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Endoplasmic reticulum-mediated unfolded protein response is an integral part of singlet oxygen signalling in plants

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SUMMARY

Singlet oxygen ($^1O_2$) is a by-product of photosynthesis that triggers a signalling pathway leading to stress acclimation or to cell death. By analyzing gene expressions in a $^1O_2$-overproducing Arabidopsis mutant (*ch1*) under different light regimes, we show here that the $^1O_2$ signalling pathway involves the endoplasmic reticulum (ER)-mediated unfolded protein response (UPR). *ch1* plants in low light exhibited a moderate activation of UPR genes, in particular *bZIP60*, and low concentrations of the UPR-inducer tunicamycin enhanced tolerance to photooxidative stress, together suggesting a role for UPR in plant acclimation to low $^1O_2$ levels. Exposure of *ch1* to high light stress ultimately leading to cell death resulted in a marked upregulation of the two UPR branches (bZIP60/IRE1 and bZIP28/bZIP17). Accordingly, mutational suppression of *bZIP60* and *bZIP28* increased plant phototolerance, and a strong UPR activation by high tunicamycin concentrations promoted high light-induced cell death. Conversely, light acclimation of *ch1* to $^1O_2$ stress put a limitation in the high light-induced expression of UPR genes, except for the gene encoding the *BIP3* chaperone, which was selectively upregulated. *BIP3* deletion enhanced Arabidopsis photosensitivity while plants treated with a chemical chaperone exhibited enhanced phototolerance. In conclusion, $^1O_2$ induces the ER-mediated UPR response that fulfils a dual role in high light stress: a moderate UPR, with selective induction of *BIP3*, is part of the acclimatory response to $^1O_2$, and a strong activation of the whole UPR is associated with cell death.

Keywords: singlet oxygen, signalling, endoplasmic reticulum, unfolded protein response, stress acclimation, programmed cell death, excess light energy.

INTRODUCTION

Plants can cope with a variety of environmental constraints and unfavourable growth conditions by reprogramming nuclear gene expression in response to organellar stimuli (Woodson and Chory, 2012). Signal molecules, such as reactive oxygen species (ROS), can be sensed directly or indirectly by the nucleus and induce genetic adjustments that lead either to stress acclimation or to programmed cell death (PCD; Gill and Tuteja, 2010; Mignolet-Spruyt *et al.*, 2016).

Among ROS, singlet oxygen ($^1O_2$) is peculiar because it is a non-redox compound produced from triplet chlorophylls inside the chloroplasts, mainly at the photosystem II (PSII) level, when light energy absorbed by the chlorophylls exceeds the capacities of photosynthesis (Krieger-Liszkay *et al.*, 2008; Triantaphylidès and Havaux, 2009). $^1O_2$ is very reactive and oxidizes macromolecules such as proteins and lipids, but it also acts as a signal molecule (Wagner *et al.*, 2004; Kim *et al.*, 2008; Triantaphylidès *et al.*, 2008; Triantaphylidès and Havaux, 2009; Laloi and Havaux, 2015). In two $^1O_2$-overproducing Arabidopsis mutants, *ch1* and *flu,* several proteins and mediators have been shown to play a role in stress acclimation and induction of PCD (Wagner *et al.*, 2004; Simkova *et al.*, 2012; Ramel *et al.*, 2013a; Laloi and Havaux, 2015; Carmody *et al.*, 2016; Chan *et al.*, 2016). In particular, phytohormones appear to be central players in the $^1O_2$-signalling network leading to PCD (Overmyer *et al.*, 2003; Danon *et al.*, 2004; Shumbe *et al.*, 2016; Beaugelin *et al.*, 2019). This indicates an integrated communication within the cell and emphasizes retrograde and interorganellar communications. In numerous signalling pathways, interorganellar signalling occurs through ROS, reactive nucleophile species and phytohormones (Kleine and Leister, 2016; Mignolet-Spruyt *et al.*, 2016).

