/pnas.1601309113

CHAPTER 3 – Cryptochrome 1a depends on blue light fluence rate to mediate osmotic stress responses in tomato

ABSTRACT – The participation of plant cryptochromes in water deficit response mechanisms has been highlighted in several reports. However, the role of tomato (*Solanum lycopersicum* L.) cryptochrome 1a (cry1a) in the blue light fluence-dependent modulation of the water deficit response remains largely elusive. The tomato *cry1a* mutant and its wild-type counterpart were grown in water (no stress) or PEG₆₀₀₀ (osmotic stress) treatments under white light (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or from low to high blue light fluence (1, 5, 10, 15 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$). We first demonstrate that under nonstress conditions *cry1a* regulates seedling growth by mechanisms that involve pigmentation, lipid peroxidation and osmoprotectant accumulation in a blue light-dependent manner. In addition, we further highlighted under osmotic stress conditions that *cry1a* increased tomato growth by reduced malondialdehyde (MDA) and proline accumulation. Although blue light is an environmental signal that influences osmotic stress responses mediated by tomato *cry1a*, specific blue light fluence rates are required during these responses. Here, we show that *CRY1a* manipulation may be a potential biotechnological target to develop a drought-tolerant tomato variety. Nevertheless, the complete understanding of this phenomenon requires further investigation.

Keywords: abiotic stress, blue light fluence, cryptochrome 1a, *cry1a* mutant, osmotic stress; *Solanum lycopersicum* L. (tomato)

1. INTRODUCTION

Plants constantly sense and respond to a wide range of environmental signals. Remarkably, among these signals, light is a key indicator of ambient conditions for plant growth and development (Casal and Yanovsky, 2004; Demarsy et al., 2018; Saijo and Loo, 2019). In addition to its prominent role during photosynthesis, light also conveys valuable information about the surrounding environment throughout the plant life cycle to control a large variety of physiological responses, a process known as photomorphogenesis (Kendrick and Kronenberg, 1994). Overall, fluctuations in aspects of the light spectrum (e.g., intensity, composition, periodicity and incidence angle) are perceived by photoreceptors, which function as photoreversible chromoproteins that sense specific wavelengths of the light spectrum (Kami et al., 2010). In higher plants, five families of photoreceptor systems exist: ultraviolet (UV)-B Resistance 8 (UVR8) receptors, receptors that are photosensitive to UV-B light (280–315 nm); cryptochromes (crys), phototropins (phot) and zeitlupes (ZTL, FKF1, and LKP2), which perceive UV-A/blue light (BL) (315–500 nm); and phytochromes (phys), photoreceptors that are sensitive to red light (600–700 nm) and far-red light (700–750 nm) (Chen and Chory, 2011; Ito et al., 2012; Jenkins, 2014; Christie et al., 2015; Mishra and Khurana, 2017). However, these sensory photoreceptors can play some specific as well as overlapping roles in the regulation of photomorphogenesis (Casal, 2000; Galvao and Fankhauser, 2015).

cry1 is the most investigated photoreceptors mediating BL photomorphogenic responses (Cashmore et al., 1999; Yu et al., 2010; Chaves et al., 2011). In fact, crys play a fundamental role in the signaling of a range of physiological responses from early root development to fruit yield (Yang et al., 2017; D'Amico-Damião et al., 2019). crys are essentially composed of an apoprotein with two prosthetic groups: a N-terminal photolyase homology-related domain and a cryptochrome C-terminal extension domain. The first domain binds two chromophores: a flavin adenine dinucleotide, which is a chromophore with photomorphogenic activity, and methenyltetrahydrofolate, which is a catalytic cofactor (Liu et al., 2011a). The first three types of plant crys were discovered in *Arabidopsis thaliana* (Cashmore et al., 1999;

Kleiner et al., 1999). The nuclear proteins cry1 and cry2 specifically act in photomorphogenic responses, such as inhibition of hypocotyl elongation, anthocyanin accumulation, cotyledon expansion and flowering (Guo et al., 1998; Cashmore et al., 1999; Yu et al., 2010). However, cry1 acts at a high BL fluence (BLF) rate (BLFR), whereas cry2 acts at low BLFR (Lin et al., 1998). On the other hand, cry3 is a DASH protein that is located in chloroplasts and mitochondria and that acts in DNA repair mechanisms (Kleine et al., 2003; Mishra and Khurana, 2017). Nevertheless, the types of crys vary among plant species (Yang et al., 2017). In tomato (*Solanum lycopersicum* L.), one of the most important vegetable crop species worldwide, four crys encoded by a multigene family have been identified: *CRY1a*, *CRY1b*, *CRY2* and *CRY3*. cry1a is both a low- and high-BLF photoreceptor that regulates photomorphogenic responses related to root development, hypocotyl elongation, pigment biosynthesis, and fruit yield in tomato (Weller et al., 2001; D'Amico-Damião et al., 2019; Fantini et al., 2019).

Although the types of crys and their responses are species specific, recent findings have shown that these photoreceptors control reactive oxygen species (ROS) homeostasis in response to BL (Consentino et al., 2015; Jourdan et al., 2015; El-Esawi et al., 2017). Coincidentally, ROS signaling pathways are involved in the transcription of genes related to biotic and abiotic stress responses (Waszczak et al., 2018; Huang et al., 2019; Saijo and Loo, 2019). In fact, the role of crys in abiotic stress responses has been highlighted, especially with respect to drought, due to redox changes that occur on photoactivation of crys and also during drought stress conditions, generating ROS and leading eventually to activation or repression of essentially similar genes, although there is a wide range of genes (specifically regulated by either BL or drought conditions) that perform unique functions (D'Amico-Damião and Carvalho, 2018). The most limiting abiotic stress to plant growth and development, drought stress reduces water content, increases turgor loss, and impairs transpiration and photosynthesis via stomatal closure, which triggers ROS production and ultimately affects the growth and yield of stressed plants (Jangid and Dwivedi, 2016). Furthermore, light quality can affect tolerance to water deficit, improving tomato plants drought tolerance by decreasing stomatal conductance thereby increasing water use efficiency (Bian et al., 2019).

However, cry-related stress responses seem to be complex because, in addition to the performance of other photoreceptor systems, they depend on the BLF and vary with plant species (Kami et al., 2010; Yang et al., 2017). For example, the control of stomatal conductance is a key drought response mechanism governed by crys in *Arabidopsis*. It has been shown that, compared with the wild type, the *cry1cry2* double mutant had a smaller stomatal opening, while *CRY1-OE* plants had a greater stomatal opening. Thus, the double mutant was more tolerant to leaf water loss, while the transgenic mutant was more susceptible (Mao et al., 2005). Interestingly, in the same study, it was verified that, as the BLF increased from 1 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the stomatal opening also increased, showing a BLF-dependent response. Similarly, regarding the inhibition of relative root length, *Brassica* (*Brassica napus*) *CRY1* overexpression (*BnCRY1-OE*) resulted in seedlings that were sensitive to osmotic stress (OS) (200, 300 and 400 mM of mannitol), while *CRY1* antisense seedlings (*BnCRY1-AS*) were more tolerant (Sharma et al., 2014). In addition, the authors found that *BnCRY1-OE* reduced the expression of genes related to drought (e.g., *LE*, *RAB18*, *NIA1*, *APX1* and *NAC*). Furthermore, when exposed to low (5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and high (25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) BLFRs, the *BnCRY1-OE* transgenic plants exhibited altered hypocotyl lengths and anthocyanin accumulation, demonstrating that cry1 controlled these responses in a light fluence-dependent manner. These reports reveal new perspectives for studying the role of crys in water deficit responses. Indeed, it is evident that crys are part of drought stress response mechanisms in plants (D'Amico-Damião and Carvalho, 2018).

Although the evidence described above suggest that cry1 mediates osmotic stress in *Arabidopsis* and *Brassica*, it remains unknown whether cry1a regulates drought responses in tomato. To test this hypothesis we performed a series of experiments using the tomato loss-of-function *cry1a* mutant under OS in seedling stage. We found that the loss of cry1a function in tomato plants grown under white light (WL) decreased seedling growth, oxidative stress and proline accumulation after OS induction. Moreover, to better understand the mechanisms underlying BL-mediated water deficit responses, we hypothesized that different photon fluxes of BL can affect the physiological responses observed under WL. Indeed, BLF altered water deficit

responses in the tomato *cry1a* mutant compared with the control genotype. All of these data are discussed in the context of our hypothesis.

2. MATERIALS AND METHODS

2.1. Plant material, growth conditions and light sources

Sterilized seeds (5% NaClO solution for 10 mins followed by thorough washing with water) of the tomato (*Solanum lycopersicum* L.) *cry1a* mutant (Weller et al. 2001) and its wild type (WT) cultivar Moneymaker, were seeded in plastic boxes that contained two layers of filter paper and that were filled with 15 mL of distilled water. The plastic boxes were maintained in a growth chamber (Model CDG-347, Fanem, Sao Paulo, Brazil) in the dark at a temperature of 25°C (SD $\pm$ 1.0°C) and relative humidity of 70% (SD $\pm$ 10.0%) until homogeneous germination (2 mm radicle) of both genotypes for four days. The growth chamber light was switched on after germination (day 5) to promote seedling cotyledon opening under a 12 h photoperiod (light : dark) for three days (Figure 1A of Appendix A). The growth chamber illumination was provided by a white light-emitting diode (LED) at 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2 of Appendix A). After cotyledon opening was induced, 20 seedlings of each genotype were transplanted separately from the plastic boxes to 300 mL plastic pots that contained five layers of filter paper and that were filled with 6 mL of distilled water (nonstress conditions) or filled with 6 mL of a polyethylene glycol 6000 (PEG₆₀₀₀; OS conditions) solution to induce a low water potential of -0.3 MPa (Vilela et al., 1992; Hajhashemi and Sofo, 2018). The conditions described above, from seed germination to PEG₆₀₀₀ treatment, were used in both experiments described below (Figure 1A of Appendix A). We performed a preliminary experiment to investigate the effects of *cry1a* mutation on OS responses in tomato seedlings. For this, three pots per treatment (nonstress or PEG₆₀₀₀ $\times$ WT or *cry1a*) were grown for seven days under the same growth chamber conditions cited above under a 12 h photoperiod (light : dark; Figure 1B of Appendix A). We further verified the role of BLF-induced signaling on *cry1a*-mediated OS responses. Thus, three pots per treatment were transported to a BL LED growth chamber that provided forced ventilation. The BL LED growth chamber consisted of five black plastic boxes (45 $\times$ 30 $\times$ 25 cm) with a cooler fan adapted to air circulation inside the chamber. On the box covers, twelve BL LED adhesive strips (max λ of 459

nm) with a length of 30 cm were installed and spaced 3 cm apart from each other for homogeneous light distribution inside the chambers. The LEDs were adjusted in each chamber to provide the following gradients of BLF: 1, 5, 10, 15 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 3 of Appendix A). To measure and guarantee the BLFRs cited above, a portable spectroradiometer (Model SpectraPen LM 500, PSI, Brno, Czech Republic) was placed at the seedlings position inside the chamber to the light source (15 cm). In all the BL treatments, the seedlings grew for seven days under an average temperature of 25°C (SD $\pm$ 3.5°C) and a relative humidity of 65% (SD $\pm$ 14.8%) under a 12 h photoperiod (light : dark; Figure 1C of Appendix A). Descriptions of the experiment steps, from seed germination to the analyses performed, are described in Figure 1 of Appendix A.

2.2. Growth analysis

Shoots (the hypocotyl) and roots were separated to measure their lengths with a digital caliper. Afterward, the aforementioned shoots and roots were washed with water and subsequently oven dried at 40°C for 48 h to determine the shoot and root dry weights using an analytical scale (Model AA-200, Denver Instrument Company, New York, USA).

2.3. Cotyledon and hypocotyl pigment analyses

Chlorophyll a+b and carotenoid contents were quantified from 25 mg of cotyledon tissue. The cotyledon tissue was added to tubes protected from light for the complete extraction of chlorophyll a+b and carotenoids in 2 mL of 80% acetone. The tubes were maintained in the darkness at 4°C for 48 h. The extracts were removed from the tubes, and the pigment contents were determined by spectrophotometry (DU-640, Beckman Coulter, Fullerton, USA) at 663 nm (chlorophyll a), 647 nm (chlorophyll b) and 470 nm (carotenoids). The chlorophyll a+b and carotenoid contents were estimated by the Lichtenthaler method (Lichtenthaler, 1987).

Anthocyanins were extracted from 45 mg of hypocotyl tissue for subsequent analysis via spectrophotometry (Peters et al., 1989). The hypocotyl samples were added to tubes that had been previously protected from light (wrapping with aluminum foil) containing 0.48 mL of extraction buffer (methanol acidified with 1% HCl), after which the tubes were maintained in darkness under 60 rpm agitation at 4°C for 36 h. Subsequently, 0.36 mL of distilled water and 0.96 mL of chloroform were added to each tube, after which the tubes were vortexed and centrifuged at 5,000 rpm at 4°C for 15 min. After centrifugation, the absorbance of the supernatant was read at 535 nm (A_{535}) via a spectrophotometer, and each sample was measured in triplicate. We measured the anthocyanin content only in the second experiment (BLF) because this response is also associated with high irradiance.

2.4. Lipid peroxidation and H₂O₂ formation

Lipid peroxidation was measured according to the content of thiobarbituric acid-reactive substances (Heath and Packer, 1968) present in the malondialdehyde (MDA) form and the content of hydrogen peroxide (H₂O₂), as determined by reaction with potassium iodide (Alexieva et al., 2001). Briefly, 300 mg of seedling (shoot and root) tissue per sample was ground in liquid nitrogen, after which the tissue and 2 mL of 0.1% trichloroacetic acid (w/v) containing 20% polyvinylpolypyrrolidone (PVPP) were mixed together. The extracts were homogenized and then transferred to 1.4 mL tubes for centrifugation at 10,000 rpm at 4°C for 15 min. The supernatants were subsequently used for both MDA and H₂O₂ determination.

To determine the MDA content, a 1 mL solution consisting of 20% trichloroacetic acid (w/v) and 0.5% 2-thiobarbituric acid (w/v) was added to 250 μ L of the supernatant. After homogenization, the mixture was incubated at 95°C for 30 min in a dry bath. The reaction was interrupted by cooling in an ice bath for 10 min, and the samples were subsequently centrifuged at 10,000 rpm at 4°C for 5 min. The absorbance of the supernatant was read at 535 (A_{535}) and 600 (A_{600}) nm on a spectrophotometer, and the MDA content was estimated using an extinction coefficient of $1.55 \times 10^{-5} \text{ mol}^{-1} \text{ cm}^{-1}$ (Gratão et al., 2012).

The H₂O₂ content was determined after the addition of 200 µL of potassium phosphate buffer (100 mM), 800 µL of potassium iodide (1 M) and 200 µL of supernatant (1:4:1 proportion, v/v/v). After homogenization, the tubes were kept on ice in the dark for 60 min. The absorbance of the supernatant was then read at 390 (A₃₉₀) nm on a spectrophotometer, and the concentration was determined using a known H₂O₂ concentration curve as a standard (Alves et al., 2017).

2.5. Compatible osmolyte contents

The proline content was determined in 125 mg of seedling (shoot and root) tissue (Bates et al., 1973). Samples from each treatment were macerated in 2.5 mL of 3% (w/v) sulfosalicylic acid. Afterward, aliquots of 1 mL taken from the resulting extract, acid ninhydrin and 99.5% (v/v) glacial acetic acid were then mixed at a 1:1:1 (v/v/v) ratio. After vortexing, the mixture was incubated at 100°C for 60 min in a water bath. The reaction was stopped by immersing the tubes in an ice bath, and 2 mL of toluene was added for phase separation. The fraction containing the chromophore group was collected, and its absorbance was read at 520 (A₅₂₀) nm on a Beckman spectrophotometer. A standard curve was used to estimate the proline content.

2.6. Statistical analyses

The mean values and standard errors (SEs) of each treatment were obtained from three replicates (pots) with twenty subreplicates (seedlings), in which at least nine seedlings were selected randomly to compose the mean values (biological replicates per pot; 9-12 for growth analysis and 9 for biochemical analysis). The experiments were repeated at least two times, and data from the most representative experiments were used. For both experiments, the experimental design was completely randomized in a 2×2 factorial scheme, and there were two genotypes (WT and *cry1a*) and two growth conditions (no stress and PEG₆₀₀₀). In the second experiment, statistical analyses were performed for each treatment of BLF. The quantitative data were subjected to a two-way analysis of variance (ANOVA) to detect significant effects of

genotype, osmotic condition and their interaction (Experiments 1 and 2), and the mean values were compared using Tukey's HSD test (significance at $p \leq 0.05$) via AgroEstat software (www.agroestat.com).

3. RESULTS

3.1. The tomato *cry1a* mutant inhibits growth under osmotic stress conditions

At the end of the experiments, seedlings with or without normal *cry1a* biological activity were analyzed to measure the impact of drought stress in tomato. *cry1a* mutant plants grown under nonstress conditions were taller than the WT and displayed elongated roots (significant genotype x osmotic condition interaction; Table 1 of Appendix A) (Figures 1A and B; Figure 2). Compared with the nonstress conditions, PEG₆₀₀₀ treatment significantly reduced the shoot and root lengths in both tomato genotypes (Figures 1A and B). Yet, this effect was significantly stronger in *cry1a* when compared to the WT. Similar results were observed in independent experiments (data not shown). On the other hand, *cry1a* appeared to modulate hypocotyl and primary root elongation under osmotic stress conditions because, in the PEG₆₀₀₀ treatment, the growth of these organs was impaired in the *cry1a* seedlings compared to the WT seedlings (shoot and root reductions of 0.2- and 0.3-fold, respectively).

As hypocotyl and root elongation could result from increased biomass accumulation, the same organs were dried and used to measure the shoot and root dry weights. Regarding shoot and root dry weights, the two genotypes responded differently to osmotic conditions (as indicated by significant genotype x osmotic condition interactions; Table 1 of Appendix A). However, we found no differences in hypocotyl or root dry weight between the genotypes grown under nonstress conditions (Figures 1C and D). In addition, the PEG₆₀₀₀ effect was not observed in the WT seedlings, whereas the *cry1a* seedlings showed reduced hypocotyl and root dry weights under PEG₆₀₀₀ conditions compared to nonstress conditions (Figures 1C and D). Under PEG₆₀₀₀ treatment, the *cry1a* seedlings was more sensitive to low water potential, as their hypocotyl and root dry weights decreased compared with those of the WT seedlings (decreases of 0.5- and 0.4-fold, respectively).

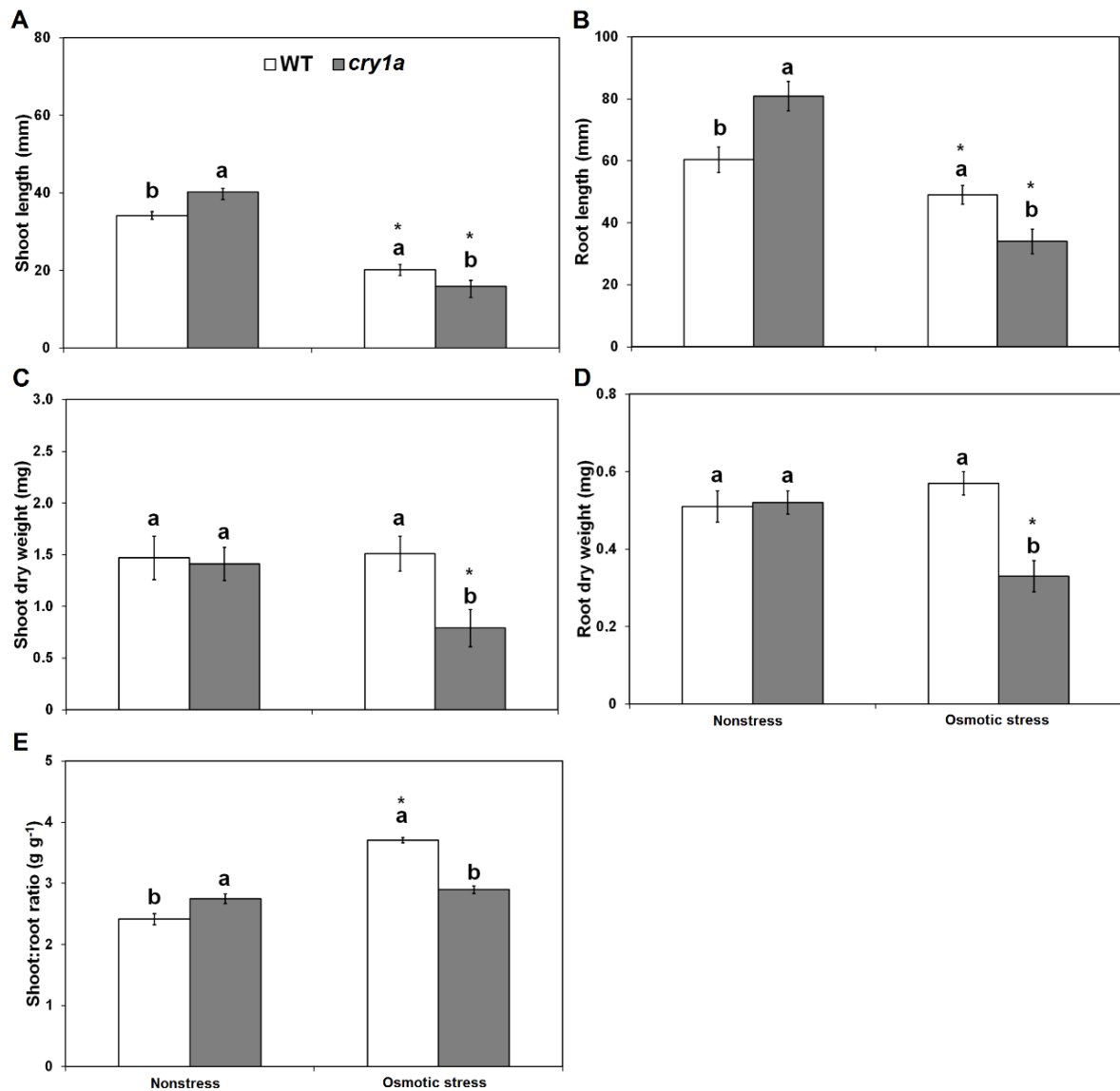


Figure 1. Growth responses of the tomato (*Solanum lycopersicum* L.) *cryptochrome 1a* mutant under osmotic stress. Biometric analysis of tomato *cry1a* mutant and wild-type (WT) seedlings grown under a 12 h white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) photoperiod under nonstress (water) or osmotic stress (PEG₆₀₀₀; -0.3 MPa) conditions: (A) shoot length; (B) root length; (C) shoot dry weight; (D) root dry weight; (E) the shoot-to-root ratio. The data are shown as the mean values of 3 pots (at least 27 seedlings). The bars represent $\pm$ SEs. The distinct letters indicate significant differences between genotypes within the same growth conditions, and the asterisks represent significant differences among growth conditions within each genotype. The mean values were compared by Tukey's test (significance at $p \leq 0.05$). *P* values for two-way ANOVA are described in Table 1 of Appendix A.



Figure 2. Picture of tomato (*Solanum lycopersicum* L.) *cry1a* mutant and wild-type (WT) seedlings grown for seven days under nonstress (water) or osmotic stress (PEG₆₀₀₀; -0.3 MPa) conditions.

3.2. Cotyledon pigmentation is altered in *cry1a* seedlings under osmotic stress

Under non-stress conditions, levels of chlorophyll a+b and carotenoid in tomato cotyledons were significantly higher in *cry1a* than in the WT (significant genotype x osmotic condition interaction; Table 2 of Appendix A) (Figures 3A and B). Compared with the nonstress conditions, PEG₆₀₀₀ treatment decreased the chlorophyll a+b and carotenoid levels by 0.4-fold and 0.3-fold, respectively, in *cry1a* cotyledons (Figures 3A and B). In contrast, WT cotyledons presented carotenoid levels that increased by 0.2-fold after OS induction, while chlorophyll a+b levels did not exhibit differences between nonstress conditions and OS conditions. Nevertheless, under OS conditions, compared with WT, *cry1a* presented greater carotenoid contents (0.3-fold).