Recently, it has been reported that chloroplastic retrograde signalling could also involve the endoplasmic reticulum (ER; Walley *et al.*, 2015; de Souza *et al.*, 2017). The

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ER is known to be the location of synthesis and folding of a large portion of the cellular proteins in eukaryotic cells. In animals, a stress-related mechanism has been extensively studied which is called unfolded protein response (UPR). In fact, UPR has been linked to numerous human diseases such as Alzheimer, Parkinson and Huntington diseases (Xiang *et al.*, 2017). In plants, the occurrence of an ER stress was initially published by Iwata and Koizumi (2005), with bZIP60 being identified as a homologue of a mammalian XBP1/yeast HAC1. Since then, the number of studies has noticeably increased, identifying various plant homologues of mammalian proteins involved in ER stress.

The UPR response in the ER is a ubiquitous mechanism taking place when unfolded and misfolded proteins aggregate inside the ER lumen, which induces a set of genes elevating the protein folding capacity in the ER (reviewed by Wan and Jiang, 2016). When the synthesis of new proteins exceeds the folding capacity, unfolded proteins accumulate in the lumen, causing ER stress. The UPR is activated when those unfolded proteins are sensed and transcription factors such as bZIP60, bZIP28 and bZIP17 are relocated to the nucleus inducing the transcription of UPR-responsive genes (Howell, 2013). They form two arms of the UPR response: one arm involves the ER-localized kinase/ribonuclease IRE1, which catalyzes the unconventional slicing of bZIP60 mRNA, and the other arm is mediated by bZIP17 and bZIP28. Those transcription factors were reported to bind to specific UPR gene promoters and hence to induce distinct UPR responses. Those genes code for ER-located effectors such as immunoglobulin binding proteins (BIP), acting as chaperones, and protein disulfide isomerases (PDI). Those proteins have a common function, namely helping the proper folding of proteins. In both animal and plant cells, PCD is induced when the UPR fails to cope with ER stress (Wang, 2001; Xu *et al.*, 2005; Williams *et al.*, 2014). The UPR mechanism has thus a dual function, as a protective system enhancing protein folding and as a PCD inducer.

A functional link between ER and chloroplasts has recently been described in a study of a mutant of the isoprenoid biosynthesis pathway (*ceh1*). This mutant accumulates 2-C-methyl-D-erythritol-2,4-cyclopyrophosphate (MEcPP) and shows an UPR transcriptional response (Walley *et al.*, 2015). Chemical treatments inducing organellar ROS production have also shown UPR activation. For instance, rotenone treatments promote anion superoxide production leading to upregulation of UPR marker genes (Ozgur *et al.*, 2015). Conversely, DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) treatments, inducing $^1O_2$ production in the chloroplast, were reported to inhibit UPR (Ozgur *et al.*, 2015). Similar to the ER-mediated UPR, mitochondria and chloroplasts can also exhibit an UPR-type response corresponding to the accumulation of proteases and chaperones in these organelles to remove damaged proteins (Callegari and Dennerlein, 2018; Dogra *et al.*, 2019).

As mentioned above, most studies have focused on elucidating molecular interactions between ER stress proteins using ER genes mutants and tunicamycin (Tm). Tm inhibits N-glycosylation of neosynthetized proteins, thus causing protein retention in the ER, resulting in ER stress. The UPR is commonly studied by analyzing the transcriptional profile of UPR marker genes under Tm treatments (Martinez and Chrispeels, 2003; Gao *et al.*, 2008; Nagashima *et al.*, 2011; Chen and Brandizzi, 2013). Phenotypical studies have mainly focused on mutants of the ER signalling in response to Tm-induced chronic ER stress (Martinez and Chrispeels, 2003; Srivastava *et al.*, 2013; Sun *et al.*, 2013) or on recovery phenotypes after ER stress on seedlings pretreated with Tm (Mishiba *et al.*, 2013; McCormack *et al.*, 2015). More recently, interest in ER stress in plants has risen rapidly, which is now seen as an integrated and finely regulated mechanism (Lu *et al.*, 2018). Long-term and chronic effects can be dissociated from recovery and transient management of ER stress. Results of recent studies describe a complex process in which the bZIP60/IRE1 UPR arm is activated during chronic ER stress, but bZIP60 is activated in an IRE1-independent manner during ER stress recovery (Guo *et al.*, 2018; Ruberti *et al.*, 2018).

The *ch1* mutant of *Arabidopsis thaliana* is highly sensitive to high light because it produces more $^1O_2$ from PSII compared with the wild-type (WT; Dall' Osto *et al.*, 2010; Ramel *et al.*, 2013a), even in low light (Shumbe *et al.*, 2017). The difference between *ch1* and WT in terms of ROS photoproduction is specific to $^1O_2$ as shown by ROS quantifications (Dall'Osto *et al.*, 2010) and the expression of ROS marker genes (Ramel *et al.*, 2013a). Non-photochemical energy quenching (NPQ) is reduced in *ch1* relative to WT, especially at high light intensities (Havaux *et al.*, 2007). However, the reduced NPQ is not a major factor in the high $^1O_2$ production by *ch1* because complete suppression of NPQ in the *ch1 npq4* double mutant did not enhance photosensitivity (Havaux *et al.*, 2007). Moreover, increased $^1O_2$ levels in *ch1* were measured under low light conditions that were not associated with different NPQ levels in *ch1* and WT.

The *ch1* mutant is able to acclimate to high light and $^1O_2$ toxicity after exposure to a moderately elevated light intensity that induces a moderate $^1O_2$ production (Ramel *et al.*, 2013a). Previous transcriptomic analyses performed on this mutant indicated the induction of the UPR transcriptional signature both in low light and in high light (Ramel *et al.*, 2013a). This prompted us to examine the possible role of the ER and of the UPR response in the $^1O_2$ signalling pathways leading to acclimation or to PCD. The results of this study show that $^1O_2$ triggers the ER-mediated UPR process, with different UPR levels inducing different physiological responses to light stress.

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RESULTS

ER stress marker genes in transcriptomic responses of Arabidopsis to $^1O_2$

In Figure 1, we analyzed various transcriptomic data related to high light and $^1O_2$ or to ER stress. Figure 1(a) shows the putative localization of proteins encoded by genes that are induced in the *ch1* mutant under high light, that is genes inducible by elevated $^1O_2$ levels (Ramel *et al.*, 2013a). A large fraction of the induced genes encodes proteins located in the cytoplasm (50%), the remaining proteins are located in mitochondria (13.1%), in chloroplasts (9.0%) and in the endomembrane system including the ER (7.4%) and the Golgi apparatus (6.6%). We also compared the transcriptome of the *ch1* mutant and WT in control conditions (Ramel *et al.*, 2013a), to determine the biological processes triggered by a chronic and low production of $^1O_2$ in *ch1*. Figure 1(b) shows the highest gene ontology enrichments in the *ch1* mutant compared with WT, which are mainly associated with responses to biotic, abiotic, and chemical stresses. Interestingly, the ER stress response is one of the major biological processes induced in the *ch1* mutant compared with WT. Thus, ER-related genes represent a substantial fraction of the genes induced by both chronic and acute $^1O_2$ photoproductions.