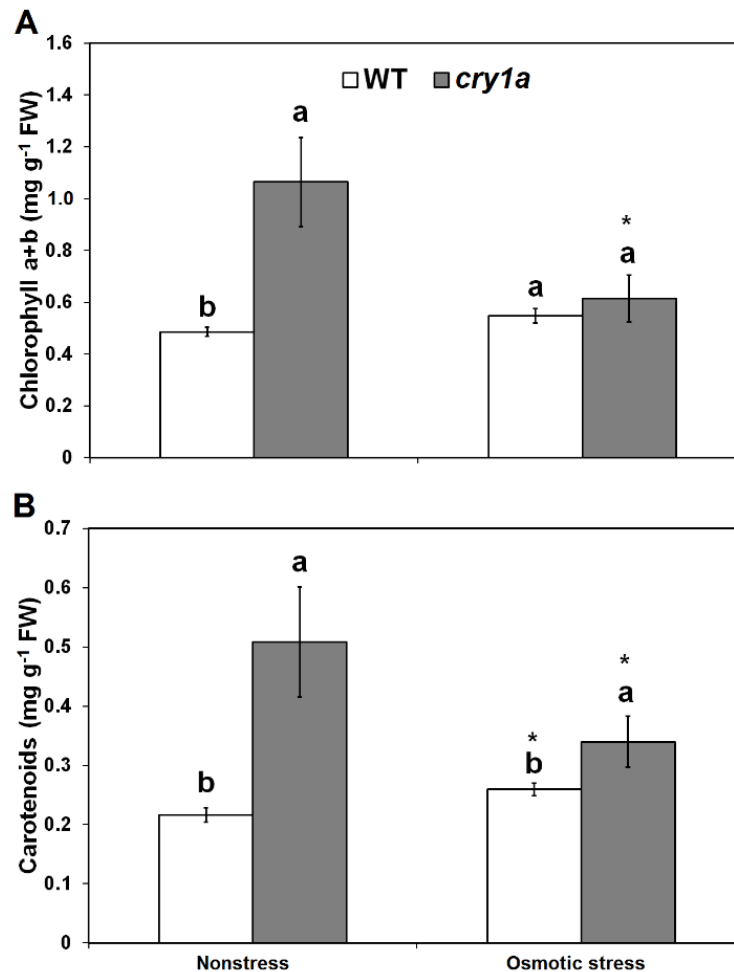


Figure 3. Osmotic stress influences the cotyledon pigmentation of the tomato (*Solanum lycopersicum* L.) *cryptochrome 1a* mutant. Cotyledon pigment content of tomato *cry1a* mutant and wild-type (WT) seedlings grown under a 12 h white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) photoperiod under nonstress (water) or osmotic stress (PEG₆₀₀₀; -0.3 MPa) conditions: (A) chlorophyll a+b; (B) carotenoids. The data are shown as the mean values of 3 pots (at least 27 seedlings). The bars represent $\pm$ SEs. The distinct letters indicate significant differences between genotypes within the same growth condition, and the asterisks represent significant differences among growth conditions within each genotype. The mean values were compared by Tukey's test (significance at $p \leq 0.05$). *P* values for two-way ANOVA are described in Table 2 of Appendix A.

3.3. Biochemical responses to osmotic stress are differentially changed in *cry1a* seedlings

The *cry1a* mutant approximately halved the concentrations of MDA and H₂O₂ compared to WT under nonstress conditions (as indicated by significant genotype x

osmotic condition interactions; Table 2 of Appendix A) (Figures 4A and B). As expected, seedlings of both genotypes accumulated higher levels of H₂O₂ and MDA when exposed to PEG₆₀₀₀ treatment compared with the nonstress conditions (Figures 4A and B). Although no apparent difference was found between the genotypes grown under PEG₆₀₀₀ treatment in terms of the H₂O₂ content, compared with the WT seedlings, the *cry1a* seedlings presented an MDA accumulation that had increased by 0.4-fold.

Notably, compared with the WT, *cry1a* showed a 0.4-fold lower proline content under nonstress conditions (Figure 4D). Moreover, a significant increase in proline content in both genotypes was verified under PEG₆₀₀₀ treatment compared with the nonstress conditions (Figure 4D). Interestingly, compared with the WT, *cry1a* accumulated 0.2-fold more proline in the PEG₆₀₀₀ treatment. These results indicate that tomato *cry1a* plays a role in proline accumulation after OS induction by low water potential (significant genotype x osmotic condition interaction; Table 2 of Appendix A).

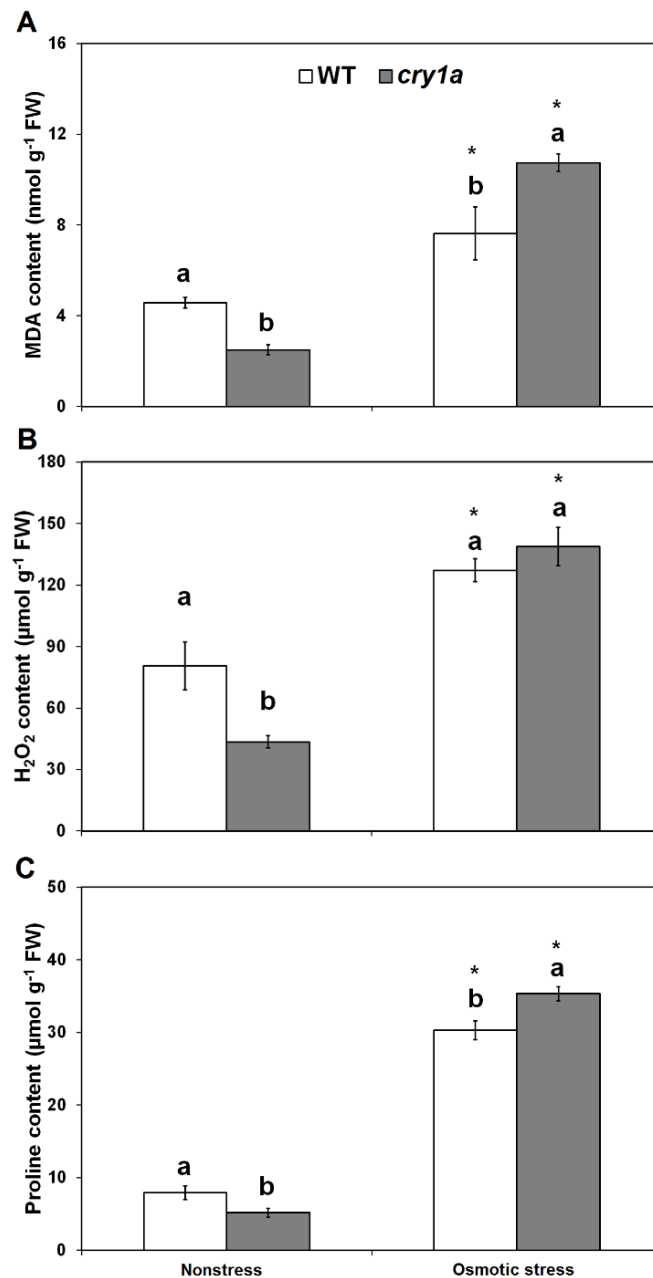


Figure 4. CRY1a loss of function alters osmotic stress responses in tomato (*Solanum lycopersicum* L.). Oxidative stress and proline assays of tomato *cry1a* mutant and wild-type (WT) seedlings grown under a 12 h white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) photoperiod under nonstress (water) or osmotic stress (PEG₆₀₀₀; -0.3 MPa) conditions: (A) malondialdehyde content; (B) hydrogen peroxide content; (C) proline content. The data are shown as the mean values of 3 pots (at least 27 seedlings). The bars represent $\pm$ SEs. The distinct letters indicate significant differences between genotypes within the same growth condition, and the asterisks represent significant differences among growth conditions within each genotype. The mean values were compared by Tukey's test (significance at $p \leq 0.05$). P values for two-way ANOVA are described in Table 2 of Appendix A.

3.4. The growth of *cry1a* seedlings under osmotic stress is affected in response to blue light fluence rate

To determine whether tomato responses to OS depend on different BLF, we tested the effects of BLFRs from 1 to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on physiological responses of *cry1a* mutant. *cry1a* overall showed longer hypocotyls compared to WT at all BLFRs when seedlings were grown under nonstress conditions (Table 3 of Appendix A; Figure 5A; Figure 6). Nevertheless, after PEG₆₀₀₀ treatment, the *cry1a* hypocotyl length was 0.3-fold longer than that of WT only at 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLF (highly significant effects of genotype and osmotic condition, $P < 0.01$; Table 3 of Appendix A). In addition, compared with WT seedlings, *cry1a* seedlings grown under nonstress conditions exhibited a greater root length (0.2-fold) at 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLF; however, the root length of the mutants decreased at 5 (0.1-fold) and 15 (0.3-fold) $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLF compared to that of WT seedlings (Figure 5D). The *cry1a* seedlings grown under PEG₆₀₀₀ treatment displayed longer roots than did the WT seedlings at 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLF (0.3-fold).

Under the nonstress conditions, the *cry1a* mutant showed a reduced shoot dry weight (0.2-fold) compared with WT seedlings at the lowest BLFR (1 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Figure 5C). However, the shoot dry weight of *cry1a* increased by 0.3-fold compared with that of the WT at 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (as indicated by a significant genotype x osmotic condition interaction at 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFR; Table 3 of Appendix A). Moreover, after PEG₆₀₀₀ treatment, the *cry1a* mutant accumulated a lower shoot biomass at 10 (0.2-fold) and 15 (0.2-fold) $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared with the WT seedlings. On the other hand, the *cry1a* root dry weight was lower than that of the WT under nonstress conditions, both at the lowest (1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and highest (15 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) BLFRs (as showed by a significant genotype x osmotic condition interaction in these BLFRs; Table 3 of Appendix A; Figure 5D). In addition, the 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLF increased the root dry weight by 0.5-fold in the *cry1a* seedlings compared with the WT seedlings under OS.

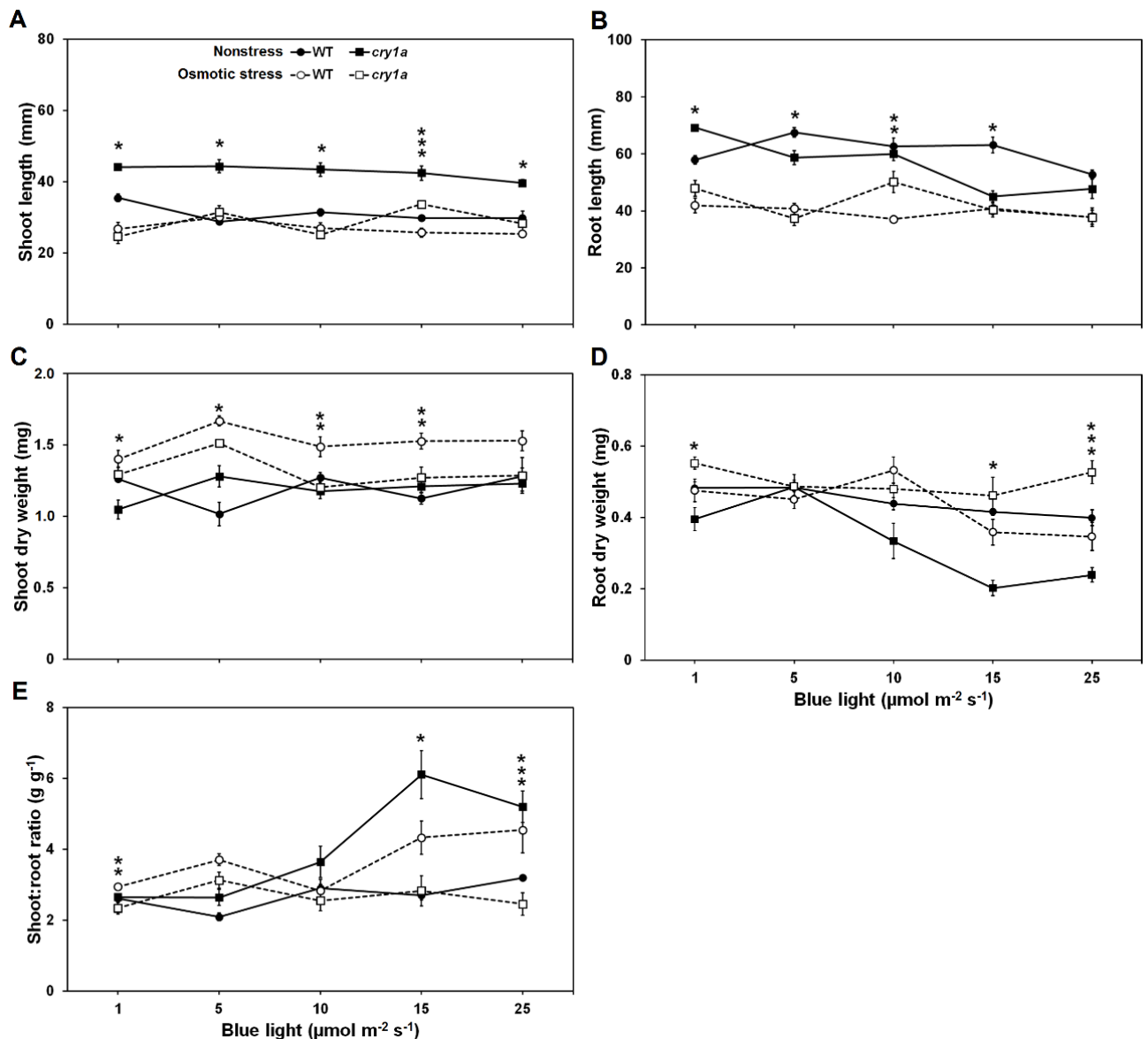


Figure 5. Blue light fluence causes cryptochrome 1a to mediate tomato (*Solanum lycopersicum* L.) growth under osmotic stress. Biometric analysis of tomato *cry1a* mutant and wild-type (WT) seedlings grown in growth chambers under blue light (12 h photoperiod) with fluence rates of 1 to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after induction of osmotic stress (PEG₆₀₀₀, -0.3 MPa). For nonstress conditions, the genotypes remained in water: (A) shoot length; (B) root length; (C) shoot dry weight; (D) root dry weight; (E) the shoot-to-root ratio. The data are shown as the mean values of 3 pots (at least 27 seedlings). The bars indicate $\pm$ SEs. The asterisks represent significant differences between the *cry1a* mutant and the WT within each blue light fluence rate and under the same growth condition (*no stress, ** osmotic stress, *** no stress and osmotic stress). The mean values were compared by Tukey's test (significance at $p \leq 0.05$). P values for two-way ANOVA are described in Table 3 of Appendix A.

Finally, a higher ratio of shoot-to-root dry weight was found in the *cry1a* seedlings compared to the WT seedlings grown under nonstress conditions at 15 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1.3- and 0.6-fold, respectively; Figure 5E). Interestingly, we observed in the PEG₆₀₀₀ treatment that the *cry1a* mutant presented a decreased ratio of shoot-to-root dry weight compared to that of the WT at the lowest (1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the highest (25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) BLFRs tested in this study.

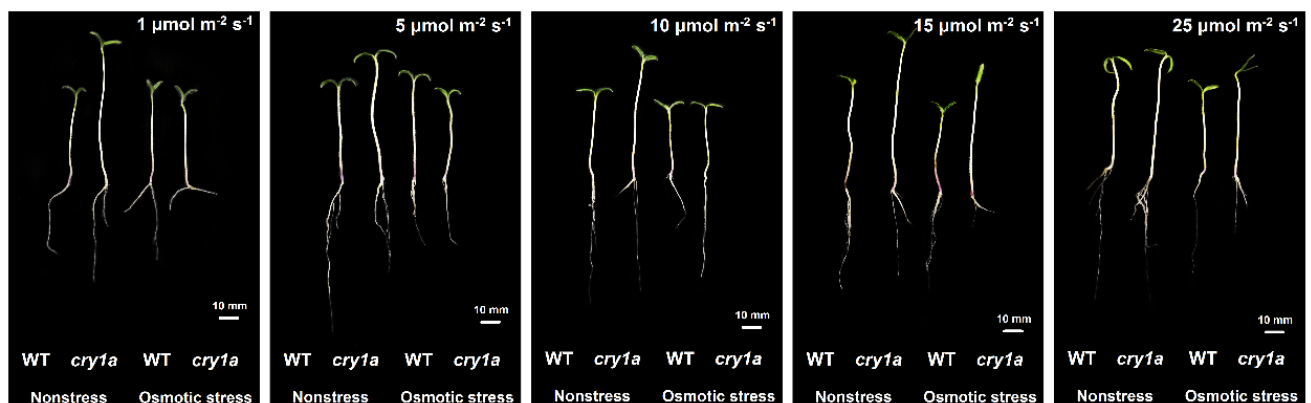


Figure 6. Pictures of tomato (*Solanum lycopersicum* L.) *cry1a* mutant and wild-type (WT) seedlings grown for seven days under nonstress (water) or osmotic stress (PEG₆₀₀₀; -0.3 MPa) conditions under blue light fluence treatments of 1, 5, 10, 15 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

3.5. Pigmentation of *cry1a* seedlings under osmotic stress are dependent on the blue light fluence rate

ANOVA revealed highly significant ($P < 0.01$) genotype x osmotic condition interactions on cotyledon pigments at the 5, 10 and 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFRs, while the other BLFRs showed only the effect of genotype on cotyledons pigments (Table 4 of Appendix A). The 1 (chlorophyll a+b content) and 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (chlorophyll a+b and carotenoid contents) BLFRs increased cotyledon pigments in *cry1a* compared to WT under nonstress conditions (Figures 7A and B). On the other hand, mutant seedlings showed reduced cotyledon pigments compared to WT seedlings at 5 (both 0.3-fold), 10 (both 0.4-fold) and 25 (both 0.3-fold) $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLF under nonstress conditions. Moreover, after PEG₆₀₀₀ treatment, *cry1a* cotyledons displayed higher chlorophyll a+b (1 and 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and carotenoid (5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) contents than did WT cotyledons at low BLFRs, whereas the 10 (carotenoids) and 15 (chlorophyll a+b and carotenoids)

$\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFRs decreased cotyledon pigments in the mutant compared with the control genotype (Figures 7A and B).

ANOVA indicated significant ($P < 0.01$) genotype $\times$ osmotic condition interactions on anthocyanin content at the 10 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFRs (Table 4 of Appendix A). As expected, compared with WT hypocotyls, *cry1a* hypocotyls accumulated reduced anthocyanin content when the seedlings were grown in nonstress conditions at the lowest (0.4-fold at 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and highest (0.4-fold at 15 and 0.7-fold at 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$) BLFs (as showed by a significant effect of genotype in these BLFRs; Table 4 of Appendix A; Figure 7C). Except for 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (no significant effects of genotype and genotype $\times$ osmotic condition interactions; Table 4 of Appendix A), for all BLFs, a lower accumulation of anthocyanins was observed in *cry1a* hypocotyls than in WT hypocotyls under PEG₆₀₀₀ treatment (Figure 7C).

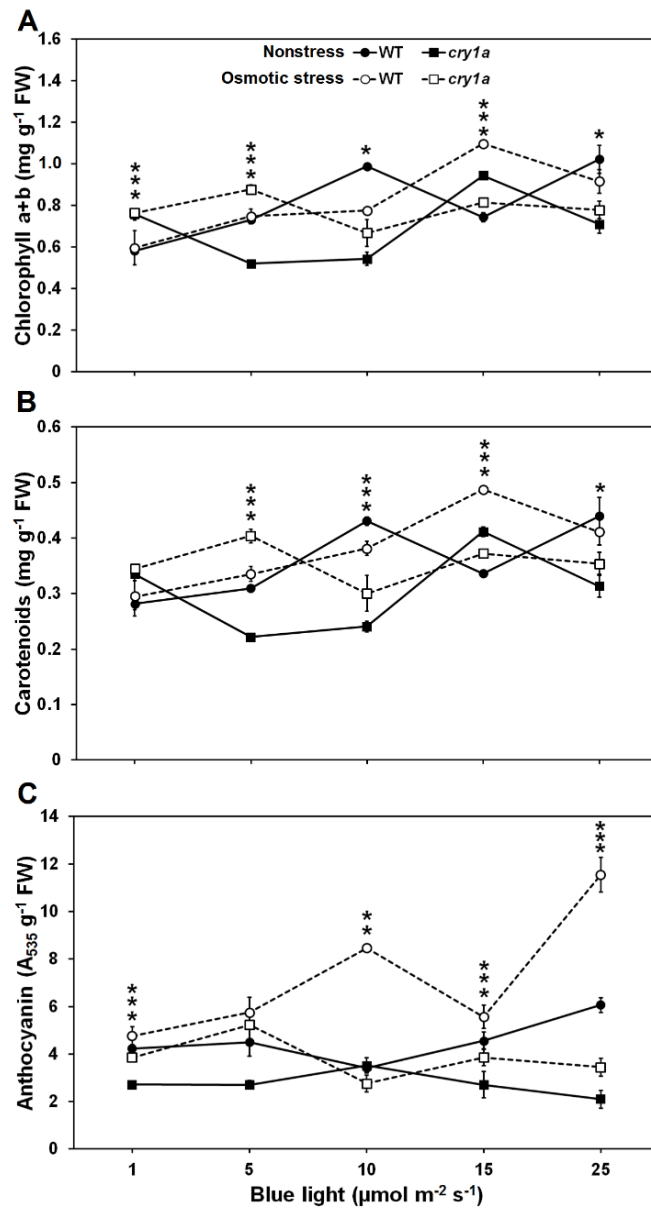


Figure 7. Cryptochrome 1a of tomato (*Solanum lycopersicum* L.) depends on blue light fluence to regulate seedling pigmentation under osmotic stress. Cotyledon and hypocotyl pigment contents of tomato *cry1a* mutant and wild-type (WT) seedlings grown in growth chambers under blue light (12 h photoperiod) with fluence rates of 1 to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after induction of osmotic stress (PEG₆₀₀₀, -0.3 MPa). As a nonstress condition, genotypes remained in water: (A) chlorophyll a+b; (B) carotenoids; (C) anthocyanins. The data are shown as the mean values of 3 pots (at least 27 seedlings). The bars indicate $\pm$ SEs. The asterisks represent significant differences between the *cry1a* mutant and WT at each blue light fluence rate and under the same growth conditions (* no stress, ** osmotic stress, *** no stress and osmotic stress). The mean values were compared by Tukey's test (significance at $p \leq 0.05$). *P* values for two-way ANOVA are described in Table 2 of Appendix A.

3.6. Blue light-dependent biochemical changes in response to osmotic stress are altered in *cry1a* mutant

Except for $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ (no significant genotype x osmotic condition interactions), ANOVA showed significant genotype x osmotic condition interactions on MDA content at all BLFs tested (Table 4 of Appendix A). In nonstress conditions, no significant difference in MDA content was found between the seedlings of both genotypes (Figure 8A). However, compared with the WT, the *cry1a* mutant exhibited a decreased H_2O_2 content at BLFRs of 5 and $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ (both 0.3-fold; Table 4 of Appendix A; Figure 8B). Compared with WT seedlings, *cry1a* seedlings showed a lower MDA content after OS induction at the 1, 15 and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ BLFRs. On the other hand, the MDA content in *cry1a* was greater than that in WT under PEG_{6000} treatment at the 5 (0.3-fold) and 10 (0.2-fold) $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFRs (Figure 8A). Indeed, the H_2O_2 content was altered under the PEG_{6000} treatment only at a high BLF (Table 4 of Appendix A). The H_2O_2 content was 0.2-fold higher at the $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ BLFR but 0.2-fold lower at the $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ BLFR in *cry1a* compared with the WT (Figure 8B).

We further studied the involvement of BLFRs in *cry1a*-related proline accumulation under PEG_{6000} treatment. Compared to WT under nonstress conditions, the proline content in *cry1a* at 1, 15 and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ BLF was 0.2-, 0.3-, and 0.3-fold lower, respectively (Table 4 of Appendix A; Figure 8C). Similarly, the proline content was lower in *cry1a* than in the WT after OS induction between BLFRs of 1 and $15 \mu\text{mol m}^{-2} \text{s}^{-1}$.