To have a better view of the ER stress response in the *ch1* mutant, we compared the expression levels of ER stress marker genes in various transcriptomic data: (1) a 10-h treatment of Arabidopsis seedlings with 10 μg ml^{-1} Tunicamycin (Tm) vs Mock treatment (Iwata *et al.*, 2010), (2) the Arabidopsis *ceh1* mutant vs WT (Xiao *et al.*, 2012), (3) the *ch1* mutant vs WT (Ramel *et al.*, 2013a), and (4) a treatment of Arabidopsis WT plants with β-cyclocitral, a β-carotene oxidation product known to act as a signalling molecule in the $^1O_2$-signalling pathway leading to acclimation (Ramel *et al.*, 2012; D'Alessandro *et al.*, 2018). The Tm treatment provides a reference transcriptome specific to ER stress and UPR activation (Figure 1c(i)). Tm is known to specifically induce defects in *N*-glycosylation leading to ER stress (Iwata *et al.*, 2010). The *ceh1* mutant is impaired in the biosynthesis of isoprenoids and accumulates MEcPP, leading to the induction of UPR-related genes (Walley *et al.*, 2015). The comparison of these expression profiles (Figure 1c(i) and (ii)) shows that the UPR genes are widely induced in *ceh1,* with no apparent specific pattern. By contrast, the *ch1* mutant shows a constitutive upregulation of numerous UPR genes (Figure 1c(iii)). The Tm treatment promotes expression of specific genes, and interestingly the same genes were induced by $^1O_2$ in *ch1*. In both Tm-treated plants and *ch1* plants, expression of *bZIP60, ERdj3B, BIP3, PDI1, PDI5, PDI6, PDI9, PDI10*, and *PDI11* is selectively increased. The similar transcriptional regulation in *ch1* and Tm-treated plants suggests a role for the ER in the response to $^1O_2$. Conversely, only a few genes were upregulated in β-cyclocitral-treated plants (Figure 1c(iv)), indicating the existence of several pathways downstream of $^1O_2$ both dependent and independent of UPR activation. In Table S1, we also examined microarray-based transcriptomic data of the Arabidopsis *flu* mutant after a transition from darkness to light (2-h illumination; op den Camp *et al.*, 2003). The *flu* mutant accumulates a chlorophyll precursor in the dark that generates high amounts of $^1O_2$ upon transfer to light (op den Camp *et al.*, 2003), leading to PCD. Some of the UPR-related genes induced in *ch1* (*BIP3, bZIP60, PDI11*) were also induced in *flu*, thus confirming their sensitivity to $^1O_2$. The fact that the other genes were not induced in *flu* could indicate that they require longer exposure to $^1O_2$ rather than 2 h or that they are specific to conditions leading to acclimation to $^1O_2$ (*ch1* in low light) rather than cell death (*flu* after dark-to-light conditions). It should be noted that the two $^1O_2$ mutants are not directly comparable because the sources of $^1O_2$ are very different (PSII in *ch1*, a chlorophyll precursor in the thylakoid membranes in *flu*), and gene expression analyses were performed on different time scales.

Unfolded protein response is activated by $^1O_2$

ER stress markers can be divided in two groups. The first group includes the ER stress sensors (Figure 2a) represented by the transcription factor *bZIP60* in both the unspliced and spliced forms, the *IRE1a* and *IRE1b* genes, coding for proteins involved in splicing of *bZIP60*, as well as *bZIP28* and *bZIP17,* also involved in the transcription of UPR-responsive genes (Ruberti *et al.*, 2015). *ANAC089* is also listed in this group; this transcription factor is activated downstream of the bZIPs, but plays a key role in the induction of ER-mediated PCD (Yang *et al.*, 2014).

We analyzed the expression of those genes in WT and in the $^1O_2$-overproducing *ch1* mutant exposed to control conditions, to high light and to acclimatory light conditions leading to increased resistance to $^1O_2$ in *ch1*. Firstly, *bZIP60* (spliced) was the only gene that exhibited higher levels of expression in the *ch1* mutant compared with in the WT in low light. As $^1O_2$ production of *ch1* in low light is small, but significantly higher than the WT levels (Shumbe *et al.*, 2017), this observation indicated that *bZIP60* is very sensitive to $^1O_2$. The expression levels of most genes increased significantly when light intensity, and therefore $^1O_2$ production, rose (Figure 2a). The only exception here was *bZIP17*. Nonetheless, under acclimatory light conditions, *bZIP17* reacted in the same way as *bZIP60* (spliced and unspliced), *IRE1a*, *IRE1b*, and *ANAC089*. Indeed, those genes have a lower expression level when plants have been acclimated to light before being subjected to high light (ACC + HL in Figure 2) than without acclimation (HL). *bZIP28* is the exception here with similar levels of expression under high light, independently of the pre-treatment (Figure 2a). To sum up, light