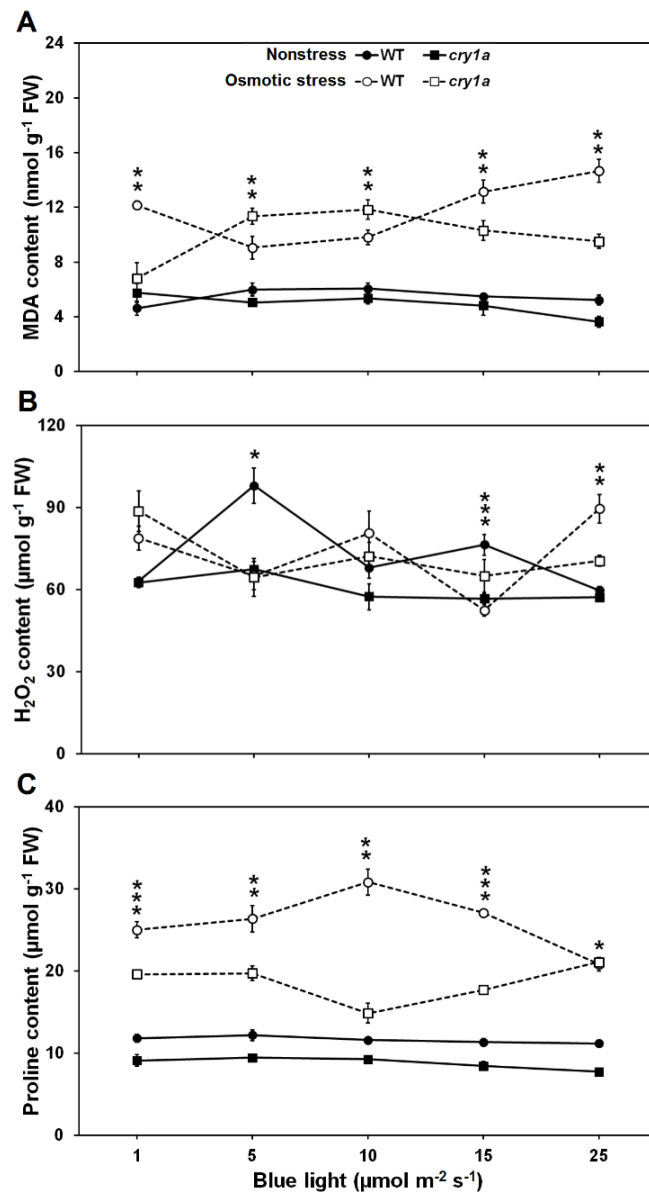


Figure 8. Blue light fluence modulates cryptochrome 1a-related osmotic stress responses in tomato (*Solanum lycopersicum* L.). Oxidative stress and proline assays of tomato *cry1a* mutant and wild-type (WT) seedlings grown in growth chambers under blue light (12 h photoperiod) with fluence rates of 1 to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after induction of osmotic stress (PEG₆₀₀₀, -0.3 MPa). As a nonstress condition, genotypes remained in water: (A) malondialdehyde content; (B) hydrogen peroxide content; (C) proline content. The data are shown as the mean values of 3 pots (at least 27 seedlings). The bars indicate $\pm$ SEs. The asterisks represent significant differences between the *cry1a* mutant and WT at each blue light fluence rate and under the same growth conditions (* no stress, ** osmotic stress, *** no stress and osmotic stress). The mean values were compared by Tukey's test (significance at $p \leq 0.05$). *P* values for two-way ANOVA are described in Table 2 of Appendix A.

4. DISCUSSION

Plant photoreceptors are key mediators of environmental stress responses. Phytochromes have been the most characterized photoreceptors in terms of abiotic stress responses (Carvalho et al., 2011; D'Amico-Damião et al., 2015; Gavassi et al., 2017). However, the emerging role of crys is remarkable (D'Amico-Damião and Carvalho, 2018), especially in the response to water deficit, one of the most critical abiotic stresses in plants (Lesk et al., 2016; González-Villagra et al., 2017; Sseremba et al., 2018). Studies often focused on the model plant species *Arabidopsis*, which has no agronomic relevance (Mao et al., 2005; Xu et al., 2009). On the other hand, tomato is an herbaceous species with a well-described genome that produces fruits with remarkable commercial interest, being one of the most important vegetable crop species worldwide (Sant'Ana and Lefsrud, 2018). Additionally, photomorphogenetic mutants of this species, such as *cry1a* mutants, have been well characterized and established (Weller et al., 2001., Liu et al., 2018a). Here, we showed that (i) *cry1a* modulates tomato seedling photomorphogenesis under nonstress conditions; (ii) BLFRs affects the *cry1a* modulation of photomorphogenic responses; (iii) the lack of *cry1a* increases OS sensitivity in the mutant; and (iv), in response to BLF signals, *cry1a* alleviates OS in tomato seedlings. A summary of the results obtained is shown in Tables 5 and 6 of Appendix A.

4.1. *cry1a* depends on blue light fluence to modulate tomato photomorphogenesis

All data discussed in this topic refer to the nonstress growth condition in order to an initial genotype characterization. Regarding the *cry1a* elongated-shoot-and-root phenotype (Figures 1A and B), *cry1a* inhibits the growth of these organs, specifically by suppressing cell elongation (Sharma et al., 2014). In fact, the control of shoot and root elongation was not a result of altered biomass accumulation in these tissues (Figures 1C and D). However, we also investigated the effects of BLF on tomato *cry1a* growth responses because these events seem to be complex, as they depend on plant

species, organ type, stage of development and BLFR (Kami et al., 2010; Yang et al., 2017; D'Amico-Damião et al., 2019; Fantini et al., 2019). The tomato mutant seedlings lacking *cry1a* exhibited a longer hypocotyl at all BLFs tested (Figure 5A). Moreover, it is well known in other species that *cry1* depends on BLF to promote primary root growth (Canamero et al., 2006). However, this effect appears to be dependent on BLFRs higher than $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in tomato (Figure 5B). We also found no differences in the shoot dry weight and root dry weight of the mutant at intermediate BLFRs, similar to the results obtained under WL experiment. In contrast, the lowest and the highest BLFRs tested could alter biomass accumulation in these organs of *cry1a* (Figures 5C and D). It is reasonable to speculate that *cry2* may act in these responses also by influencing tomato shoot and root growth depending on the BLFRs (Fantini et al., 2019). Furthermore, *cry1a* strongly reduced the root dry weight at the highest BLF used as a result of an enhanced shoot-to-root ratio (Figure 5E).

Previous studies have elucidated the role of *cry1a* in the pigmentation of tomato cotyledons and hypocotyls (Ninu et al., 1999; Weller et al., 2001; Fantini et al., 2019). We verified that *cry1a* negatively regulates tomato cotyledon pigmentation because *cry1a* seedlings showed higher chlorophyll and carotenoid contents compared to WT seedlings (Figures 3A and B). In other species, the expression of the *AtCLH1* (upregulated) and *AtCLH2* (downregulated) genes, which are related to chlorophyll catabolism, is regulated by *cry1* (Banaś et al., 2011). Furthermore, carotenoids are nonenzymatic antioxidants that quench singlet oxygen in chloroplasts (DellaPenna and Barry, 2006; Ramel et al., 2012) and exhibit increased synthesis in the *cry1a* (Figure 3B), indicating a prominent role of this photoreceptor in carotenoid accumulation in tomato seedlings. However, specific BLFs are crucial for *cry1a*-mediated cotyledon pigmentation, since the BLF-treated mutant exhibited different behavior in comparison with the WT. Moreover, the *cry1a* cotyledons increased pigment contents at BLFRs of 1 and $15 \mu\text{mol m}^{-2} \text{s}^{-1}$, while other BLFRs tested decreased the pigmentation of *cry1a* cotyledons (Figures 7A and B). In tomato, *CRY1a* is strongly related to the BL-dependent promotion of anthocyanin content, as evidenced by a reduced accumulation in the *cry1a* mutant hypocotyl (Liu et al., 2018b). Interestingly, we did not find any differences in anthocyanin content between *cry1a* and WT at intermediate BLFRs

(Figure 7C), although we confirmed the previous behavior highlighted for the *cry1a* mutant after testing the other BLFRs.

Plants alter their ROS metabolism to acclimate and adapt to environmental fluctuations (Waszczak et al., 2018). Moreover, relatively high levels of ROS can induce a series of defense mechanisms, such as antioxidant activity and accumulation of osmoprotectants (Ashraf and Foolad, 2007; Foyer, 2018). Recently, several studies have addressed the role of *crys* in regulating the expression of genes whose products are involved in ROS scavenging, H₂O₂ signaling and ROS formation during the *crys* photocycle (Chen et al., 2013; Consentino et al., 2015; Jourdan et al., 2015; El-Esawi et al., 2017). In this sense, the lipid peroxidation, ROS formation and proline content in tomato mutant seedlings were reduced compared to those in the WT seedlings (Figure 4), demonstrating a positive role of *cry1a* in these responses. Indeed, ROS signaling is modulated upstream by the activity of *crys* and CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) in Arabidopsis (Chen et al., 2013). Moreover, the interaction between *cry1* and COP1 depends on BLF (Liu et al., 2011b). Except in response to the 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFR, the H₂O₂ and proline contents decreased in *cry1a* compared with WT under all BLFs tested (Figures 8B and C), similar to the results obtained under WL (Figures 4B and C). However, the lipid peroxidation modulated by *cry1a* appears to be independent of BLF-related signaling because no difference was found in the MDA content between genotypes grown under BL (Figure 8A).

4.2. Blue light fluence signals *cry1a* to alleviate osmotic stress in tomato

The emerging role of *crys* in water deficit acclimation has been highlighted in several plant species (D'Amico-Damião and Carvalho, 2018). Specifically, the lack of *cry1* in Arabidopsis and Brassica results in more efficient growth and water relation mechanisms that provide tolerance to OS (Mao et al., 2005; Xu et al., 2009; Sharma et al., 2014). Otherwise, tomato *cry1a* appears to positively regulate seedling growth under OS conditions considering the reduced elongation and biomass accumulation of the *cry1a* mutant under PEG₆₀₀₀ treatment (Figure 1). Moreover, *cry1a* requires a BLFR

between 10 and 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to promote shoot biomass accumulation under OS conditions, since the shoot dry weight accumulation of the *cry1a* mutant was impaired (Figure 5C). However, *cry1a* inhibited seedling elongation within the same BLF range, as the mutant showed greater hypocotyl and root length after OS induction (Figures 5A and B). Although *cry1a* promotes root growth under OS (Figures 1B and D) *cry1a* allocated less biomass to this organ at the highest BLFR (Figure 5D) and produced more shoot growth than root growth (Figure 5E). Nevertheless, the mutation also decreased the shoot-to-root ratio at the lowest BLFR tested (Figure 5E), which seems to be a shade-avoidance response due to the low BLF perceived primarily by *cry1* (Keller et al., 2011). In addition, abiotic stress-responsive genes are regulated by BL in tomato, and their expression triggers physiological responses depending on the BLF (Lopez et al., 2012; Omidvar and Fellner, 2015; El-Esawi et al., 2017).

The mechanisms underlying water deficit responses can alter plant pigment contents (Jangid and Dwivedi, 2016; Dalal and Tripathy, 2018). *crys* are known to modulate chlorophyll and carotenoid biosynthesis in a BL-dependent manner (Facella et al., 2008; Banaś et al., 2011). Interestingly, although no changes have been observed in chlorophyll (Figure 3A), *cry1a* negatively modulated carotenoid biosynthesis under OS conditions, as high levels of this pigment were observed in the mutant (Figure 3B). Indeed, *cry1a* is required during OS to protect cotyledon carotenoid damage, which could indicate a relatively effective mechanism against stressful conditions (Ramel et al., 2012; Bandurska et al., 2013). However, this mechanism involves a BLFR lower than 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to increase *cry1a* carotenoid and chlorophyll levels (Figures 7A and B) according to the aforementioned results (Figure 3). The 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BLFR appears to be a threshold for *cry1a* to negatively or positively regulate cotyledon pigmentation, because above this BLFR, the pigment levels were severely reduced in the mutant after OS induction (Figures 7A and B). In fact, the differences observed when *cry1a* plants were grown under WL and BL could be a photoprotective response of these pigments against high light stress caused by the high energy of BL wavelengths (Demmig-Adams and Adams, 1992; Demarsy et al., 2018) given the relevant role of *crys* for photoprotection (Weston et al., 2000). In addition to carotenoids and chlorophyll, anthocyanins are strongly related to high-

radiation-stress responses, since BL comprises highly energetic short wavelengths that influence anthocyanin accumulation (Steyn et al., 2002; Johkan et al., 2010). Moreover, the role of anthocyanins in response to other abiotic stresses, such as drought (González-Villagra et al., 2017), has been highlighted due to their high activity as a nonenzymatic antioxidant (Petrussa et al., 2013). To separate OS from high-radiation stress, we decided to investigate the anthocyanin accumulation in tomato seedlings. Using BLF tests, we verified that under OS, *cry1a* also positively regulates the anthocyanin content (Figure 7C).

crys participate in ROS metabolism (Chen et al., 2013; Zhou et al., 2018). Although no changes in H_2O_2 content were observed under OS conditions (Figure 4B), BLFRs higher than $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ seemed to affect ROS formation in the *cry1a* seedlings (Figure 8B). ROS signaling under BL was approximately 2.5-fold greater in transgenic Arabidopsis roots that overexpressed *CRY1* than in the *cry1cry2* double mutant roots (El-Esawi et al., 2017). In addition, it has been shown that ROS-responsive genes (*APX2*, *ZAT10* and *SIB1*) are regulated in large part by *cry1* (Chen et al., 2013). For instance, H_2O_2 may act as a signaling molecule at low concentrations (Waszczak et al., 2018), while at higher levels, it is a harmful form of ROS that leads to MDA formation, an indicator of lipid peroxidation (Foyer, 2018; Huang et al., 2019). In general, *cry1a* negatively regulated lipid peroxidation under PEG₆₀₀₀ treatment because the mutant had a high MDA content (Figure 4A). Notably, BLFRs between 5 and $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ caused the MDA content to increase in the mutant after OS induction, although this response was different under the other BLFs used on *cry1a* tomato (Figure 8A). In this way, osmoprotectants scavenge ROS and play a role in osmotic adjustment during lipid peroxidation (Ashraf and Foolad, 2007), highlighting proline accumulation, which is a well-known response during OS in tomato (Jangid and Dwivedi, 2016; Ferreira-Júnior et al., 2018). In addition, a recent report highlighted that light controls salt-induced proline accumulation via ELONGATED HYPOCOTYL 5 in Arabidopsis (Kovács et al., 2019). The proline content was greater in the mutant seedlings than in the WT seedlings, showing that *cry1a* negatively regulates this response under OS conditions (Figure 4C). Nevertheless, BLFRs below $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ reduced the proline accumulation in the *cry1a* mutant compared with the WT (Figure

8C). Interestingly, BL signaling caused responses that were opposite those obtained in seedlings grown under WL. It is possible that other photoreceptors interact with cry1a during OS to modulate proline accumulation and that these molecules are activated by wavelengths other under WL. For example, strong candidates would be tomato phytochromes, as mutants defective in *phya*, *phyb1* and *phyb2* show alterations in proline accumulation under water deficit (Gavassi et al., 2017).

crys influence the diverse physiological processes in plants (Kendrick and Kronenberg, 1994; Cashmore et al., 1999; Kami et al., 2010; Chaves et al., 2011). The roles of cry BL photoreceptors have been increasingly attributed to OS acclimation (D'Amico-Damião and Carvalho, 2018). In addition, the use of *cry1* mutants and transgenics in water deficit studies has revealed phenotypes of interest for genetic improvement of osmotic tolerance (Mao et al., 2005; Xu et al., 2009; Sharma et al., 2014). However, little is known about the OS responses mediated by tomato crys. Therefore, to further elucidate the mechanisms involved, we encourage the use of *cry2* single mutants and *cry1acry2* double mutants. However, given the role of *cry1a* in the metabolism of phytohormones, secondary metabolites and even other photoreceptors, the pathways by which *cry1a* regulates plant growth during water deficit are very complex (Casal, 2000; Facella et al., 2012a; Facella et al., 2012b; Liu et al., 2018a; Liu et al., 2018b), thus opening many avenues to be explored, mainly those by which *cry1a* modulates stress tolerance.

5. CONCLUSIONS

We showed that tomato cry1a has a role in seedling growth and the metabolic response to water deficit (Tables 5 and 6 of Appendix A). The functional cry1a protects the seedlings from detrimental effects of OS in tomato. In addition, we found that BLF applied to tomato seedlings influences cry1a-regulated OS responses. However, cry1a requires specific BLFRs to mediate the growth, pigmentation and oxidative stress of tomato seedlings under water deficit. Genetic manipulation of the agricultural traits of tomato *CRY1a* is a potential tool for developing more-drought-tolerant materials.

CRedit author statement

Victor D'Amico-Damião: conceptualization, methodology, validation, formal analysis, investigation, writing - original draft, writing - review & editing. **José Clebson Barbosa Lúcio:** methodology, investigation. **Reginaldo Oliveira:** methodology, investigation. **Lucas Aparecido Gaion:** validation, formal analysis. **Rafael Ferreira Barreto:** methodology, investigation. **Rogério Falleiros Carvalho:** conceptualization, validation, writing - original draft, writing - review & editing, supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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6. REFERENCES

- Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. **Plant, Cell & Environment** 24:1337–1344. doi: 10.1046/j.1365-3040.2001.00778.x
- Alves LR, Monteiro CC, Carvalho RF, Ribeiro PC, Tezotto T, Azevedo RA, Gratão PL (2017) Cadmium stress related to root-to-shoot communication depends on ethylene and auxin in tomato plants. **Environmental and Experimental Botany** 134:102–115. doi: 10.1016/j.envexpbot.2016.11.008
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. **Environmental and Experimental Botany** 59:206–216. doi: 10.1016/j.envexpbot.2005.12.006
- Banaś AK, Łabuz J, Sztatelman O, Gabryś H, Fiedor L (2011) Expression of enzymes involved in chlorophyll catabolism in *Arabidopsis* is light controlled. **Plant Physiology** 57:1497–1504. doi: 10.1104/pp.111.185504
- Bandurska H, Niedziela J, Chadzinikolau T (2013) Separate and combined responses to water deficit and UV-B radiation. **Plant Science** 213:98–105. doi: 10.1016/j.plantsci.2013.09.003
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. **Plant Soil** 39:205–207. doi: 10.1007/BF00018060
- Bian Z, Zhang X, Wang Y, Lu C (2019). Improving drought tolerance by altering the photosynthetic rate and stomatal aperture via green light in tomato (*Solanum lycopersicum* L.) seedlings under drought conditions. **Environmental and Experimental Botany** 167:103844. doi: 10.1016/j.envexpbot.2019.103844
- Canamero RC, Bakrim N, Bouly JP, Garay A, Dudkin EE, Habricot Y, Ahmad M (2006) Cryptochrome photoreceptors cry1 and cry2 antagonistically regulate primary root elongation in *Arabidopsis thaliana*. **Planta** 224:995–1003. doi: 10.1007/s00425-006-0280-6
- Carvalho RF, Campos ML, Azevedo RA (2011) The role of phytochromes in stress tolerance. In: Ahmad P, Azooz MM, Prasad MNV (Eds.) **Salt Stress in Plants**. New York: Springer, p. 283–299. doi: 10.1007/978-1-4614-6108-1_12
- Casal JJ, Yanovsky MJ (2004) Regulation of gene expression by light. **International Journal of Developmental Biology** 49:501–511. doi: 10.1387/ijdb.051973jc

Casal JJ (2000) Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. **Photochemistry and Photobiology** 71:1–11. doi: 10.1562/0031-8655(2000)0710001PCPPII2.0.CO2

Cashmore AR, Jarillo JA, Wu YJ, Liu D (1999) Cryptochromes: blue light receptors for plants and animals. **Science** 284:760–765. doi: 10.1126/science.284.5415.760

Chaves I, Pokorný R, Byrdin M, Hoang N, Ritz T, Brettel K, Essen LO, van der Horst GTJ, Batschauer A, Ahmad M (2011) The cryptochromes: blue light photoreceptors in plants and animals. **Annual Review of Plant Biology** 62:335–364. doi: 10.1146/annurev-arplant-042110-103759

Chen D, Xu G, Tang W, Jing Y, Ji Q, Fei Z, Lin R (2013) Antagonistic basic helix-loop-helix/bZIP transcription factors form transcriptional modules that integrate light and reactive oxygen species signaling in *Arabidopsis*. **The Plant Cell** 25:1657–1673. doi: 10.1105/tpc.112.104869

Chen M, Chory J (2011) Phytochrome signaling mechanisms and the control of plant development. **Trends in Cell Biology** 21:664–671. doi: 10.1016/j.tcb. 2011.07.002

Christie JM, Blackwood L, Petersen J, Sullivan S (2015) Plant flavoprotein photoreceptors. **Plant and Cell Physiology** 56:401–413. doi: 10.1093/pcp/pcu196

Consentino L, Lambert S, Martino C, Jourdan N, Bouchet PE, Witczak J, Castello P, El-Esawi M, Corbineau F, d'Harlingue A, Ahmad M (2015). Blue-light dependent reactive oxygen species formation by *Arabidopsis* cryptochrome may define a novel evolutionarily conserved signaling mechanism. **New Phytologist** 206:1450–1462. doi: 10.1111/nph.13341

D'Amico-Damião V, Carvalho RF (2018) Cryptochrome-related abiotic stress responses in plants. **Frontiers in Plant Science** 9:1897. doi: 10.3389/fpls.2018.01897

D'Amico-Damião V, Cruz FJR, Gavassi MA, Santos DMM, Melo HC, Carvalho RF (2015) Photomorphogenic modulation of water stress in tomato (*Solanum lycopersicum* L.): the role of phytochromes A, B1, and B2. **The Journal of Horticultural Science and Biotechnology** 90:25–30. doi: 10.1080/14620316.2015.11513149

D'Amico-Damião V, dos Santos JC, Girotto NC, Carvalho RF (2019) Cryptochrome 1a influences source-sink partitioning during different stages of growth in tomato. **Theoretical and Experimental Plant Physiology** 31:295–302. doi: 10.1007/s40626-019-00141-1

Dalal VK, Tripathy BC (2018) Water-stress induced downsizing of light-harvesting antenna complex protects developing rice seedlings from photo-oxidative damage. **Scientific Reports** 8:5955. doi: 10.1038/s41598-017-14419-4

DellaPenna D, Pogson BJ (2006) Vitamin synthesis in plants: tocopherols and carotenoids. **Annual Review of Plant Biology** 57:711–738. doi: 10.1146/annurev.arplant.56.032604.144301

Demarsy E, Goldschmidt-Clermont M, Ulm R (2018) Coping with ‘dark sides of the sun’ through photoreceptor signaling. **Trends in Plant Science** 23:260–271. doi: 10.1016/j.tplants.2017.11.007

Demmig-Adams B, Adams III, WW (1992) Photoprotection and other responses of plants to high light stress. **Annual Review of Plant Biology** 43:599–626. doi: 10.1146/annurev.pp.43.060192.003123

El-Esawi M, Arthaut LD, Jourdan N, d’Harlingue A, Link J, Martino CF, Ahmad M (2017) Blue-light induced biosynthesis of ROS contributes to the signaling mechanism of Arabidopsis cryptochrome. **Scientific Reports** 7:13875. doi: 10.1038/s41598-017-13832-z

Facella P, Daddiego L, Giuliano G, Perrotta G (2012a). Gibberellin and auxin influence the diurnal transcription pattern of photoreceptor genes via CRY1a in tomato. **PLoS One** 7:e30121. doi: 10.1371/journal.pone.0030121

Facella P, Daddiego L, Perrotta G (2012b). CRY1a influences the diurnal transcription of photoreceptor genes in tomato plants after gibberellin treatment. **Plant Signaling & Behavior** 7:1034–1036. doi: 10.4161/psb.20657

Facella P, Lopez L, Carbone F, Galbraith DW, Giuliano G, Perrotta G (2008) Diurnal and circadian rhythms in the tomato transcriptome and their modulation by cryptochrome photoreceptors. **PLoS One** 3:e2798. doi: 10.1371/journal.pone.0002798

Fantini E, Sulli M, Zhang L, Aprea G, Jiménez-Gómez JM, Bendahmane A, Perrotta G, Giuliano G, Facella P (2019) Pivotal roles of cryptochromes 1a and 2 in tomato development and physiology. **Plant Physiology** 179:732–748. doi: 10.1104/pp.18.00793

Ferreira Júnior DC, Gaion LA, Sousa Júnior GS, Santos DMM, Carvalho RF (2018) Drought-induced proline synthesis depends on root-to-shoot communication mediated by light perception. **Acta Physiologiae Plantarum** 40:15. doi: 10.1007/s11738-017-2591-6

Foyer CH (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. **Environmental and Experimental Botany** 154:134–142. doi: 10.1016/j.envexpbot.2018.05.003

Galvão VC, Fankhauser C (2015) Sensing the light environment in plants: photoreceptors and early signaling steps. **Current Opinion in Neurobiology** 34:46–53. doi: 10.1016/j.conb.2015.01.013

Gavassi MA, Monteiro CC, Campos ML, Melo HC, Carvalho RF (2017) Phytochromes are key regulators of abiotic stress responses in tomato. **Scientia Horticulturae** 222:126–135. doi: 10.1016/j.scienta.2017.04.035

González-Villagra J, Kurepin LV, Reyes-Díaz MM (2017) Evaluating the involvement and interaction of abscisic acid and miRNA156 in the induction of anthocyanin biosynthesis in drought-stressed plants. **Planta** 246:299–312. doi: 10.1007/s00425-017-2711-y

Gratão PL, Monteiro CC, Carvalho RF, Tezotto T, Piotto FA, Peres LEP, Azevedo RA (2012) Biochemical dissection of *diageotropica* and *never ripe* tomato mutants to Cd-stressful conditions. **Plant Physiology and Biochemistry** 56:79–96. doi: 10.1016/j.plaphy.2012.04.009

Guo H, Yang H, Mockler TC, Lin C (1998) Regulation of flowering time by *Arabidopsis* photoreceptors. **Science** 279:1360–1363. doi: 10.1126/science.279.5355.1360

Hajhashemi S, Sofo A (2018) The effect of polyethylene glycol-induced drought stress on photosynthesis, carbohydrates and cell membrane in *Stevia rebaudiana* grown in greenhouse. **Acta Physiologiae Plantarum** 40:142. doi: 10.1007/s11738-018-2722-8

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts, kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics** 125:189–198. doi: 10.1016/0003-9861(68)90654-1

Huang H, Ullah F, Zhou DX, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. **Frontiers in Plant Science** 10:800. doi: 10.3389/fpls.2019.00800

Ito S, Song YH, Imaizumi T (2012) LOV domain-containing f-box proteins: light-dependent protein degradation modules in *Arabidopsis*. **Molecular Plant** 5:573–582. doi: 10.1093/mp/sss013

Jangid KK, Dwivedi P (2016) Physiological responses of drought stress in tomato: a review. **International Journal of Agriculture, Environment and Biotechnology** 9:53. doi: 10.5958/2230-732X.2016.00009.7

Jenkins GI (2014) The UV-B photoreceptor UVR8: from structure to physiology. **The Plant Cell** 26:21–37. doi: 10.1105/tpc.113.119446

Johkan M, Shoji K, Goto F, Hashida SN, Yoshihara T (2010) Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. **HortScience** 45:1809–1814. doi: 10.21273/HORTSCI.45.12.1809

Jourdan N, Martino C, El-Esawi M, Witczak J, Bouchet PE, d'Harlingue A, Ahmad M (2015) Blue-light dependent ROS formation by Arabidopsis cryptochrome-2 may contribute toward its signaling role. **Plant Signaling & Behavior** 10:e1042647. doi: 10.1080/15592324.2015.1042647

Kami C, Lorrain S, Hornitschek P, Fankhauser C (2010) Light-regulated plant growth and development. In: Timmermans MCP (Ed.) **Current Topics in Developmental Biology**. San Diego: Elsevier Academic Press Inc, p. 29–66. doi: 10.1016/S0070-2153(10)91002-8

Keller MM, Jaillais Y, Pedmale UV, Moreno JE, Chory J, Ballaré CL (2011) Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades. **The Plant Journal** 67:195–207. doi: 10.1111/j.1365-313X.2011.04598.x

Kendrick RE, Kronenberg GHM (Eds.) (1994) Photomorphogenesis in Plants. Dordrecht: Kluwer Academic Publishers, 864p. doi: 10.1007/978-94-011-1884-2

Kleine T, Lockhart P, Batschauer A (2003) An *Arabidopsis* protein closely related to *Synechocystis* cryptochrome is targeted to organelles. **The Plant Journal** 35:93–103. doi: 10.1046/j.1365-313X.2003.01787.x

Kleiner O, Kircher S, Harter K, Batschauer A (1999) Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. **The Plant Journal** 19:289–296. doi: 10.1046/j.1365-313X.1999.00535.x

Kovács H, Aleksza D, Baba AI, Hajdu A, Király AM, Zsigmond L, Tóth SZ, Kozma-Bognár L, Szabados L (2019). Light control of salt-induced proline accumulation is mediated by ELONGATED HYPOCOTYL 5 in *Arabidopsis*. **Frontiers in Plant Science** 10:1584. doi: 10.3389/fpls.2019.01584

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. **Nature** 529:84–87. doi: 10.1038/nature16467

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In.: Colowick SP, Kaplan NO (Eds.) **Methods in Enzymology**. New York: Academic Press, p. 350–382. doi: 10.1016/0076-6879(87)48036-1

Lin C, Yang H, Guo H, Mockler T, Chen J, Cashmore AR (1998) Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light receptor cryptochrome 2. **Proceedings of the National Academy of Sciences, USA** 95:2686–2690. doi: 10.1073/pnas.95.5.2686

Liu CC, Ahammed GJ, Wang GT, Xu CJ, Chen KS, Zhou YH, Yu JQ (2018a). Tomato *CRY1a* plays a critical role in the regulation of phytohormone homeostasis, plant development, and carotenoid metabolism in fruits. **Plant, Cell & Environment** 41:354–366. doi: 10.1111/pce.13092

Liu CC, Chi C, Jin LJ, Zhu J, Yu JQ, Zhou YH (2018b). The bZip transcription factor *HY5* mediates *CRY1a*-induced anthocyanin biosynthesis in tomato. **Plant, Cell & Environment** 41:1762–1775. doi: 10.1111/pce.13171

Liu H, Liu B, Zhao C, Pepper M, Lin C (2011a). The action mechanisms of plant cryptochromes. **Trends in Plant Science** 16:684–691. doi: 10.1016/j.tplants.2011.09.002

Liu B, Zuo Z, Liu H, Liu X, Lin C (2011b). *Arabidopsis* cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. **Genes & Development** 25:1029–1034. doi: 10.1101/gad.2025011

Lopez L, Carbone F, Bianco L, Giuliano G, Facella P, Perrotta G (2012) Tomato plants overexpressing cryptochrome 2 reveal altered expression of energy and stress-related gene products in response to diurnal cues. **Plant, Cell & Environment** 35:994–1012. doi: 10.1111/j.1365-3040.2011.02467.x

Mao J, Zhang YC, Sang Y, Li QH, Yang HQ (2005) A role for *Arabidopsis* cryptochromes and COP1 in the regulation of stomatal opening. **Proceedings of the National Academy of Sciences, USA** 102:12270–12275. doi: 10.1073/pnas.0501011102

Mishra S, Khurana JP (2017) Emerging roles and new paradigms in signaling mechanisms of plant cryptochromes. **Critical Reviews in Plant Sciences** 36:89–115. doi: 10.1080/07352689.2017.1348725

Ninu L, Ahmad M, Miarelli C, Cashmore AR, Giuliano G (1999) Cryptochrome 1 controls tomato development in response to blue light. **The Plant Journal** 18:551–556. doi: 10.1046/j.1365-313X.1999.00466.x

Omidvar V, Fellner M (2015) DNA methylation and transcriptomic changes in response to different lights and stresses in *7B-1* male-sterile tomato. **PLoS One** 10:e0121864. doi: 10.1371/journal.pone.0121864

Peters JL, Van Tuinen A, Adamse P, Kendrick RE, Koornneef M (1989) High pigment mutants of tomato exhibit high sensitivity for phytochrome action. **Journal of Plant Physiology** 134:661–666. doi: 10.1016/S0176-1617(89)80024-0

Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S, Vianello A (2013) Plant flavonoids—biosynthesis, transport and involvement in stress responses. **International Journal of Molecular Sciences** 14:14950–14973. doi: 10.3390/ijms140714950

Ramel F, Mialoundama AS, Havaux M (2012) Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants. **Journal of Experimental Botany** 64:799–805. doi: 10.1093/jxb/ers223

Saijo Y, Loo EPI (2019) Plant immunity in signal integration between biotic and abiotic stress responses. **New Phytologist** 225:87–104. doi: 10.1111/nph.15989

Sant'Ana DVP, Lefsrud M (2018) Tomato proteomics: tomato as a model for crop proteomics. **Scientia Horticulturae**. 239:224–233. doi: 10.1016/j.scienta.2018.05.041

Sharma P, Chatterjee M, Burman N, Khurana JP (2014) Cryptochrome 1 regulates growth and development in *Brassica* through alteration in the expression of genes involved in light, phytohormone and stress signalling. **Plant, Cell & Environment** 37:961–977. doi: 10.1111/pce.12212

Sseremba G, Tongoona P, Eleblu J, Danquah EY, Kizito EB (2018) Heritability of drought resistance in *Solanum aethiopicum* Shum group and combining ability of genotypes for drought tolerance and recovery. **Scientia Horticulturae** 240:213–220. doi: 10.1016/j.scienta.2018.06.028

Steyn WJ, W SJE, Holcroft DM, Jacobs G (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. **New Phytologist** 155:349–361. doi: 10.1046/j.1469-8137.2002.00482.x

Vilela FA, Doni Filho L, Siqueira EL (1992) Tabela de potencial osmótico em função da concentração de polietileno glicol 6000 e da temperatura. **Pesquisa Agropecuária Brasileira** 26:1957–1968.

Waszczak C, Carmody M, Kangasjärvi J (2018) Reactive oxygen species in plant signaling. **Annual Review of Plant Biology** 69:209–236. doi: 10.1146/annurev-arplant-042817-040322

Weller JL, Perrotta G, Schreuder MEL, van Tuinen A, Koornneef M, Giuliano G, Kendrick RE (2001) Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. **The Plant Journal** 25:427–440. doi: 10.1046/j.1365-313x.2001.00978.x

Weston E, Thorogood K, Vinti G, López-Juez E (2000) Light quantity controls leaf-cell and chloroplast development in *Arabidopsis thaliana* wild type and blue-light-perception mutants. **Planta** 211:807–815. doi: 10.1007/s004250000392

Xu P, Xiang Y, Zhu H, Xu H, Zhang Z, Zhang C, Zhang L, Ma Z (2009) Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. **Plant Physiology** 149:760–774. doi: 10.1104/pp.108.132217

Yang Z, Liu B, Su J, Liao J, Lin C, Oka Y (2017) Cryptochromes orchestrate transcription regulation of diverse blue light responses in plants. **Photochemistry and Photobiology** 93:112–127. doi: 10.1111/php.12663

Yu X, Liu H, Klejnot J, Lin C (2010) The cryptochrome blue light receptors. **The Arabidopsis Book**. 8:e0135. doi: 10.1199/tab.0135

Zhou T, Meng L, Ma Y, Liu Q, Zhang Y, Yang Z, Yang D, Bian M (2018) Overexpression of sweet sorghum cryptochrome 1a confers hypersensitivity to blue light, abscisic acid and salinity in *Arabidopsis*. **Plant Cell Reports** 37:251–264. doi: 10.1007/s00299-017-2227-8

CHAPTER 4 – Cryptochrome 1a of tomato mediates long-distance signaling of soil water deficit

ABSTRACT – Although the blue light photoreceptors cryptochromes mediate the expression of genes related to reactive oxygen species, whether cryptochrome 1a (*cry1a*) regulates local and long-distance signaling of water deficit in tomato (*Solanum lycopersicum* L.) is unknown. Thus the *cry1a* tomato mutant and its wild-type (WT) were reciprocally grafted (WT/WT; *cry1a/cry1a*; WT/*cry1a*; *cry1a*/WT; as scion/rootstock) or grown on their own roots (WT and *cry1a*) under irrigated and water deficit conditions. Plant growth, pigmentation, oxidative stress, water relations, stomatal characteristics and leaf gas exchange were measured. WT and *cry1a* plants grew similarly under irrigated conditions, whereas *cry1a* plants had less root biomass and length and higher tissue malondialdehyde concentrations under water deficit. Despite greater oxidative stress, *cry1a* maintained chlorophyll and carotenoid concentrations in drying soil. Lower stomatal density of *cry1a* likely increased its leaf relative water content (RWC). In grafted plants, scion genotype largely determined shoot and root biomass accumulation irrespective of water deficit. In chimeric plants grown in drying soil, *cry1a* rootstocks increased RWC while WT rootstocks maintained photosynthesis of *cry1a* scions. Manipulating tomato *CRY1a* may enhance plant drought tolerance by altering leaf pigmentation and gas exchange during soil drying via local and long-distance effects.

Keywords: abiotic stress, *cry1a* mutant, drought, shoot-to-root signaling, *Solanum lycopersicum* L. (tomato), water deficit

1. INTRODUCTION

Soil water deficit decreases crop yields by restricting plant growth and development (Hussain et al., 2018) and changing the expression of thousands of genes (Dubois et al., 2017). Mild soil drying can induce partial stomatal closure to maintain leaf water status without affecting photosynthesis (A), thereby increasing instantaneous water use efficiency (WUE), the ratio of A to transpiration (E). However, prolonged soil drying usually decreases leaf water potential, thereby limiting A via both stomatal and non-stomatal mechanisms (Jangid and Dwivedi, 2016). Reactive oxygen species (ROS) generated during photosynthetic processes can damage cellular membranes, stimulating the upregulation of antioxidant system components (Schneider et al., 2019). Furthermore, drought affects the biosynthesis and degradation of photosynthetic pigments, although enhanced carotenoid content can act as an antioxidant in photosynthetic membrane lipids, augmenting plant drought tolerance (Sharma et al., 2012). Thus soil drying affects plant physiological, biochemical and morphological responses. However, many environmental factors also alter plant responses to drought stress.

Incident light is utilized in the photosynthetic process to split water molecules at photosystem II, which undergoes severe changes during drought stress (Pinheiro and Chaves, 2011). For example, high light stress could aggravate the effects of water deficit, increasing ROS formation if electron transport rate is compromised (Sharma et al., 2012). Furthermore, light quality can affect tolerance to water deficit, as adding green (530 nm) light to a control treatment of red (660 nm) and blue (450 nm) LEDs improved tomato (*Solanum lycopersicum* L.) drought tolerance by decreasing stomatal conductance without limiting photosynthesis, thereby increasing instantaneous water use efficiency (Bian et al., 2019). In addition, using LED lights with peaks in the blue and red spectral regions enhanced chlorophyll a/b ratio in pepper (*Capsicum annuum* L.) seedlings compared to growing plants with compact fluorescent lamps (peaks at green and red spectral regions), thereby increasing electron transport rate while decreasing non-photochemical quenching during water deficit (Hoffmann et al., 2015).

While light quality can enhance drought tolerance by affecting photosynthesis, it is also important to consider its photomorphogenetic role.

Photomorphogenesis is the process by which light modulates plant growth and development from seed germination to senescence. Higher plants encode photoreceptor proteins sensitive to changes in the light quality, quantity, duration and direction. Currently, five plant photoreceptor families are described: UV-B resistance locus 8 (ultraviolet-B light photoreceptor); cryptochromes (crys), phototropins and zeaxanthins (ultraviolet-A/blue light photoreceptors); and phytochromes (red/far-red light photoreceptor) (Galvão and Fankhauser, 2015). Of these, crys photoreceptors (and other) regulate a wide range of physiological and developmental processes, from seed germination to fruiting. In *Arabidopsis thaliana*, three crys were identified (cry1, cry2, and cry3) and characterized (cry1 and cry2) as mediators of seedling de-etiolation, anthocyanin accumulation and cotyledon expansion as well as circadian rhythm and photoperiod-dependent flowering (Yu et al., 2010; Chaves et al., 2011). In tomato, one of the most important vegetables in the world, four cry genes within a multigene family were identified: *CRY1a*, *CRY1b*, *CRY2* and *CRY3*. The nuclear proteins cry1a and cry2 photoreceptors (responsive to both a low- and high-blue light fluence rates) are active in the photomorphogenic responses, while it is not clear if the *CRY1b* gene encodes a functional photoreceptor in tomato. Expression of *CRY3* encodes a DASH protein located in semi-autonomous organelles, that acts in DNA repair mechanisms. Furthermore, cry1a regulates photomorphogenic responses related to early root growth, hypocotyl and stem elongation, pigments biosynthesis and fruit yield (Weller et al., 2001; D'Amico-Damião et al., 2019; Fantini et al., 2019). Several papers have started to investigate whether these cry-mediated effects alter plant drought tolerance.

Changes in gene expression, biochemical responses and stomatal opening might be involved in crys mediating plant drought stress responses (D'Amico-Damião and Carvalho, 2018). For example, crys up-regulate (eg *RD29A*, *ADH1* and *ABA2* in *Arabidopsis*) and down-regulate (eg *LEA*, *RAB18*, *NIA1*, *APX1* and *NAC* in *Brassica napus*) the expression of ABA/stress-responsive genes (Xu et al., 2009; Sharma et al., 2014). Moreover, in tomato grown under optimal conditions (available water and white light), *cry1a* mutant leaves had 20% higher ABA concentrations than wild type plants

(Liu et al., 2018a), which may enhance drought tolerance by improving the regulation of water status via stomatal closure, lessening the risk of plant water deficit (D'Amico-Damião et al., 2015; Bertolino et al., 2019). However, the physiological responses of *cry1a* plants to soil water deficit has not been investigated.

Grafting techniques have been widely used to understand local and long-distance regulation of plant drought stress responses (Albacete et al. 2015; Thomas and Frank, 2019). For example, soil water deficit causes the plant hormone ABA to accumulate throughout the plant, and reciprocal grafting experiments using wild-type and ABA-deficient mutants can resolve the relative importance of roots and shoots in regulating plant responses (Li et al., 2018). Since *cry1* positively affects Arabidopsis root growth by decreasing *cry1* mutant root extension growth under blue light (Canamero et al 2006), it might alter plant drought responses by affecting water uptake. Furthermore, since the *cry1a* mutant enhances foliar ABA accumulation of tomato (Liu et al. 2018a) which might affect stomatal regulation of plant water status, we hypothesized that *cry1a* is involved in long-distance signaling of plant drought stress responses. In other species, cryptochrome-mediated water deficit responses have investigated *cry1*, but tomato has not been investigated. Since *cry2* has been associated with flowering and fruiting in tomato but *CRY2* is unstable under high blue light fluences in Arabidopsis, whereas tomato *cry1a* controls several physiological and developmental process, our efforts focused on the tomato *cry1a* mutant. For the first time, we explored the role of the tomato *CRY1a* gene on plant water stress responses using the photomorphogenetic mutant *cry1a* and grafting to understand the role of this photoreceptor in long-distance signaling (Albacete et al. 2015; Thomas and Frank, 2019). Thus we grew own-rooted and reciprocally grafted *cry1a* and WT tomato plants under well-watered conditions and in drying soil, to evaluate biomass accumulation, plant water relations, stomatal anatomy and biochemical responses.

2. MATERIALS AND METHODS

2.1. Plant material, growth condition and grafting technique

Sterilized seeds (5% NaClO solution for 10 mins followed by thorough washing with water) of the tomato (*Solanum lycopersicum* L.) *cry1a* mutant (Weller et al. 2001) and its wild type (WT; cv. Moneymaker) were used. Seeds were placed in trays containing a substrate (1:1 mixture of pine bark base:expanded vermiculite) supplemented with 1g L⁻¹ of NPK fertilizer 10:10:10 and 4 g L⁻¹ of lime. The plants were grown in a naturally lit greenhouse under an average temperature of 27°C (SD ± 2.37°C) and a relative humidity of 60% (SD ± 13%), under a 12 h/12 h (light/dark) photoperiod at photosynthetic photon flux density (PPFD) of 450–700 µmol m⁻² s⁻¹. Fifteen days after sowing (DAS) on the same substrate, the plants were transferred to 200 mL pots, and grafting performed.

Fifteen-day old plants were grafted by the splice method with the aid of a scalpel blade and grafting clips, to obtain the following graft combinations: WT/WT, *cry1a/cry1a*, WT/*cry1a*, *cry1a*/WT (scion/rootstock) (Figure 1 of Appendix B). Immediately after grafting, the basal quarter of the pots was submerged in water (in a floating moist chamber at 25°C ± 2°C and a high relative humidity: 88% ± 10%) under a 12 h/12 h (light/dark) photoperiod at 45 µmol m⁻² s⁻¹ supplied by white light LEDs, until the graft union had completely healed (30 DAS). During this period, own-rooted plants of *cry1a* and WT genotypes remained in the same conditions as grafted plants. After the graft union had healed, own-rooted and grafted plants were transferred to 2.8 L pots containing the same substrate, where they were irrigated daily until the beginning of the water deficit (40 DAS).

2.2. Water deficit treatment

Preliminary tests with the same pot, substrate and growth conditions aimed to determine soil water holding capacity and plant responses to soil drying. Pots were uniformly filled with the equivalent of 500 g of substrate. After irrigating the substrate

to the drip point, the pots were allowed to drain overnight to determine the drained capacity (1.74 g g^{-1}). Furthermore, plant evapotranspiration was monitored daily (gravimetrically) to estimate soil water availability. After suspending irrigation for ten days, soil moisture had declined to 30% of field capacity (0.53 g g^{-1}) while the irrigated treatment (receiving 500 mL daily, split between early morning and late afternoon) maintained values close field capacity. At the end of the experiment, the substrate was oven dried (105°C for 24 h) to determine the soil moisture. At 50 DAS, the own-rooted and grafted plants were harvested for analysis described below. For further biochemical analyzes, plants were immediately freeze-dried in liquid nitrogen and stored at -80°C .

2.3. Growth analysis

Plant height and maximum root length were determined using a graduated ruler. Shoots and roots were separated, the roots washed to remove the substrate and oven dried at 60°C for 72 hours to measure their dry weights using an analytical scale (Model AA-200, Denver Instrument Company, New York, USA).

2.4. Leaf pigments content

Chlorophyll a+b and carotenoids pigments were extracted (acetone 80%) from the fifth fully expanded leaf (25 mg) and determined by spectrophotometry (DU-640, Beckman Coulter, Fullerton, USA) at 663 nm (chlorophyll a), 647 nm (chlorophyll b) and 470 nm (carotenoids).

2.5. Lipid peroxidation and H_2O_2 content

Lipid peroxidation was evaluated from the fifth fully expanded leaf (500 mg) and roots (700 mg) according to the content of thiobarbituric acid-reactive substances present in the malondialdehyde (MDA) form and the content of hydrogen peroxide (H_2O_2), as determined by reaction with potassium iodide. MDA content was estimated

using an extinction coefficient of $1.55 \times 10^{-5} \text{ mol}^{-1} \text{ cm}^{-1}$. H_2O_2 content was determined using a known concentration curve as a standard (Gaion et al., 2018).

2.6. Leaf relative water content

The relative water content (RWC) was determined with discs (5 mm diameter) of fifth fully expanded leaf, approximately 200 mg of fresh weight, according to the following equation: $(\text{FW}-\text{DW})/(\text{TW}-\text{DW}) \times 100$ (FW, fresh weight; DW, dry weight; TW, turgid weight). The discs were immediately weighed to determine the fresh weight (FW) and subsequently the discs were placed in petri dishes with deionized water for 6 h to determine the turgid weight (TW). Then, the discs were dried at 60°C for 48 h to obtain the dry weight (DW).

2.7. Stomatal measurements

Stomatal density was obtained from the fifth fully expanded leaf using “super glue” to obtain impressions of paradermal sections of the abaxial epidermis using a glass slides microscope and were counted using an optical microscope with micrometric ruler (Gaion et al., 2018). Stomatal pore area was obtained from the same glass slides microscope impressions, and digitized using an optical microscope and a video camera coupled to a microcomputer (IM50, Leica, Copenhagen, Denmark). After images digitization, the stomatal pore area was measured using a Photoshop image processing software (CS5 Extended, Adobe Systems, San Jose, USA).

2.8. Leaf gas exchange

Photosynthesis (A) and leaf transpiration (E) of the fifth fully expanded leaf was measured between 9 and 11 am using a gas exchange system (LCpro, Analytical Development Co., Hoddeston, UK) illuminated with a blue-red LED light source supplying $1,200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD (Gaion et al., 2018). The water use efficiency (WUE) was calculated by dividing A by E .