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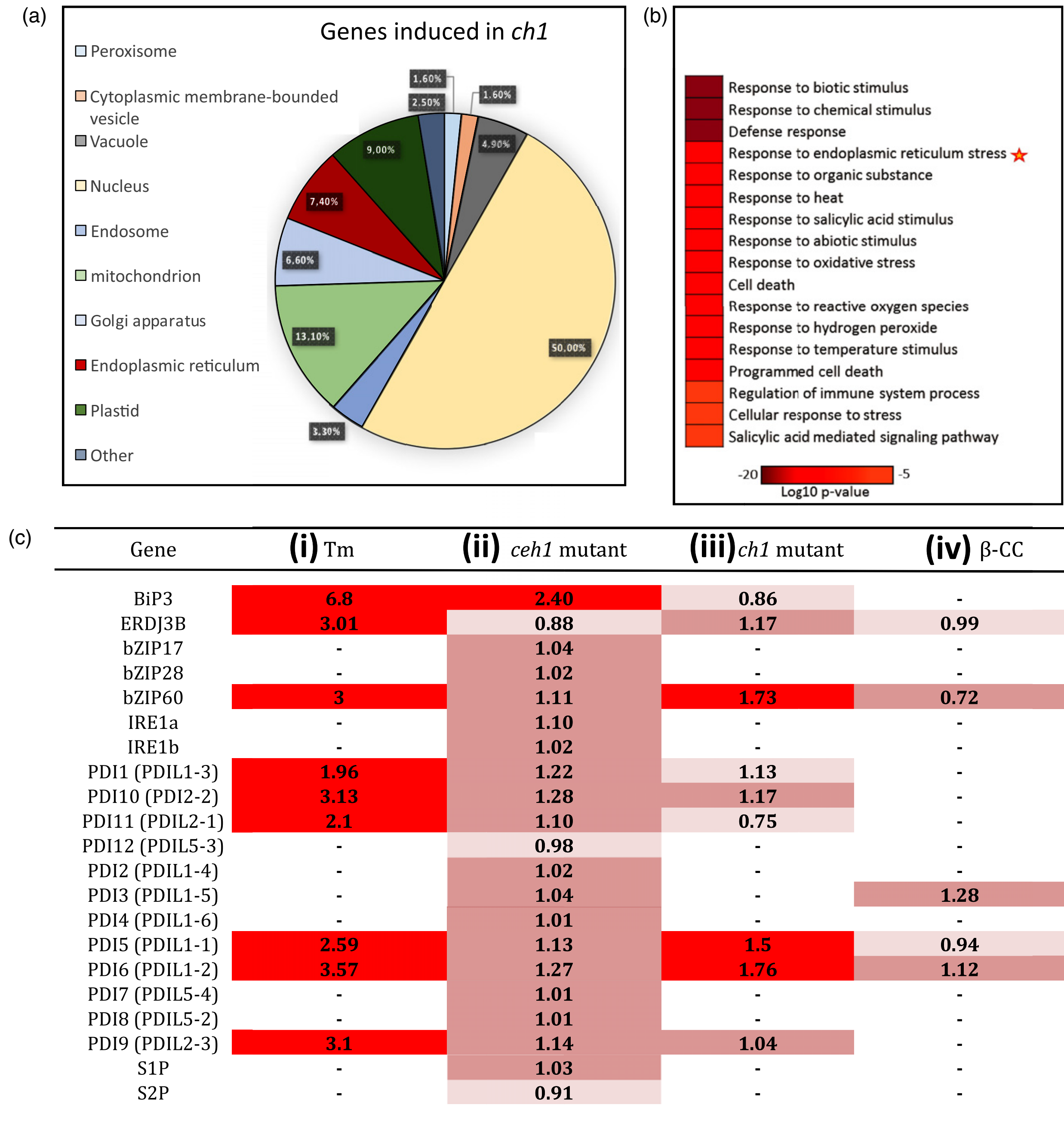

Gene	(i) Tm	(ii) *ceh1* mutant	(iii) *ch1* mutant	(iv) β-CC
BiP3	6.8	2.40	0.86	-
ERDJ3B	3.01	0.88	1.17	0.99
bZIP17	-	1.04	-	-
bZIP28	-	1.02	-	-
bZIP60	3	1.11	1.73	0.72
IRE1a	-	1.10	-	-
IRE1b	-	1.02	-	-
PDI1 (PDIL1-3)	1.96	1.22	1.13	-
PDI10 (PDI2-2)	3.13	1.28	1.17	-
PDI11 (PDIL2-1)	2.1	1.10	0.75	-
PDI12 (PDIL5-3)	-	0.98	-	-
PDI2 (PDIL1-4)	-	1.02	-	-
PDI3 (PDIL1-5)	-	1.04	-	1.28
PDI4 (PDIL1-6)	-	1.01	-	-
PDI5 (PDIL1-1)	2.59	1.13	1.5	0.94
PDI6 (PDIL1-2)	3.57	1.27	1.76	1.12
PDI7 (PDIL5-4)	-	1.01	-	-
PDI8 (PDIL5-2)	-	1.01	-	-
PDI9 (PDIL2-3)	3.1	1.14	1.04	-
S1P	-	1.03	-	-
S2P	-	0.91	-	-