2.9. Data analyses

The experimental design used was completely randomized with five biological replicates (plants) per treatment, except for leaf gas exchange measurements which were with three biological replicates per treatment. Except for the growth analysis, all measurements comprised 3 technical replicates per leaf. Furthermore, stomatal measurements were performed with 10 technical replicates (randomly selected fields of view) per leaf. Each experiment was repeated at least three times, with data from a representative experiment reported. Own-rooted plants were compared in a 2×2 factorial scheme, consisting of two genotypes (WT and *cry1a*) and two conditions (irrigated and water deficit), using two-way analysis of variance (ANOVA) to determine the main effects of genotype, watering treatment and their interaction (Experiment 1). The grafted plants (Experiment 2) were compared in a 4×2 factorial scheme, comprising four graft combinations (WT/WT, *cry1a/cry1a*, WT/*cry1a* and *cry1a*/WT) and two conditions (irrigated and water deficit) using two-way ANOVA. Furthermore, a three-way ANOVA determined the main effects of rootstock, scion and water treatment. Mean values were compared using Tukey's HSD test (significance at $p \leq 0.05$) via AgroEstat software (www.agroestat.com). Linear regressions determined significant ($P < 0.05$) relationships between variables (r^2 and P -values shown; Table 1 of Appendix B) combining data from both own-rooted and grafted plants.

3. RESULTS

3.1. Response of own-rooted tomato plants to water deficit

The *cry1a* mutant was 20% taller than the WT in both irrigated and water deficit conditions (Figure 1A). Water deficit decreased plant height by 10%, averaged across both genotypes (Figure 1E). Although taller, the *cry1a* mutant had 13% lower shoot biomass in both irrigated and water deficit conditions (Figure 1C). Water deficit decreased shoot biomass by 24%, averaged across both genotypes. The *cry1a* mutant also had less root biomass and length (22 and 15% respectively) than WT plants (Figures 1B and D). Although water deficit decreased root biomass by 11%, maximum root length increased by 12% (averaged across genotypes). The decrease in root biomass seemed accentuated in *cry1a* while the increase in root length seemed greater in WT plants (Figures 1B and D). Thus *cry1a* plants prioritized shoot elongation over shoot biomass accumulation, and had a diminished root system, but otherwise responded similarly to water deficit as WT plants (Figure 2C of Appendix B).

In irrigated conditions, *cry1a* leaves had 29% lower pigment (chlorophyll a+b and carotenoid) contents than WT plants (Figures 2A and B). The two genotypes responded differently to water deficit (as indicated by significant genotype x water interaction), as *cry1a* maintained pigment content but WT plants decreased both chlorophyll a+b and carotenoid contents by 44% (Figures 2A and B). Thus water deficit decreased pigment content of WT plants, but *cry1a* plants maintained pigment content.

Shoot and root malondialdehyde (MDA) content of the *cry1a* mutant was higher (63% and 20% respectively) than WT plants in both irrigated and water deficit conditions (Figures 2C and E). Although there were no significant genotypic effects on shoot hydrogen peroxide (H₂O₂) content (Figure 2D), *cry1a* mutant exhibited 15% lower root H₂O₂ content compared to WT plants (averaged across water availabilities; Figure 2F). Soil water deficit significantly increased whole plant MDA and H₂O₂ contents to a similar extent in both genotypes (no significant genotype x water interaction). Thus MDA accumulation of *cry1a* plants was greater than in WT plants, with similar shoot and root H₂O₂ contents of both genotypes.

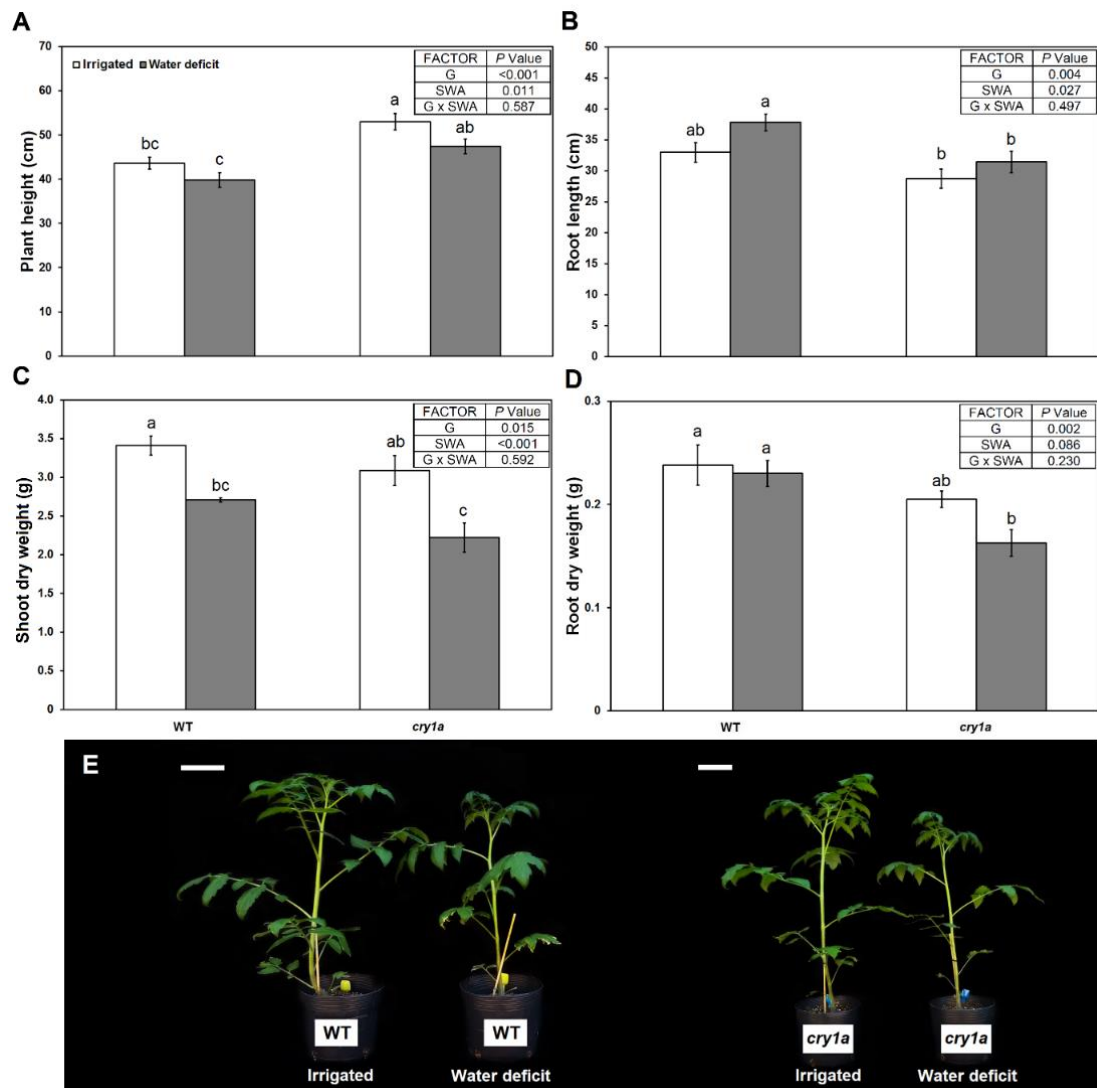


Figure 1. Growth analysis of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) plant height; (B) root length; (C) shoot dry weight; (D) root dry weight. The data are shown as the mean values of 5 plants of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the treatments. The mean values were compared using the Tukey's HSD test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of genotype (G), soil water availability (SWA), and their interactions (G x SWA) after ANOVA. (E) Tomato cv. Moneymaker phenotypes of own-rooted plants of *cry1a* mutant and wild-type (WT) submitted to water deficit treatment or daily irrigated treatment. Pictures were digitally and exhibited followed by correspondent scale bar (10 cm).

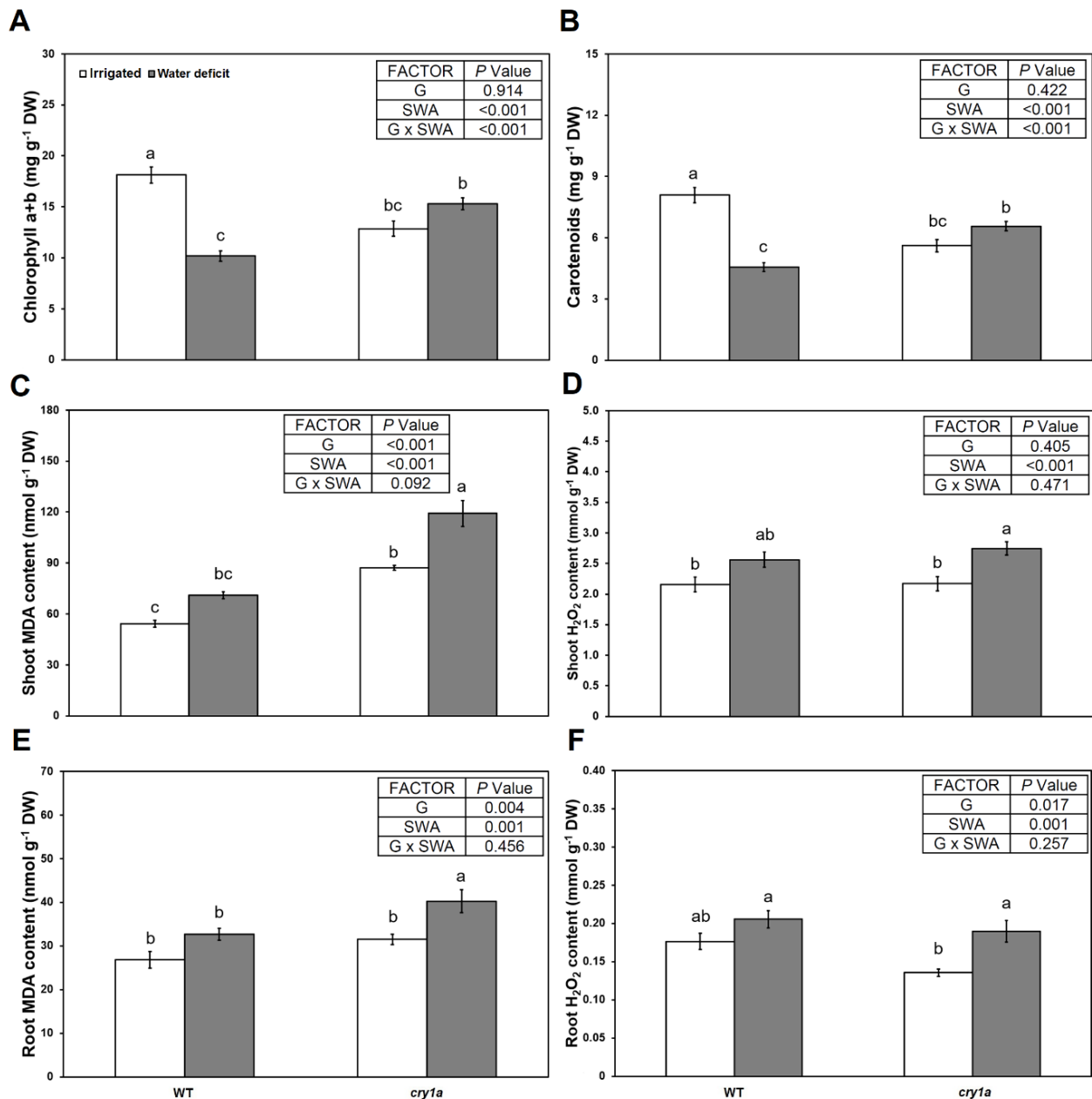


Figure 2. Biochemical analysis of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) chlorophyll a+b; (B) carotenoids; (C) shoot malondialdehyde content; (D) shoot hydrogen peroxide content; (E) root malondialdehyde content; (F) root hydrogen peroxide content. The data are shown as the mean values of 5 plants (3 technical replicates) of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the treatments. The mean values were compared using the Tukey's HSD test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of genotype (G), soil water availability (SWA), and their interactions (G x SWA) after ANOVA.

Leaf relative water content (RWC) of *cry1a* was higher than the WT irrespective of soil moisture (Figure 3A). Both genotypes responded similarly to water deficit (as indicated by no significant genotype x water interaction), which decreased leaf RWC by 5%. Stomatal density of *cry1a* mutant was 31% lower than the WT in both irrigated and water deficit conditions (Figure 3B). Water deficit increased stomatal density to a similar extent (by 21%) in both genotypes (no significant genotype x water interaction). Stomatal pore area of both genotypes was similar, with soil water deficit decreasing it by 75% respectively (averaged across genotypes; Figures 3C and D). Thus the greater leaf RWC of *cry1a* plants was associated with decreased stomatal density, but otherwise the two genotypes responded similarly to water deficit.

Under well-watered conditions, photosynthesis (*A*) and transpiration (*E*) of *cry1a* mutant was 14% and 21% less respectively than WT plants (Figures 4A and B). Soil water deficit decreased *A* similarly in both genotypes by 13%, but had less effect on *E* of *cry1a* (significant genotype x water interaction), since *E* of well-watered *cry1a* plants was lower (Figure 4B). Water use efficiency (WUE = ratio of *A/E*) was higher in WT plants, and increased similarly under water deficit by 34% (averaged across genotypes; Figures 4A and C). Both genotypes showed similar photosynthetic and WUE responses to water deficit.

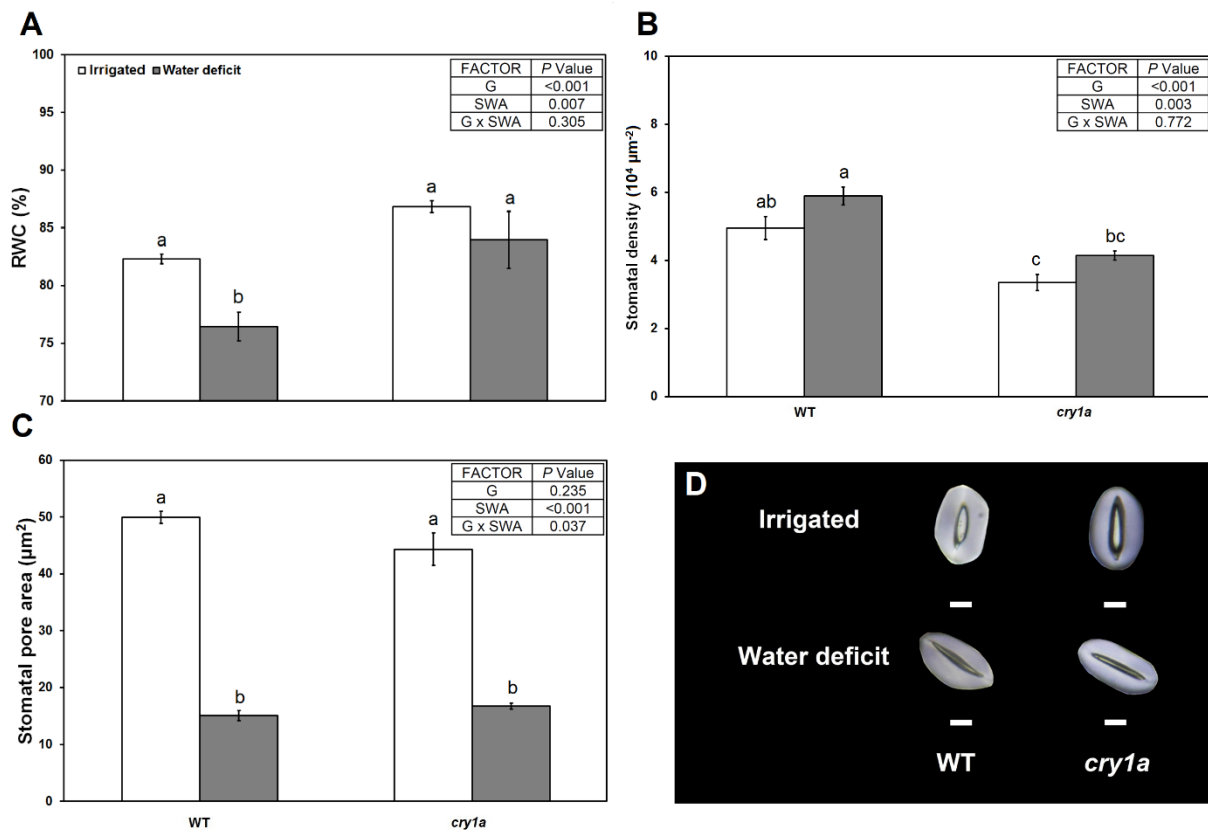


Figure 3. Water relations and stomatal measurements of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) relative water content (RWC); (B) stomatal density; (C) stomatal pore area. The data are shown as the mean values of 5 plants (10 technical replicates) of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the treatments. The mean values were compared using the Tukey's HSD test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of genotype (G), soil water availability (SWA), and their interactions (G x SWA) after ANOVA. (D) Representative images of leaf abaxial epidermis stomata from *cry1a* and WT plants grown under irrigated condition or subjected to water deficit condition. Scale bars: 10 μ m.

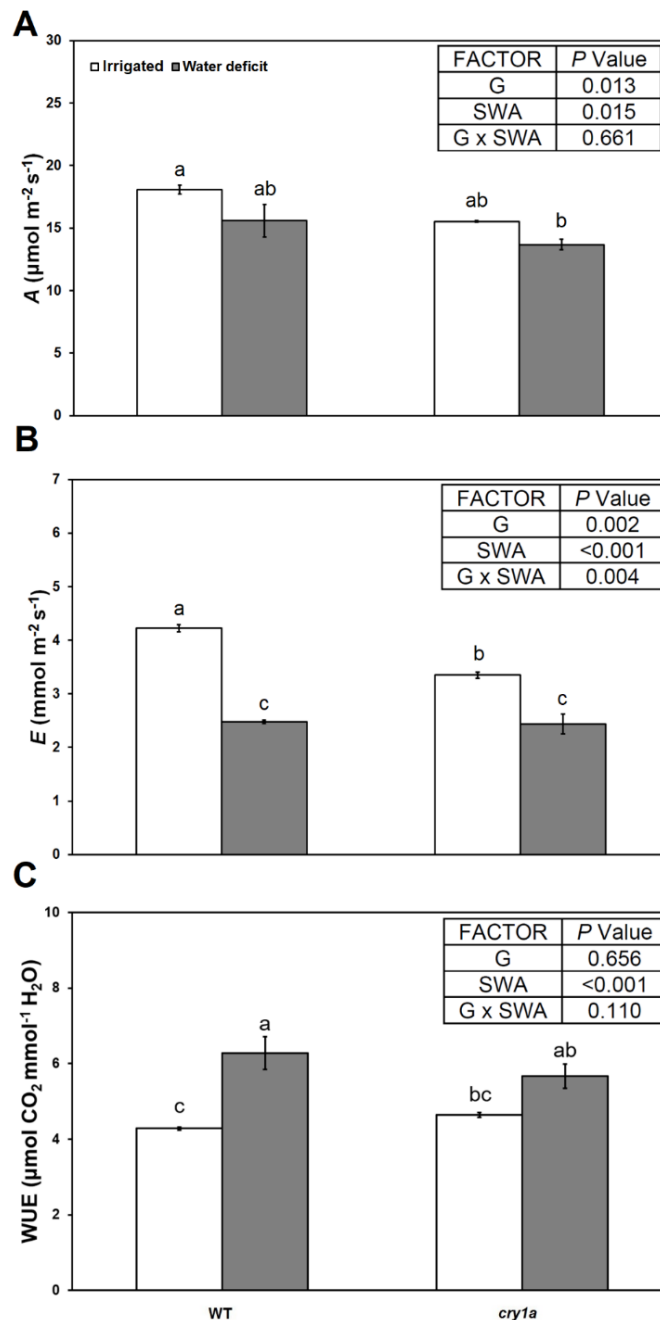


Figure 4. Gas exchange of the fifth fully expanded leaf of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) photosynthesis (A); (B) leaf transpiration (E); (C) water use efficiency (WUE). The data are shown as the mean values of 3 plants (3 technical replicates) of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the treatments. The mean values were compared using the Tukey's HSD test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of genotype (G), soil water availability (SWA), and their interactions (G x SWA) after ANOVA.

3.2. Responses of reciprocally grafted plants to water deficit

Plant height was determined by scion, but not rootstock genotype (Figures 5A and E), with *cry1a* scions 14% taller (averaged over both rootstocks). Nevertheless, a WT rootstock enhanced height of a *cry1a* scion (by 12% compared to *cry1a* self-grafts) while a *cry1a* rootstock enhanced height of a WT scion (by 23% compared to WT self-grafts), as indicated by a significant scion x rootstock interaction. Soil drying decreased plant height by 17% (averaged over all graft combinations) independently of scion or rootstock. WT scions were 21% heavier than *cry1a* scions (independent of the rootstock). Soil drying decreased shoot dry weight by 21% (averaged over all graft combinations) independently of scion or rootstock (Figure 5C). Thus scion stem elongation of reciprocally grafted plants was increased by a chimeric rootstock, but otherwise rootstock genotype did not affect scion biomass accumulation or response to soil drying.

Scion and rootstock had no significant effect on root length (Figure 5B), but soil drying stimulated root length by 11% (averaged across all graft combinations). Scion determined root dry weight, with WT scions resulting in 46% heavier roots independent of rootstock (Figure 5D). Root systems of all graft combinations responded similarly to soil drying.

While soil water deficit decreased pigment content of WT scions by 16% independent of rootstock, pigment content of *cry1a* scions depended on the rootstock (Figures 6A and B). Soil drying decreased pigment content of *cry1a*/WT plants (as indicated by significant scion x rootstock x water interactions). Thus the rootstock affected pigment responses of *cry1a* scions to water deficit, with a WT rootstock phenotypically reverting the response of *cry1a* self-grafts (*cry1a/cry1a* maintained pigment content).

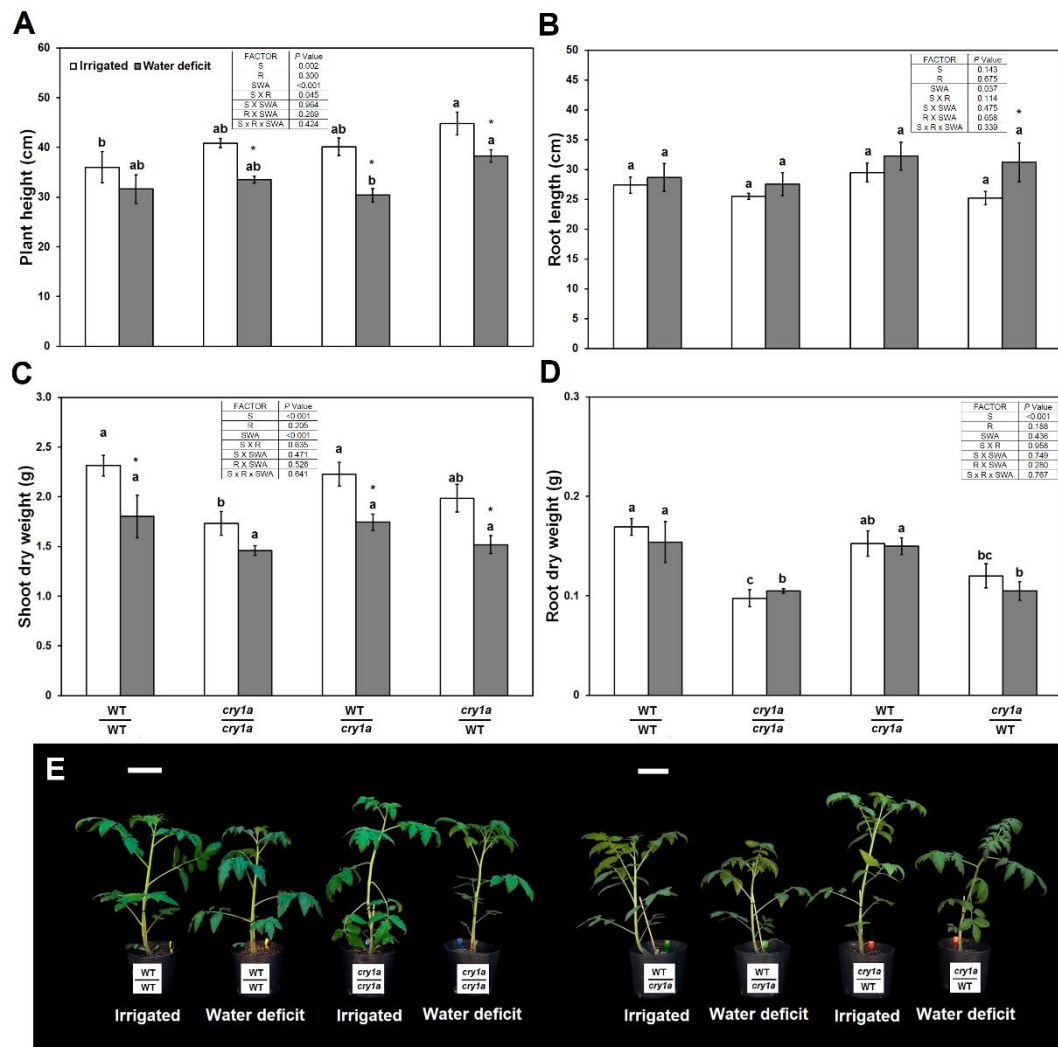


Figure 5. Growth analysis of self-grafts and heterografts of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) plant height; (B) root length; (C) shoot dry weight; (D) root dry weight. The data are shown as the mean values of 5 plants of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the graft combinations within the same growth condition, and the asterisks represent significant differences between the growth conditions in the same graft combination. The mean values were compared using the Tukey's test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of scion (S), rootstock (R), soil water availability (SWA), and their interactions (S x R; S x SWA; R x SWA; S x R x SWA) after ANOVA. (E) Tomato cv. Moneymaker phenotypes of reciprocal grafted plants of *cry1a* mutant and wild-type (WT) submitted to water deficit treatment or daily irrigated treatment. The genotype below represents the rootstock and the genotype above represents the scion. Pictures were digitally and exhibited followed by correspondent scale bar (10 cm).

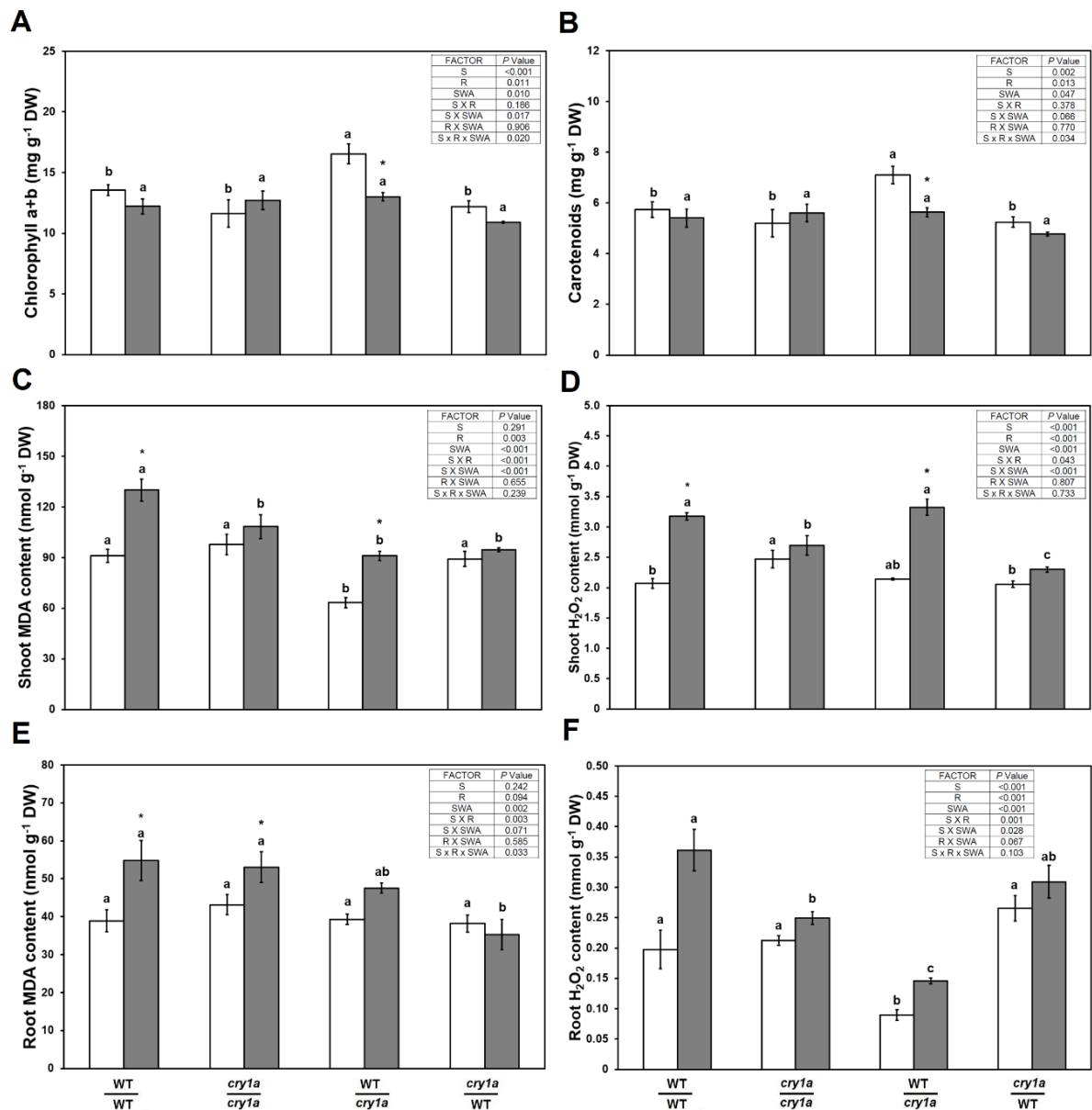


Figure 6. Biochemical analysis of self-grafts and heterografts of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) chlorophyll a+b; (B) carotenoids; (C) shoot malondialdehyde content; (D) shoot hydrogen peroxide content; (E) root malondialdehyde content; (F) root hydrogen peroxide content. The data are shown as the mean values of 5 plants (3 technical replicates) of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the graft combinations within the same growth condition, and the asterisks represent significant differences between the growth conditions in the same graft combination. The mean values were compared using the Tukey's test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of scion (S), rootstock (R), soil water availability (SWA), and their interactions (S x R; S x SWA; R x SWA; S x R x SWA) after ANOVA.

Under well-watered conditions, shoot hydrogen peroxide (H_2O_2) content was similar in all graft combinations, but soil drying elicited scion-dependent responses (Figure 6D). Thus soil drying increased shoot H_2O_2 content of WT scions by 30% independent of rootstock, while there was no change in *cry1a* self-grafts and *cry1a*/WT plants. These changes in ROS generation were only partially reflected in shoot malondialdehyde (MDA) accumulation. Plants grown on a *cry1a* rootstock generally had 11% lower shoot MDA concentrations (Figure 6C). Soil drying increased MDA concentrations of WT scions by 43% (averaged over both rootstocks), but had no effect in *cry1a* scions. Thus scion affected shoot H_2O_2 content while rootstock affected shoot H_2O_2 and MDA accumulation, with scion determining the response of shoot H_2O_2 and MDA content to soil drying.

WT rootstocks had 63% higher root H_2O_2 content than *cry1a* rootstocks independent of soil water availability, but scion genotype also had a significant effect on *cry1a* rootstocks (Figure 6F). Thus a WT scion approximately halved root H_2O_2 content of *cry1a* rootstocks independent of soil water availability. Soil drying increased root H_2O_2 content of all graft combinations similarly. Under well-watered conditions, root MDA concentration was similar in all graft combinations, but soil drying increased root MDA accumulation by 20% (averaged across all graft combinations; Figure 6E). Soil water deficit had variable effects on root MDA content in the different graft combinations (as indicated by significant scion x rootstock x water interactions). Root MDA content of WT self-grafted plants increased by 41% under soil drying, but did not change in *cry1a*/WT plants. Thus *cry1a* rootstocks had lower root H_2O_2 contents in drying soil, but this did not affect MDA accumulation.

Leaf relative water content (RWC) was primarily rootstock-dependent (Figure 7A), with scions on *cry1a* rootstocks showing 4% higher RWC (averaged over both soil water availabilities). Nevertheless, the response of RWC to soil drying was scion-dependent, with soil drying decreasing RWC of WT scions by 3% while *cry1a* scions maintained RWC, as indicated by a significant scion x soil drying interaction. Maintenance of leaf RWC in *cry1a* scions was associated with their 18% lower stomatal density independent of soil water availability or rootstock (Figure 7B). Stomatal pore area was determined by scion, but not the rootstock (Figures 7C and D), and was 16%

lower in *cry1a* scions than WT scions (averaged over both rootstocks). A *cry1a* rootstock decreased stomatal pore area of a WT scion by 26%, independent of soil water availability. Soil drying decreased stomatal pore area of all graft combinations, but to a greater extent in WT scions than *cry1a* scions (significant scion x soil water availability interaction). Thus maintenance of leaf water status in *cry1a* scions was rootstock-independent and attributed to their decreased stomatal density.

Under well-watered conditions, photosynthesis (*A*) and transpiration (*E*) of WT/*cry1a* plants was 36% and 21% more than WT self-grafts (Figures 8A and B). Soil water deficit restricted leaf gas exchange, with effects of both scion and rootstock. WT self-grafts showed the greatest restriction, with a limited response in *cry1a*/WT plants. In drying soil, a *cry1a* rootstock enhanced *A* and *E* of a WT scion, while a WT rootstock enhanced *A* and *E* of a *cry1a* scion. Soil drying increased WUE by 7% (averaged across all graft combinations; Figure 8C), with the most prominent response in self-grafted *cry1a* plants. Rootstock did not affect WUE response to soil drying, but plants with a *cry1a* rootstock generally had a higher WUE.

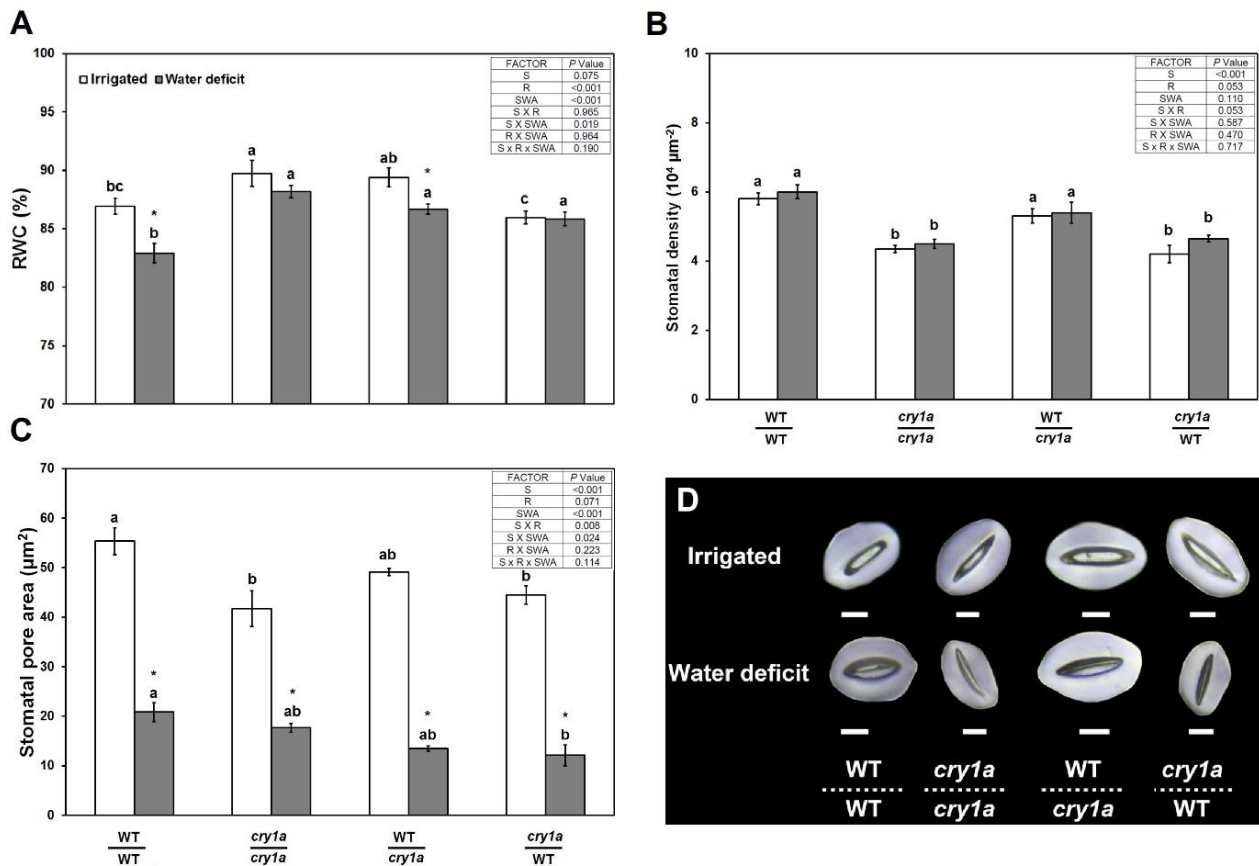


Figure 7. Water relations and stomatal measurements of self-grafts and heterografts of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) relative water content (RWC); (B) stomatal density; (C) stomatal pore area. The data are shown as the mean values of 5 plants (10 technical replicates) of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the graft combinations within the same growth condition, and the asterisks represent significant differences between the growth conditions in the same graft combination. The mean values were compared using the Tukey's test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of scion (S), rootstock (R), soil water availability (SWA), and their interactions (S x R; S x SWA; R x SWA; S x R x SWA) after ANOVA. (D) Representative images of leaf abaxial epidermis stomata from self-grafts and heterografts of *cry1a* and WT plants grown under irrigated condition or subjected to water deficit condition. Scale bars: 10 μ m.

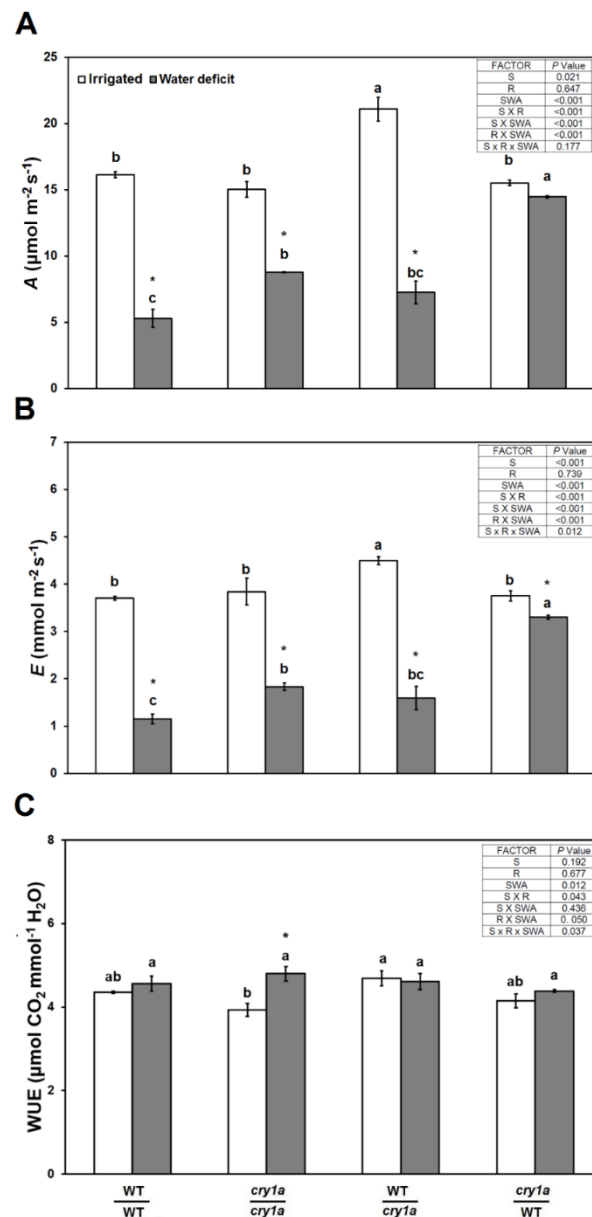


Figure 8. Gas exchange of the fifth fully expanded leaf of self-grafts and heterografts of tomato *cry1a* mutant and wild-type (WT) grown under irrigated or water deficit conditions: (A) photosynthesis (A); (B) leaf transpiration (E); (C) water use efficiency (WUE). The data are shown as the mean values of 3 plants (3 technical replicates) of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the graft combinations within the same growth condition, and the asterisks represent significant differences between the growth conditions in the same graft combination. The mean values were compared using the Tukey's test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of scion (S), rootstock (R), soil water availability (SWA), and their interactions (S x R; S x SWA; R x SWA; S x R x SWA) after ANOVA.

3.3. Correlations between plant variables

Since shoot dry weight was correlated with photosynthesis (A) across both experiments (Table 1 of Appendix B), correlation analysis attempted to explain variation in A . A declined linearly with transpiration (E) as the soil dried (Figure 9B), suggesting a stomatal limitation. However, A was not correlated with leaf relative water content (RWC; Figure 9A), suggesting non-hydraulic mediation of stomatal conductance. Although A was not correlated with chlorophyll content (Figure 9D) as mutant scions maintained A even with reduced foliar pigment content, it declined linearly with hydrogen peroxide (H_2O_2) content (Figure 9C), suggesting a further non-stomatal limitation.

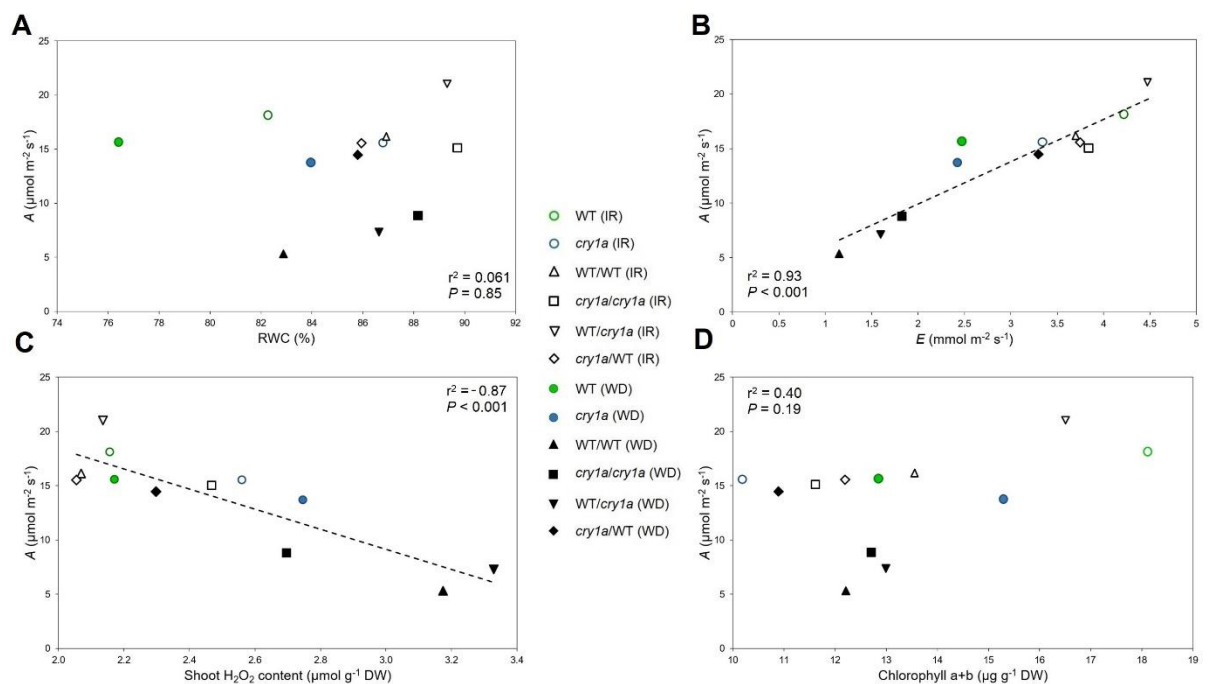


Figure 9. Relationships between leaf photosynthesis (A) and (A) relative water content (RWC); (B) transpiration (E); (C) hydrogen peroxide (H_2O_2) content; (D) chlorophyll a+b in own-rooted, self-grafts and heterografs (indicated as scion/rootstock) of tomato *cry1a* mutant and wild-type (WT) grown under irrigated (IR) or water deficit (WD) conditions. Each point represents a soil water availability treatment x genotype or graft combination. Linear correlations (r^2 and P -values) were fitted by dashed lines when $P < 0.05$.

4. DISCUSSION

While previous work characterized the effects of tomato *CRY1a* under optimal conditions (Liu et al., 2018a; D'Amico-Damião et al., 2019), for the first time we demonstrate this gene is also involved in regulating leaf pigmentation, root lipid peroxidation and leaf gas exchange responses to water deficit. Whereas water deficit decreases pigment (chlorophyll and carotenoid) concentrations of WT plants, the *cry1a* mutant maintains pigment levels. Moreover, the *CRY1a* photoreceptor modulates long-distance signaling of water deficit, with scion x rootstock x water availability interactions affecting leaf pigments and leaf transpiration (*E*), and also root malondialdehyde (MDA) content of grafted plants. While further work is needed to understand the mechanistic basis of this regulation, *CRY1a* mediation of root-shoot communication of soil water deficit does not seem hydraulically regulated, since relative water content (RWC) was not correlated with any other variable, such as leaf gas exchange measurements (Figure 9A). Physiological responses of own-rooted and reciprocally grafted plants are discussed in turn.

4.1. *cry1a* photoreceptor positively regulates tomato growth

As expected, soil drying decreased stem elongation, shoot and root biomass accumulation but enhanced primary root length (Figure 1), with an attenuated root growth response in *cry1a*. Altered source-sink partitioning (D'Amico-Damião et al., 2019) might explain this response, with *cry1a* showing greater shoot growth than root growth compared to WT plants independent of soil water availability (Figure 2A of Appendix B). However, these morphological responses were generally independent of soil drying (no genotype x soil water availability interactions), indicating that *CRY1a* positively regulates root extension and biomass accumulation in tomato. Repeated periods of soil drying and re-watering seem necessary to accentuate root biomass accumulation of tomato in response to soil water deficit (Mingo et al., 2004). Although *cry1a* plants grew taller independent of soil moisture, they accumulated less shoot biomass and leaf area (Figure 2B of Appendix B). However, such growth inhibition did

not seem to be hydraulically-mediated, as *cry1a* plants had a higher leaf RWC (Figure 3A), especially in drying soil. Decreased *E* of *cry1a* plants (Figure 4B), especially when well-watered, was caused by their lower stomatal density (Figure 3B) as stomatal pore area was similar to WT plants independent of soil moisture (Figures 3C and D). Similarly, lower stomatal density of the *Arabidopsis atdtm1* mutant was associated with increased relative water content (Zhao et al., 2017). While the lower stomatal density (and leaf *E*) of *cry1a* under well-watered conditions might delay soil moisture depletion, ultimately both genotypes showed similar morphological (Figure 1) and physiological (Figure 4) responses to soil drying.

Blue light activates *crys* to positively regulate downstream expression of the *HY5* (*LONG HYPOCOTYL 5*) gene, encoding the well-characterized light signaling transcription factor; *HY5* a photomorphogenesis promotor (Su et al., 2017). Under optimal soil moisture and light conditions, tomato lines overexpressing *OFPs* (*OVATE FAMILY PROTEINS*) upregulated foliar *HY5* and *CAT2* (*CATALASE 2*) expression, thereby increasing total chlorophyll while decreasing hydrogen peroxide (H_2O_2) and MDA contents (Zhou et al., 2019). When grown under blue light in well-watered conditions, *cry1a* mutant and *SICRY1a* overexpressing lines had lower and higher *SIHY5* transcript levels than WT plants, respectively (Liu et al., 2018b). Taken together, these results are consistent with the biochemical (leaf pigments and shoot MDA concentrations) responses of well-watered own-rooted *cry1a* plants grown in a naturally lit greenhouse (Figure 2). In contrast, since drought gradually increases *HY5* expression (Song et al., 2017), *SIHY5* overexpressing lines decreased H_2O_2 and MDA accumulation compared to WT, as their enzymatic antioxidant defense systems (eg SUPEROXIDE DISMUTASE, SOD; and CAT) were enhanced (Han et al., 2020). Thus the functional *SICRY1a* in the WT likely positively regulates the expression of *SIHY5*, thereby promoting the activity of antioxidant enzymes thus decreasing membrane lipid peroxidation during soil drying.

Nevertheless, leaf pigment (carotenoid and chlorophyll) responses of the two genotypes to soil water deficit greatly differed (Figures 2A and B). Under well-watered conditions, the *cry1a* mutant had lower pigment concentrations than WT plants, but higher concentrations under soil water deficit. Soil drying significantly decreased

pigment concentrations of WT plants but maintained (or tended to increase) concentrations in *cry1a*. Drought concentrated the leaf pigments in thinner *cry1a* leaves (Liu et al., 2018a; D'Amico-Damião et al., 2019), by reducing chloroplast ultrastructure volume in fully expanded tomato leaves (Mäkelä et al., 2000). In other words, genotypic differences in leaf structure accentuated soil drying effects, resulting in divergent pigment responses to drought in WT (decreased) and *cry1a* (maintained) plants.