Figure 1. Analysis of the transcriptome of the Arabidopsis *ch1* mutant in comparison with other transcriptomes. (a) Cellular localization of proteins coded by genes induced in *ch1* plants (leaves) exposed to high light stress ($\log_2 > 0.5$) relative to *ch1* grown under standard conditions (data from Ramel et al., 2013a). (b) Control conditions (data from Ramel et al., 2013a). (c) Comparative transcriptomic analysis of endoplasmic reticulum (ER) stress-related genes induced (i) by Tm (data from Iwata et al., 2010), in (ii) the Arabidopsis *ceh1* mutant (Walley et al., 2015), (iii) the *ch1* mutant (Ramel et al., 2013a), and (iv) β-CC-treated Arabidopsis WT plants (Ramel et al., 2012). Data are in $\log_2$.

conditions (HL) leading to cell death in *ch1* induces expression of UPR-related genes while acclimation to $^1O_2$ (ACC + HL) appears to require a downregulation of the ER stress sensors. *bZIP28* has a peculiar profile of expression, being induced by both high light stress and acclimation conditions. Taken together, those results indicated correlation between the global induction of the UPR sensors and the occurrence of cell death.

The second group of ER stress markers includes UPR genes that code for protein involved in the protein folding process in the ER lumen, and that are called UPR effectors (Figure 2b) containing chaperones and PDIs. *ERdj3B* is

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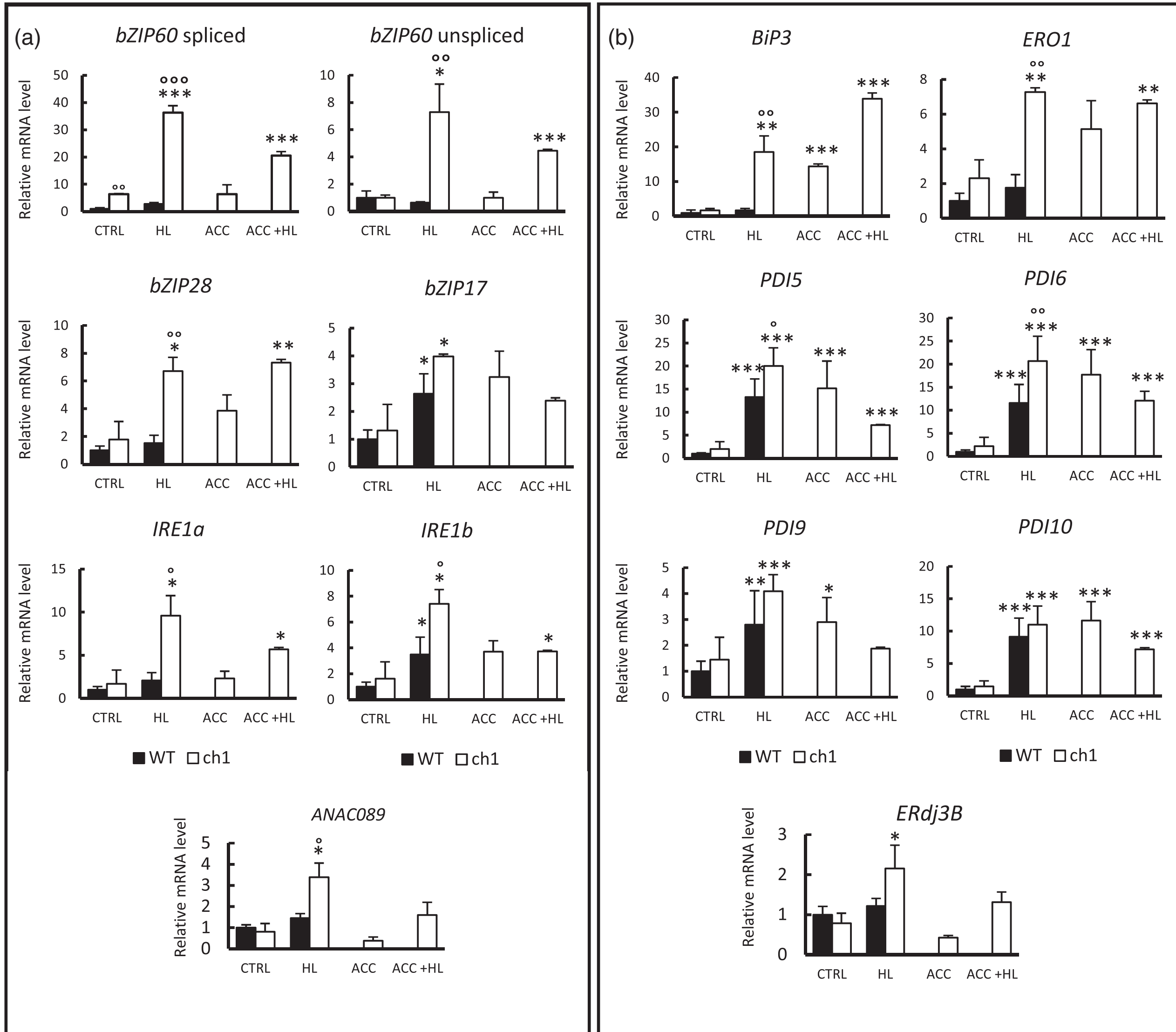