Although greater light absorption suggested by higher chlorophyll concentrations of *cry1a* plants growing in drying soil did not enhance shoot H₂O₂ (a representative ROS) content relative to WT plants (Figure 2D), greater oxidative damage (measured as MDA accumulation) occurred (Figure 2C) perhaps because other reactive molecules were generated. Indeed, crys modulate ROS homeostasis in a blue light dependent manner, which implies the expression of genes whose products are related to ROS scavenging, H₂O₂ signaling and ROS formation (Consentino et al., 2015; D'Amico-Damião and Carvalho, 2018). Nevertheless, high light exposure of low-light adapted *cry1a* plants resulted in less photoinhibition than the WT (Kraslevski et al., 2020), indicating complex relationships between ROS generation and oxidative damage. However, these molecular responses may not be specifically light-mediated, as root H₂O₂ content of *cry1a* and WT plants was similar in drying soil, yet *cry1a* roots accumulated greater MDA concentrations in drying soil. Nevertheless, following cry activation, ROS signaling in the apex of the primary root of transgenic Arabidopsis that overexpressed *CRY1* was approximately 2.5-fold greater than *cry1cry2* double mutant roots (El-Esawi et al., 2017). Irrespective of the sources of oxidative stress (excess light absorption or soil drying), greater oxidative damage of *cry1a* plants growing in drying soil (Figures 2C and E) occurred even at higher leaf water status (Figure 3A), indicating *CRY1a* is involved in mediating tolerance to oxidative stress.

Despite greater oxidative damage of *cry1a* plants growing in drying soil (Figure 2C), leaf photosynthesis (*A*) was comparable to WT plants (Figure 4A). Although *A* of *cry1a* plants was 13% lower than WT plants (averaged across soil moisture treatments), both genotypes decreased *A* similarly in response to soil drying (no genotype x soil moisture interaction) as transpiration decreased. Thus diminished *A* of

cry1a could be explained by decreased stomatal density limiting internal CO₂ concentration (C_i) even in well-watered plants (Bertolino et al., 2019), with drought-induced stomatal closure (Figure 3C) imposing an additional limitation. While further A- C_i analysis is required to distinguish the physiological importance of stomatal and non-stomatal limitation of A in these genotypes, they maintained similar leaf water use efficiency (WUE) independent of soil water availability.

4.2. Importance of tomato *cry1a* in regulating long-distance-signaling of water deficit

Differential shoot and root growth of own-rooted *cry1a* and WT plants (Liu et al., 2018a; D'Amico-Damião et al., 2019; Figure 1) seemed entirely scion-mediated, as reciprocal grafting experiments revealed no rootstock effects (Figure 5). However, when WT *Nicotiana attenuata* scions were grafted onto photoreceptor-silenced rootstocks, only phyB1 and B2 (but not *cry2*) delayed shoot growth both in field and glasshouse growth conditions (Oh et al., 2018), indicating that photoreceptor levels in the roots can exert systemic effects on shoot growth. Greater biomass of WT scions increased root biomass independent of rootstock, with similar assimilate partitioning across all graft combinations (Figure 2F of Appendix B). Despite these differences in root biomass, root extension of all graft combinations was similar within a soil moisture treatment (Figure 5B), indicating limited shoot-to-root signaling mediated by *cry1a*. Increased, or maintenance of, root biomass allocation with soil drying in tomato (Mingo et al., 2004) results from greater shoot (than root) sensitivity to water deficit, and seemed independent of location (root or shoot) of the *CRY1a* gene.

However, *cry1a* mediated root-shoot communication determining leaf pigment and root malondialdehyde (MDA) concentrations as the soil dried, since there were significant scion, rootstock and water interactions (Figures 6A, B and E). Grafting *per se* (independent of rootstock genotype) prevented *cry1a* scions upregulating shoot MDA and hydrogen peroxide (H₂O₂) concentrations in response to water deficit (cf. Figures C and D; Figures 6C and D), consistent with transcriptome profiles of tomato under biotic stress strongly differing (6% of all genes) between own-rooted and self-

grafted plants (Spanò et al., 2020). In addition, self-grafts from two different grapevine genotypes result in the differential expression of genes involved in oxidative stress (eg *GLUTATHIONE S-TRANSFERASES*, *ASCORBATE OXIDASE*, *POLYPHENOL OXIDASE*, and *PEROXIDASE* genes) compared to heterografts (Cookson et al., 2014), indicating complex oxidative responses may account for inconsistent results between own-rooted and grafted plants.

Nevertheless, a *cry1a* rootstock enhanced leaf pigment concentrations of WT scions, but only in well-watered plants (Figures 6A and B). However, this extra light-absorbing capacity did not enhance foliar oxidative stress (Figures 6C and D). Greater root MDA accumulation of own-rooted *cry1a* plants under water deficit (Figure 2E) did not occur in grafted plants, independent of the scion. However, a *cry1a* scion decreased drought-induced root MDA accumulation of WT rootstocks (Figure 6E) despite similar rootstock H₂O₂ levels (Figure 6F). Furthermore, a WT scion decreased root H₂O₂ levels of *cry1a* rootstock independent of soil moisture. While drought-induced H₂O₂ generation can cause lipid peroxidation (Huang et al., 2019), it is also involved in long-distance signaling (via second messengers) of essential physiological processes that regulate tomato drought stress responses (Waszczak et al., 2018), e.g. root growth (Ivanchenko et al., 2013) and stomatal closure (Ahammed et al., 2020). HY5 is a candidate molecule to regulate H₂O₂ root-shoot communication by interacting with CRY1a activity in tomato (Liu et al., 2018b; Han et al., 2020), since local activity (shoot or root) of HY5 transcription factor (protein) mediates downstream mobile signals from shoots to roots in *Arabidopsis* regulating plant growth (Burko et al., 2020), and is likely important in *CRY1a* mediated root-shoot communication in tomato (Figure 10).

Decreased stomatal density of *cry1a* (Figure 3B) was not rescued by grafting onto a WT rootstock (Figure 7B), yet *cry1a*/WT plants had a lower relative water content (RWC) than self-grafted *cry1a* plants. This was likely because *cry1a*/WT plants transpired more, which increased photosynthesis (A; Figures 8A and B) irrespective of soil moisture. For instance, lower RWC of the tomato *not* (*notabilis*; ABA-deficient) mutant under water deficit was related to its enhanced transpiration rate (Yuan et al., 2010). Whether a WT rootstock can enhance whole plant hydraulic conductance of

cry1a requires further investigation. In contrast, neither rootstock or soil drying altered stomatal density of WT scions, nor did rootstock affect the responses of stomatal pore area to drying soil. Indeed, the stomatal density of reciprocally grafted seedlings of a drought-tolerant and a drought-sensitive tomato genotypes was primarily (but not rootstock) determined across both soil water availability (watered or drought) conditions (Zhang et al., 2019). Nevertheless, WT scions on a *cry1a* rootstock showed an attenuated RWC response to soil drying, despite similar stomatal closure to WT self-grafts. Again, rootstock-mediated changes in whole plant hydraulic conductance seem necessary to explain the regulation of leaf gas exchange in these graft combinations (de Ollas et al., 2018).

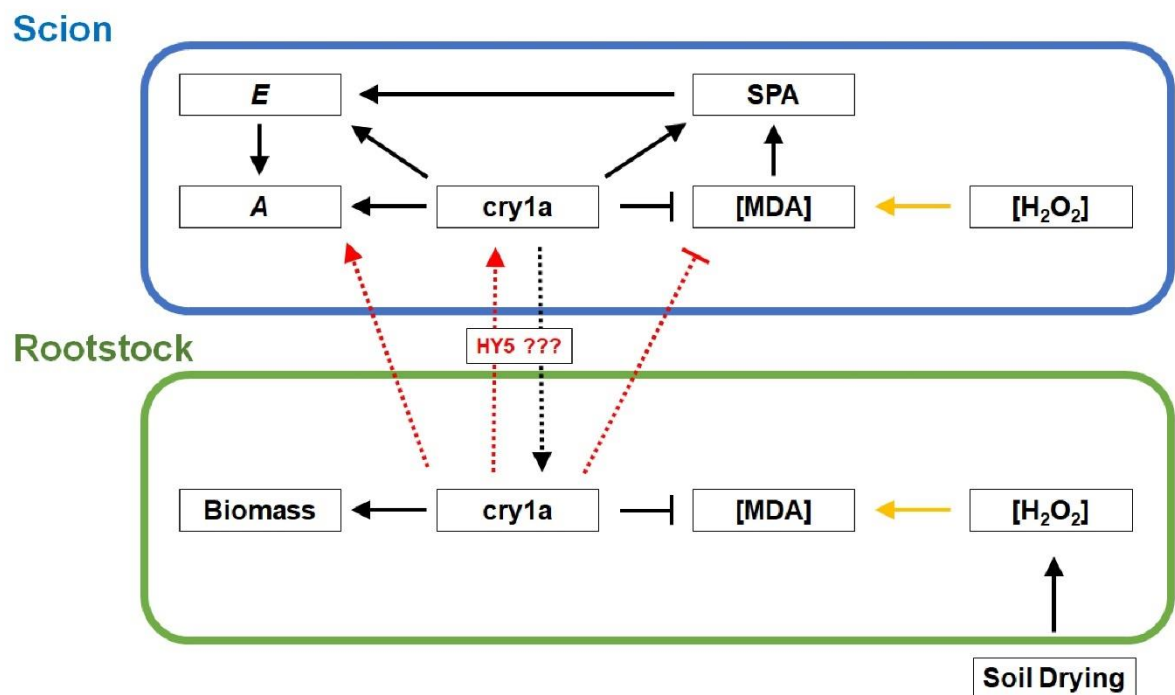


Figure 10. Schematic figure of tomato *cry1a* effects on stomatal (stomatal pore area = SPA), oxidative stress (H₂O₂ and MDA concentrations), leaf gas exchange (A, E) and root biomass responses of grafted plants. Positive effects are represented by lines ending in an arrow, and negative effects are represented by lines ending in a bar. Root-shoot communication (as scion x rootstock x soil water availability interactions) is represented by dashed lines (red, root-to-shoot; black, shoot-to-root). Relationships within oxidative stress pathways are indicated by a yellow line, with the putative role of the HY5 transcription factor mediating unknown (???) downstream mobile signals indicated.

5. CONCLUSIONS

In conclusion, although own-rooted plants were not directly compared with grafted plants, *CRY1a* appears to have both local and long-distance roles in regulating leaf pigmentation and gas exchange during water deficit responses (Figure 10). Thus own-rooted *cry1a* plants maintained foliar pigment concentrations in drying soil, possibly related to maintenance of leaf RWC due to lower stomatal density (a local effect). Furthermore, a WT rootstock maintained *cry1a* transpiration under water deficit, independently of changes in stomatal density or pore area or leaf water status, which enhanced photosynthesis (a long-distance effect). While soil drying causes stomatal limitation of photosynthesis that seems independent of leaf water status (Figure 9A), further work is needed to understand how *CRY1a* downregulates photosynthesis, especially since greater lipid peroxidation occurred in own-rooted *cry1a* plants and photosynthesis declined linearly with shoot H₂O₂ concentration.

Author contributions

V.D.D., I.C.D. and R.F.C. designed, interpreted, wrote and approved the manuscript for publication. V.D.D., R.O. and J.C.B.L. performed the experiments and collected the data. V.D.D. and D.R.R. performed the leaf gas exchange experiments and collected the data. V.D.D., I.C.D. and R.F.C. analyzed the results and constructed the figures.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version.

6. REFERENCES

- Albacete A, Martínez-Andújar C, Martínez-Pérez A, Thompson AJ, Dodd IC, Pérez-Alfocea F (2015) Unravelling rootstock × scion interactions to improve food security. **Journal of Experimental Botany** 66:2211–2226. doi: 10.1093/jxb/erv027
- Ahammed GJ, Li X, Yang Y, Liu C, Zhou G, Wan H, Cheng Y (2020) Tomato *WRKY81* acts as a negative regulator for drought tolerance by modulating guard cell H₂O₂–mediated stomatal closure. **Environmental and Experimental Botany** 171:103960. doi: 10.1016/j.envexpbot.2019.103960
- Bertolino LT, Caine RS, Gray JE (2019) Impact of stomatal density and morphology on water-use efficiency in a changing world. **Frontiers in Plant Science** 10:225. doi: 10.3389/fpls.2019.00225
- Bian Z, Zhang X, Wang Y, Lu C (2019) Improving drought tolerance by altering the photosynthetic rate and stomatal aperture via green light in tomato (*Solanum lycopersicum* L.) seedlings under drought conditions. **Environmental and Experimental Botany** 167:103844. doi: 10.1016/j.envexpbot.2019.103844
- Burko Y, Gaillochet C, Seluzicki A, Chory J, Busch W (2020) Local HY5 activity mediates hypocotyl growth and shoot-to-root communication. **Plant Communications** 1:100078. doi: 10.1016/j.xplc.2020.100078
- Canamero RC, Bakrim N, Bouly JP, Garay A, Dudkin EE, Habricot Y, Ahmad M (2006) Cryptochrome photoreceptors cry1 and cry2 antagonistically regulate primary root elongation in *Arabidopsis thaliana*. **Planta** 224:995–1003. doi: 10.1007/s00425-006-0280-6
- Chaves I, Pokorny R, Byrdin M, Hoang N, Ritz T, Brettel K, Essen LO, van der Horst GTJ, Batschauer A, Ahmad M (2011) The cryptochromes: blue light photoreceptors in plants and animals. **Annual Review of Plant Biology** 62:335–364. doi: 10.1146/annurev-arplant-042110-103759
- Consentino L, Lambert S, Martino C, Jourdan N, Bouchet PE, Witczak J, Castello P, El-Esawi M, Corbineau F, d'Harlingue A, Ahmad M (2015) Blue-light dependent reactive oxygen species formation by *Arabidopsis* cryptochrome may define a novel evolutionarily conserved signaling mechanism. **New Phytologist** 206:1450–1462. doi: 10.1111/nph.13341

Cookson SJ, Clemente Moreno MJ, Hevin C, Nyamba Mendome LZ, Delrot S, Magnin N, Trossat-Magnin C, Ollat N (2014) Heterografting with nonself rootstocks induces genes involved in stress responses at the graft interface when compared with autografted controls. **Journal of Experimental Botany** 65:2473–2481. doi: 10.1093/jxb/eru145

D'Amico-Damião V, Carvalho RF (2018) Cryptochrome-related abiotic stress responses in plants. **Frontiers in Plant Science** 9:1897. doi: 10.3389/fpls.2018.01897

D'Amico-Damião V, Cruz FJR, Gavassi MA, Santos DMM, Melo HC, Carvalho RF (2015) Photomorphogenic modulation of water stress in tomato (*Solanum lycopersicum* L.): the role of phytochromes A, B1, and B2. **The Journal of Horticultural Science and Biotechnology** 90:25–30. doi: 10.1080/14620316.2015.11513149

D'Amico-Damião V, dos Santos JC, Girotto NC, Carvalho RF (2019) Cryptochrome 1a influences source-sink partitioning during different stages of growth in tomato. **Theoretical and Experimental Plant Physiology** 31:295–302. doi: 10.1007/s40626-019-00141-1

de Ollas C, Arbona V, Gómez-Cadenas A, Dodd IC (2018) Attenuated accumulation of jasmonates modifies stomatal responses to water deficit. **Journal of Experimental Botany** 69:2103–2116. doi: 10.1093/jxb/ery045

Dubois M, Claeys H, van den Broeck L, Inzé D (2017) Time of day determines Arabidopsis transcriptome and growth dynamics under mild drought. **Plant, Cell & Environment** 40:180–189. doi: 10.1111/pce.12809

El-Esawi M, Arthaut LD, Jourdan N, d'Harlingue A, Link J, Martino CF, Ahmad M (2017) Blue-light induced biosynthesis of ROS contributes to the signaling mechanism of Arabidopsis cryptochrome. **Scientific Reports** 7:13875. doi: 10.1038/s41598-017-13832-z

Fantini E, Sulli M, Zhang L, Aprea G, Jiménez-Gómez JM, Bendahmane A, Perrotta G, Giuliano G, Facella P (2019) Pivotal roles of cryptochromes 1a and 2 in tomato development and physiology. **Plant Physiology** 179:732–748. doi: 10.1104/pp.18.00793

Gaion LA, Monteiro CC, Cruz FJR, Rossatto DR, Lopez-Diaz I, Carrera E, Lima JE, Peres LEP, Carvalho RF (2018) Constitutive gibberellin response in grafted tomato modulates root-to-shoot signaling under drought stress. **Journal of Plant Physiology** 221:11–21. doi: 10.1016/j.jplph.2017.12.003

Galvão VC, Fankhauser C (2015) Sensing the light environment in plants: photoreceptors and early signaling steps. **Current Opinion in Neurobiology** 34:46–53. doi: 10.1016/j.conb.2015.01.013

Han N, Fan S, Zhang T, Sun H, Zhu Y, Gong H, Guo J (2020) *SIHY5* is a necessary regulator of the cold acclimation response in tomato. **Plant Growth Regulation** 91:1–12. doi: 10.1007/s10725-020-00583-7

Hoffmann AM, Noga G, Hunsche M (2015) Acclimations to light quality on plant and leaf level affect the vulnerability of pepper (*Capsicum annuum* L.) to water deficit. **Journal of plant research** 128:295–306. doi: 10.1007/s10265-014-0698-z

Huang H, Ullah F, Zhou DX, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. **Frontiers in Plant Science** 10:800. doi: 10.3389/fpls.2019.00800

Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L (2018) Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. **Frontiers in Plant Science** 9:393. doi: 10.3389/fpls.2018.00393

Ivanchenko MG, den Os D, Monshausen GB, Dubrovsky JG, Bednářová A, Krishnan N (2013) Auxin increases the hydrogen peroxide (H₂O₂) concentration in tomato (*Solanum lycopersicum*) root tips while inhibiting root growth. **Annals of Botany** 112:1107–1116. doi: 10.1093/aob/mct181

Jangid KK, Dwivedi P (2016) Physiological responses of drought stress in tomato: a review. **International Journal of Agriculture, Environment and Biotechnology** 9:53. doi: 10.5958/2230-732X.2016.00009.7

Kreslavski VD, Strokin VV, Pashkovskiy PP, Balakhnina TI, Voloshin RA, Alwasel S, Kosobryukhova AA, Allakhverdiev, SI (2020) Deficiencies in phytochromes A and B and cryptochrome 1 affect the resistance of the photosynthetic apparatus to high-intensity light in *Solanum lycopersicum*. **Journal of Photochemistry and Photobiology B: Biology** 210:111976. doi: 10.1016/j.jphotobiol.2020.111976

Li W, de Ollas C, Dodd IC (2018) Long-distance ABA transport can mediate distal tissue responses by affecting local ABA concentrations. **Journal of Integrative Plant Biology** 60:16–33. doi: 10.1111/jipb.12605

Liu CC, Ahammed GJ, Wang GT, Xu CJ, Chen KS, Zhou YH, Yu JQ (2018a) Tomato *CRY1a* plays a critical role in the regulation of phytohormone homeostasis, plant development, and carotenoid metabolism in fruits. **Plant, Cell & Environment** 41:354–366. doi: 10.1111/pce.13092

Liu CC, Chi C, Jin LJ, Zhu J, Yu JQ, Zhou YH (2018b) The bZip transcription factor *HY5* mediates *CRY1a*-induced anthocyanin biosynthesis in tomato. **Plant, Cell & Environment** 41:1762–1775. doi: 10.1111/pce.13171

Mäkelä P, Kärkkäinen J, Somersalo S (2000) Effect of glycinebetaine on chloroplast ultrastructure, chlorophyll and protein content, and RuBPCO activities in tomato grown under drought or salinity. **Biologia Plantarum** 43:471–475. doi: 10.1023/A:1026712426180

Mingo DM, Theobald JC, Bacon MA, Davies WJ, Dodd IC (2004) Biomass allocation in tomato (*Lycopersicon esculentum*) plants grown under partial rootzone drying: enhancement of root growth. **Functional Plant Biology** 31:971–978. doi: 10.1071/FP04020

Oh Y, Fragoso V, Guzzonato F, Kim SG, Park CM, Baldwin IT (2018) Root-expressed phytochromes B1 and B2, but not PhyA and Cry2, regulate shoot growth in nature. **Plant, Cell & Environment** 41:2577–2588. doi: 10.1111/pce.13341

Pinheiro C, Chaves MM (2011) Photosynthesis and drought: can we make metabolic connections from available data?. **Journal of Experimental Botany** 62:869–882. doi: 10.1093/jxb/erq340

Schneider JR, Caverzan A, Chavarria G (2019) Water deficit stress, ROS involvement, and plant performance. **Archives of Agronomy and Soil Science** 65:1160–1181. doi: 10.1080/03650340.2018.1556789

Sharma P, Chatterjee M, Burman N, Khurana JP (2014) Cryptochrome 1 regulates growth and development in *Brassica* through alteration in the expression of genes involved in light, phytohormone and stress signalling. **Plant, Cell & Environment** 37:961–977. doi: 10.1111/pce.12212

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **Journal of Botany** 2012:217037. Doi: 10.1155/2012/217037

Spanò R, Ferrara M, Montemurro C, Mulè G, Gallitelli D, Mascia T (2020) Grafting alters tomato transcriptome and enhances tolerance to an airborne virus infection. **Scientific reports** 10:1–13. Doi: 10.1038/s41598-020-59421-5

Song K, Kim HC, Shin S, Kim KH, Moon JC, Kim JY, Lee BM (2017) Transcriptome analysis of flowering time genes under drought stress in maize leaves. **Frontiers in Plant Science** 8:267. Doi: 10.3389/fpls.2017.00267

Su J, Liu B, Liao J, Yang Z, Lin C, Oka Y (2017) Coordination of cryptochrome and phytochrome signals in the regulation of plant light responses. **Agronomy** 7:25. Doi: 10.3390/agronomy7010025

Thomas HR, Frank MH (2019) Connecting the pieces: uncovering the molecular basis for long-distance communication through plant grafting. **New Phytologist** 223:582–589. doi: 10.1111/nph.15772

Waszczak C, Carmody M, Kangasjärvi J (2018) Reactive oxygen species in plant signaling. **Annual Review of Plant Biology** 69:209–236. doi: 10.1146/annurev-arplant-042817-040322

Weller JL, Perrotta G, Schreuder MEL, van Tuinen A, Koornneef M, Giuliano G, Kendrick RE (2001) Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. **The Plant Journal** 25:427–440. doi: 10.1046/j.1365-313x.2001.00978.x

Xu P, Xiang Y, Zhu H, Xu H, Zhang Z, Zhang C, Zhang L, Ma Z (2009) Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. **Plant Physiology** 149:760–774. doi: 10.1104/pp.108.132217

Yu X, Liu H, Klejnot J, Lin C (2010) The cryptochrome blue light receptors. **The Arabidopsis Book** 8:e0135. doi: 10.1199/tab.0135

Yuan GF, Jia CG, Li Z, Sun B, Zhang LP, Liu N, Wang QM (2010) Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. **Scientia Horticulturae** 126:103–108. doi: 10.1016/j.scienta.2010.06.014

Zhang Z, Cao B, Li N, Chen Z, Xu K (2019) Comparative transcriptome analysis of the regulation of ABA signaling genes in different rootstock grafted tomato seedlings under drought stress. **Environmental and Experimental Botany** 166:103814. doi: 10.1016/j.envexpbot.2019.103814

Zhao L, Li Y, Xie Q, Wu Y (2017) Loss of *CDKC; 2* increases both cell division and drought tolerance in *Arabidopsis thaliana*. **The Plant Journal** 91:816–828. doi: 10.1111/tpj.13609

Zhou S, Chen X, Li F, Feng P, Hu G, Chen G, Xie Q, Hu, Z (2019) Overexpression of *SIOFP20* in tomato affects plant growth, chlorophyll accumulation and leaf senescence. **Frontiers in Plant Science** 10:1510. doi: 10.3389/fpls.2019.01510

APPENDICES

Appendix A. Supplementary material for chapter 3

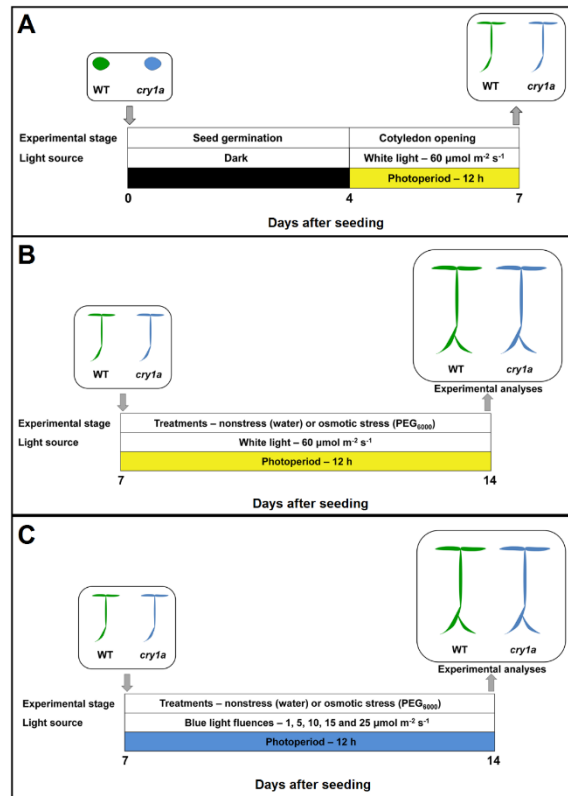


Figure 1. Summary of experimental stages from seed germination to performed analyses in tomato (*Solanum lycopersicum* L.) *cry1a* mutant and wild-type (WT) seedlings. (A) Seeds of both genotypes were germinated in the dark for four days. To promote cotyledon opening, the germinated seeds remained in a growth chamber for three days under a white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) photoperiod of 12 h (light : dark). After this period, the experimental treatments were applied. (B) After cotyledon opening, osmotic stress was induced in some *cry1a* mutant and WT seedlings (seven days after seeding) with polyethylene glycol 6000 (PEG₆₀₀₀, -0.3 MPa). The other *cry1a* mutant and WT seedlings were treated with distilled water as a nonstress condition. The treated seedlings were maintained in a growth chamber under a white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) photoperiod of 12 h (light : dark) for seven days. Experimental analyses were performed seven days after the treatments were applied. (C) After cotyledon opening, osmotic stress was induced in some *cry1a* mutant and WT seedlings (seven days after seeding) with polyethylene glycol 6000 (PEG₆₀₀₀, -0.3 MPa). The other *cry1a* mutant and WT seedlings were treated with distilled water as a nonstress condition. In addition to the osmotic stress treatment, blue light fluence treatments were applied. Seedlings treated with osmotic stress were grown for seven days in growth chambers under a 12 h (light : dark) blue light photoperiod, with fluence rates of 1, 5, 10, 15 and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$. Experimental analyses were performed seven days after osmotic stress and after blue light fluence treatments were applied.