Figure 2. RT-qPCR analysis of *UPR* gene expression under various light conditions. Wild-type (WT) (black bars) and *ch1* plants (white bars) grown under standard conditions (CTRL) were exposed to high light stress for 8 h (HL). Acc + HL = *ch1* plants exposed to high light stress after light acclimation (Acc). Panel (a) shows genes encoding UPR sensors. Panel (b) shows UPR effectors. Data are expressed relative to WT CTRL and are mean values of 3 separate experiments + SD. Student's *t*-test: *, **, ***; different from control conditions at $P < 0.05$, 0.01 and 0.001, respectively. o, oo and ooo, *ch1* values different WT values at $P < 0.05$, 0.01 and 0.001, respectively.

another UPR biomarker gene that can be classified in this category. Both *BIP3* and *ERdj3B* are influenced by *bZIP28* and *bZIP60*. *BIP3* is more dependent on *bZIP60*, while *ERdj3B* exhibits a stronger dependence on *bZIP28* (Ruberti *et al*., 2018). This group of genes can be divided into two subgroups according to their expression profile. The first subgroup concerns *BIP3, ERO1*and *ERdj3B*. Those genes were not significantly expressed in WT plants in high light. Conversely, in *ch1,* their expression was strongly upregulated in high light. The second subgroup is represented by *PDI5*, *PDI6*, *PDI9*, and *PDI10*, which exhibited comparable levels of expression in WT and *ch1* both in low and high light conditions. Acclimation of *ch1* to light (i.e. to $^1O_2$) led to a downregulation of the expression of the four PDI genes in high light. *ERdj3B* is also downregulated during acclimation. Interestingly *BIP3* and *ERO1* are two genes not downregulated by the acclimation treatment. In fact, *BIP3* expression was even maximal in *ch1* plants acclimated and then exposed to high light.