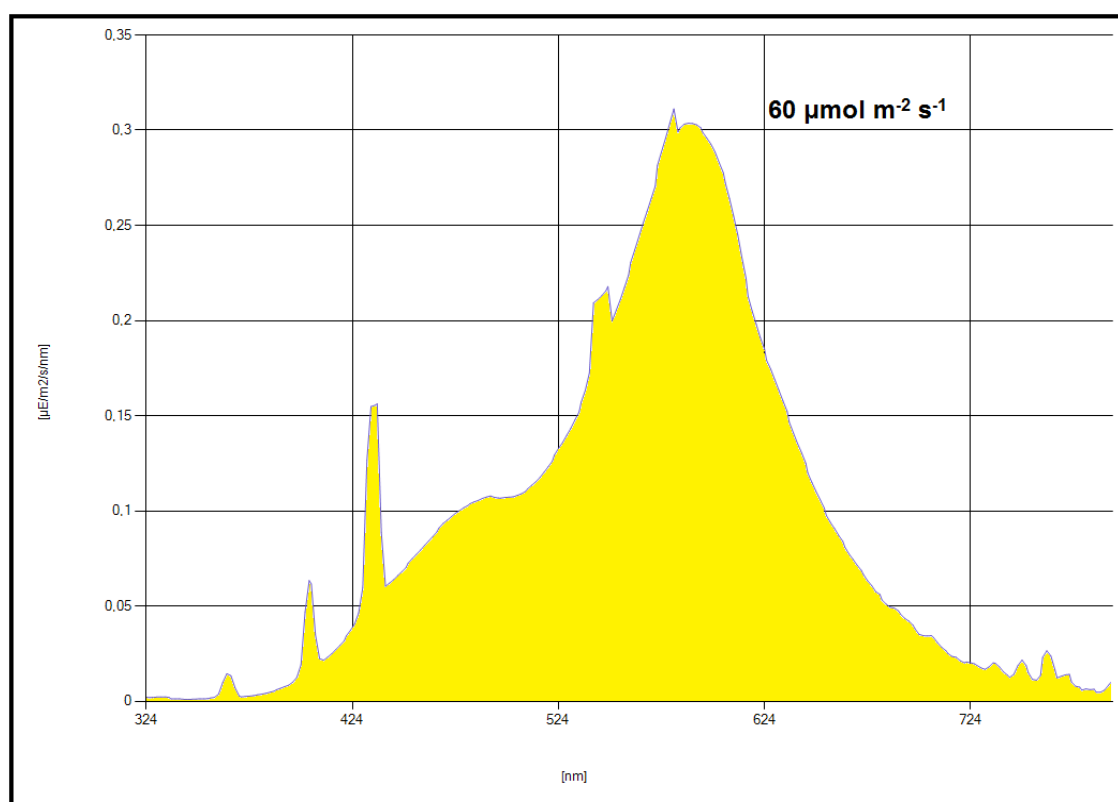


Figure 2. Light spectral curve of the white LEDs in the growth chamber, as measured with a portable spectroradiometer (SpectraPen LM 500, PSI, Brno, Czech Republic). The white light fluence was equal to a photon flux energy of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$.

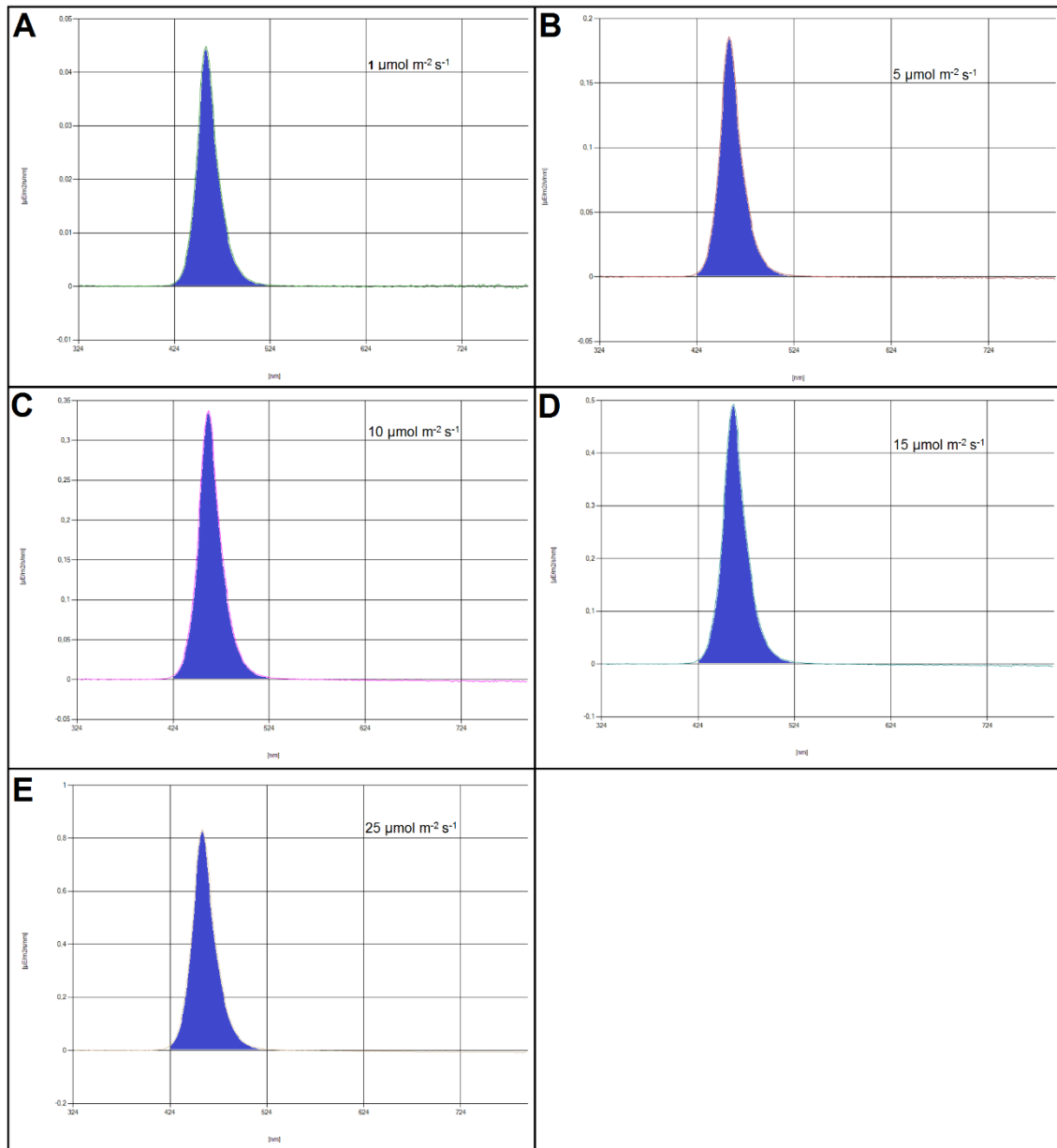


Figure 3. Light spectral curve of the blue LEDs in the growth chamber, as measured with a portable spectroradiometer (SpectraPen LM 500, PSI, Brno, Czech Republic). (A) Photon flux energy of 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light; (B) Photon flux energy of 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light; (C) Photon flux energy of 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light; (D) Photon flux energy of 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light; (E) Photon flux energy of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light.

Table 1. Two-way ANOVA of growth parameters in the osmotic stress experimente 1 (white light LEDs). *P* values are presented for each main effect or interaction.

Effect or Interaction	Shoot length (Fig. 1A)	Root length (Fig. 1B)	Shoot dry weight (Fig. 1C)	Root dry weight (Fig. 1D)
Genotype (G)	0.08	0.49	<0.01	0.04
Osmotic condition (OC)	<0.01	<0.01	0.06	0.09
G x OC	<0.01	<0.01	0.02	0.03

Table 2. Two-way ANOVA of biochemical parameters in the osmotic stress experimente 1 (white light LEDs). *P* values are presented for each main effect or interaction.

Effect or Interaction	Chlorophyll (Fig. 3A)	Carotenoids (Fig. 3B)	MDA (Fig. 4A)	H ₂ O ₂ (Fig. 4B)	Proline (Fig. 4C)
Genotype (G)	<0.01	<0.01	0.41	0.11	0.17
Osmotic condition (OC)	<0.01	<0.01	<0.01	<0.01	<0.01
G x OC	<0.01	<0.01	<0.01	<0.01	<0.01

Table 3. Two-way ANOVA of growth parameters in the osmotic stress experimente 2 (blue light LEDs). *P* values are presented for each main effect or interaction within each blue light fluence.

Blue light fluence	Effect or Interaction	Shoot length (Fig. 5A)	Root length (Fig. 5B)	Shoot dry weight (Fig. 5C)	Root dry weight (Fig. 5D)	Shoot-to-root ratio (Fig. 5E)
1 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	0.07	<0.01	0.02	0.83	0.04
	Osmotic condition (OC)	<0.01	<0.01	<0.01	0.02	0.93
	G x OC	<0.01	0.23	0.35	0.01	0.02
5 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	0.02	0.38	0.46	0,94
	Osmotic condition (OC)	<0.01	<0.01	<0.01	0.54	<0.01
	G x OC	<0.01	0.25	<0.01	0.51	0,02
10 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	0.09	<0.01	0.08	0.48
	Osmotic condition (OC)	<0.01	<0.01	0.04	0.02	0.10
	G x OC	<0.01	0.02	0.10	0.53	0.14
15 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	<0.01	0.16	0.13	0.08
	Osmotic condition (OC)	<0.01	<0.01	<0.01	0.02	0.12
	G x OC	0.10	<0.01	0.02	<0.01	<0.01
25 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	0.38	0.11	0.74	0.92
	Osmotic condition (OC)	<0.01	<0.01	0.01	<0.01	0.14
	G x OC	0.06	0.38	0.27	<0.01	<0.01

Table 4. Two-way ANOVA of biochemical parameters in the osmotic stress experimente 2 (blue light LEDs). *P* values are presented for each main effect or interaction within each blue light fluence.

Blue light fluence	Effect or Interaction	Chlorophylls (Fig. 7A)	Carotenoids (Fig. 7B)	Anthocyanins (Fig. 7C)	MDA (Fig. 8A)	H ₂ O ₂ (Fig. 8B)	Proline (Fig. 8C)
1 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	0.03	<0.01	0.02	0.31	<0.01
	Osmotic condition (OC)	0.82	0.57	<0.01	<0.01	<0.01	<0.01
	G x OC	0.93	0.94	0.22	<0.01	0.28	0.07
5 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	0.12	0.35	0.07	0.28	0.02	<0.01
	Osmotic condition (OC)	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
	G x OC	<0.01	<0.01	0.28	0.03	0.02	0.09
10 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	<0.01	<0.01	0.25	0.13	<0.01
	Osmotic condition (OC)	0.26	0.79	<0.01	<0.01	0.04	<0.01
	G x OC	<0.01	0.02	<0.01	0.03	0.85	<0.01
15 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	0.01	0.01	<0.01	0.03	0.38	<0.01
	Osmotic condition (OC)	<0.01	<0.01	0.04	<0.01	0.08	<0.01
	G x OC	<0.01	<0.01	0.88	0.14	<0.01	<0.01
25 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Genotype (G)	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
	Osmotic condition (OC)	0.73	0.82	<0.01	<0.01	<0.01	<0.01
	G x OC	0.14	0.20	<0.01	0.01	0.02	<0.01

Table 5. Summarized results of experimental analyses performed on tomato (*Solanum lycopersicum* L.) *cry1a* mutant and WT seedlings under white light.

Growth condition	Analyses									
	Growth		Dry weight			Pigments content		Oxidative stress		Compatible osmolyte
	Shoot	Root	Shoot	Root	Ratio	Chlorophyll	Carotenoids	MDA	H ₂ O ₂	Proline
Nonstress	↑	↑	●	●	↑	↑	↑	↓	↓	↓
Osmotic stress	↓	↓	↓	↓	↓	●	↑	↑	●	↑

The arrows indicate a decreased (↓) or increased (↑) response of *cry1a* mutant seedlings relative to wild-type (WT) seedlings under the same growth conditions (no stress or osmotic stress). No differences between the *cry1a* mutant and WT are indicated by circles (●).

Table 6. Summarized results of experimental analyses performed on tomato (*Solanum lycopersicum* L.) *cry1a* mutant and WT seedlings under blue light fluence rates of 1 to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Seedlings under blue light fluence rates of 1 to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$												
Blue light fluences	Growth condition	Analyses										
		Growth		Dry weight			Pigments content			Oxidative stress		Compatible osmolyte
		Shoot	Root	Shoot	Root	Ratio	Chlorophyll	Carotenoids	Anthocyanin	MDA	H ₂ O ₂	Proline
1 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Nonstress	↑	↑	↓	↓	●	↑	●	↓	●	●	↓
	Osmotic stress	●	●	●	●	↓	↑	●	↓	↓	●	↓
5 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Nonstress	↑	↓	↑	●	●	↓	↓	●	●	↓	●
	Osmotic stress	●	●	●	●	●	↑	↑	●	↑	●	↓
10 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Nonstress	↑	●	●	●	●	↓	↓	●	●	●	●
	Osmotic stress	●	↑	↓	●	●	●	↓	↓	↑	●	↓
15 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Nonstress	↑	↓	●	↓	↑	↑	↑	↓	●	↓	↓
	Osmotic stress	↑	●	↓	●	●	↓	↓	↓	↓	↑	↓
25 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Nonstress	↑	●	●	↓	↑	↓	↓	↓	●	●	↓
	Osmotic stress	●	●	●	↑	↓	●	●	↓	↓	↓	●

The arrows indicate a decreased (↓) or increased (↑) response of *cry1a* mutant seedlings relative to wild-type (WT) seedlings at each blue light fluence under the same growth conditions (no stress or osmotic stress). No differences between the *cry1a* mutant and WT are indicated by circles (●).

Appendix B. Supplementary material for chapter 4

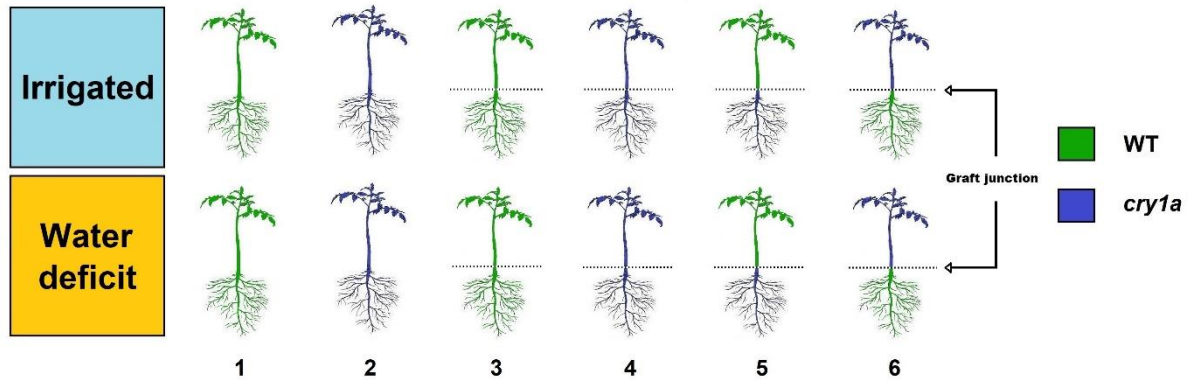


Figure 1. Reciprocal grafting of tomato (*Solanum lycopersicum* L.) *cry1a* mutant and wild-type (WT) rootstock/scion combinations under different growth conditions to study cryptochrome 1a signaling during water deficit: (1) WT, (2) *cry1a*, (3) WT/WT, (4) *cry1a/cry1a*, (5) WT/*cry1a* and (6) *cry1a*/WT.

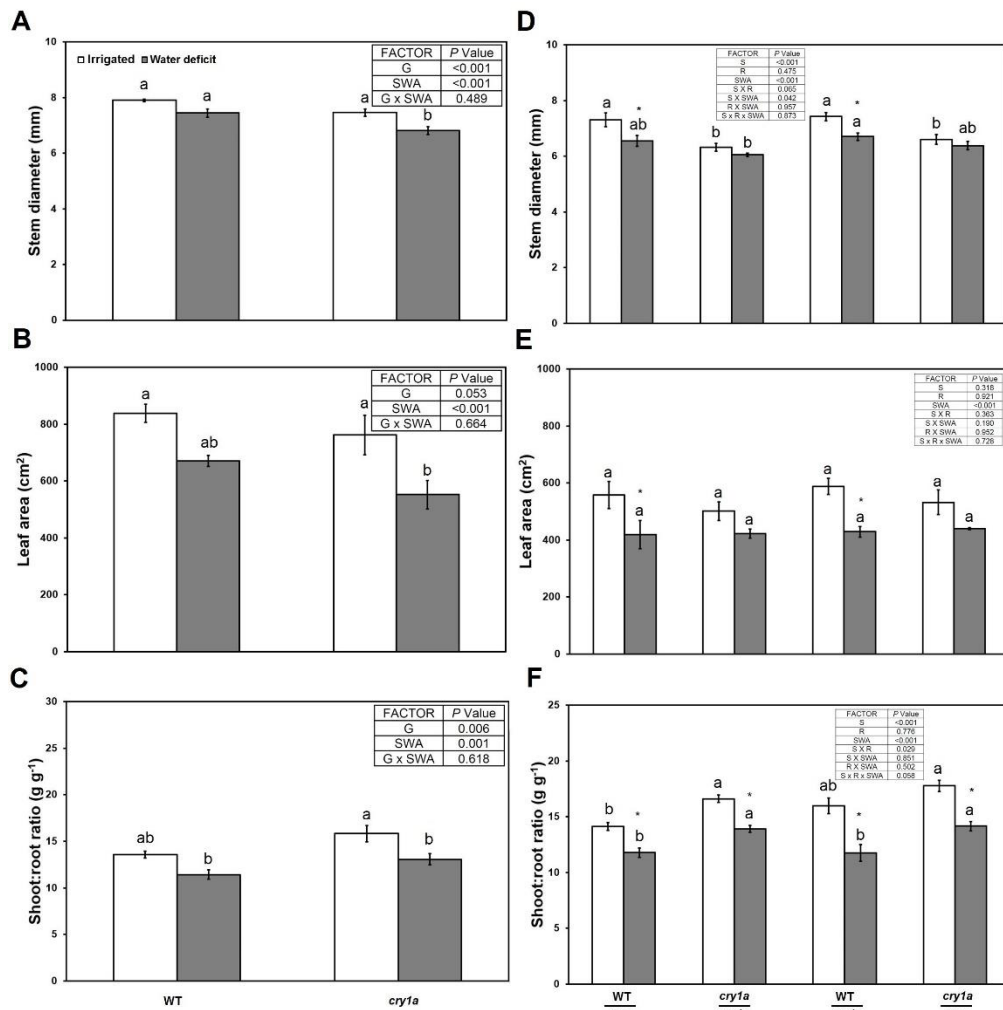


Figure 2. Supplementary growth analysis of tomato *cry1a* mutant and wild-type (WT): (A) stem diameter; (B) leaf area; (C) shoot:root dry weight ratio, and reciprocal grafts combination of *cry1a* and WT: (D) stem diameter; (E) leaf area; (F) shoot:root dry weight ratio, grown under irrigated or water deficit conditions. The stem diameter was measured with a digital caliper in the base of the stem. The total leaf area was evaluated using a leaf area meter (L-3100, Li-Cor, Lincoln, USA). The shoot to root ratio was calculated dividing shoot dry weight by root dry weight. The data are shown as the mean values of 5 plants of each treatment and the bars represent $\pm$ SEs. The distinct letters above the bars indicate significant differences between the plants (genotypes or graft combinations) within the same growth condition, and the asterisks represent significant differences between the growth conditions in the same plant (genotypes or graft combinations). The mean values were compared using the Tukey's test (significance at $p \leq 0.05$). The tables summarize the significance (P -values) of genotype (G), soil water availability (SWA), and their interactions (G x SWA) after ANOVA or scion (S), rootstock (R), soil water availability (SWA), and their interactions (S x R; S x SWA; R x SWA; S x R x SWA) after ANOVA.

Table 1. Linear correlation coefficient between parameters with r^2 and asterisks for P values.

	Chlorophyll a+b	Shoot MDA	Root MDA	Shoot H ₂ O ₂	Root H ₂ O ₂	Stomatal density	Stomatal pore area	RWC	A	E	Shoot DW
Shoot MDA	-0.39										
Root MDA	-0.27	0.74**									
Shoot H ₂ O ₂	-0.23	0.58*	0.71*								
Root H ₂ O ₂	-0.53	0.62*	0.41	0.26							
Stomatal density	0.28	0.13	0.22	0.076	-0.086						
Stomatal pore area	0.30	-0.61*	-0.39	-0.59*	-0.27	-0.14					
RWC	-0.14	-0.6	0.36	0.065	0.0087	-0.40	0.36				
A	0.40	-0.75**	-0.79**	-0.87***	-0.55	-0.20	0.66*	0.061			
E	0.33	-0.75**	-0.69*	-0.83***	-0.38	-0.27	0.79**	0.29	0.93***		
Shoot DW	0.43	-0.70*	-0.80**	-0.40	-0.49	-0.16	0.47	-0.42	0.58*	0.45	
Root DW	0.43	0.64*	-0.74**	-0.28	-0.62*	-0.030	0.12	-0.56	0.44	0.22	0.90***

Linear correlation coefficient between parameters with r^2 and asterisks for P values in own-rooted, reciprocal and self-grafted tomato plants grown under irrigated or water deficit condition. Text in bold highlights significant correlations plotted in figures 1–8. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.