Salicylate is required for high light-induced cell death and induction of ER stress genes in *ch1*

As shown in Figure 3(a–c), salicylate is required for the development of high light-induced cell death in *ch1*. The double mutant *ch1 sid2*, deficient in salicylate biosynthesis, was much more resistant to high light than *ch1*, with

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no leaf bleaching (Figure 3a) and reduced lipid peroxidation (Figure 3a (bottom) and 3b). Accumulation of hydroxy fatty acids and of lipid peroxides has been previously shown to be associated with the onset of PCD in plant leaves (Beaugelin *et al.*, 2019). This was further confirmed here by visualizing the extent of cell death by Trypan blue staining (Figure 3c). Leaves from the *ch1 sid2* double mutant exhibited much less dead cells than *ch1* leaves. Furthermore, the expression of ER stress sensors (*bZIP60, IRE1a, IRE1b, ANAC089, ERdj3B*) was noticeably reduced in high light-treated double mutant plants compared with *ch1* plants (Figure 3d). The expression levels measured in *ch1 sid2* leaves were roughly similar to the levels measured in control *ch1* leaves not exposed to high light. As shown in Figure 2, UPR-related genes are expressed similarly in *ch1* and *WT* under control conditions, except bZIP60 (spliced) which was more induced in *ch1*. These results confirmed the correlation between the occurrence of cell death and ER stress-related gene expression and highlight the role of salicylic acid in the induction of $^1O_2$-induced ER stress (Ruberti *et al.*, 2018). In contrast, the expression of the UPR effector gene *PDI5* was unaffected by the change in salicylate. This differential sensitivity of *PDI5* and *PDI6* to salicylate remains to be explored.

The chaperones BIP3 and TUDCA protect plants against photooxidative damage

BIP3 was found to be the most induced gene in *ch1* plants acclimated to light stress (Figure 2b), suggesting a possible involvement in photoresistance. A knock-out *bip3* mutant was then exposed to high light stress. *bip3* plants exhibited an increased sensitivity to high light compared with WT, which was associated with higher levels of lipid peroxidation measured by autoluminescence imaging and high-performance liquid chromatography (HPLC) analysis of hydroxy octadecatrienoic acid (HOTE) resulting from the oxidation of linolenic acid, the major fatty acid in Arabidopsis leaves (Figure 4a,b).

Tauroursodeoxycholic acid (TUDCA) is a chemical chaperone that can mitigate ER stress (Uppala *et al.*, 2017). This compound has been rather extensively studied in mammals in which it stabilizes misfolded proteins and polypeptides and plays a role in protein transport and degradation (Phillips *et al.*, 2008; Lee *et al.*, 2010; Drack *et al.*, 2012). In plants, TUDCA has been shown to alleviate ER stress and cell death caused by Tm and dithiothreitol (Watanabe and Lam, 2008). As shown in Figure 5, Arabidopsis plants sprayed with 100 or 250 μM TUDCA before high light stress exhibited an increased phototolerance compared with water-treated plants. Visual symptoms of photodamage (leaf bleaching, flaccidness) were reduced by TUDCA (Figure 5a). Moreover, lipid peroxidation, as measured by autoluminescence imaging (Figure 5b) and HOTE levels (Figure 5c), was lowered. The results shown in Figures 4(a,b) and 5 proved the role of natural or synthetic 'anti-ER stress' chaperones (BIP3 or TUDCA, respectively) in the tolerance of Arabidopsis to high light stress. Therefore, we can hypothesize that UPR effectors contribute to diminishing ER stress and hence sensitivity to high light stress.

Induction of ER stress can either induce or reduce tolerance to high light stress depending on the level of UPR activation

Plants were treated with low (1 μg ml^{-1}) or high (10 μg ml^{-1}) concentration of Tm in a unique pulse of 5 h before high light exposure. The high light stress was performed until photooxidative damage is observed. In the experiments with a 1 μg ml^{-1} Tm pre-treatment, symptoms appeared in plants after 30 h of high light exposure. Conversely, in the 10 μg ml^{-1} Tm pre-treatment, symptoms of stress appeared in Tm-treated plants already after 26 h of high light exposure. Therefore, it is likely that high Tm concentrations and the consequent ER stress enhance high light-induced oxidative damage.

The low Tm treatment brought about a small increase in the expression of the ER-related genes *bZIP60* (spliced), *BIP3* and *ERdj3B* (Figures 6c and S1). indicating low ER stress and UPR response at this concentration. High light induced the expression of those genes, and this induction was not perturbed by Tm treatments. In contrast with those genes, the low Tm treatment decreased *bZIP28* expression both in low light and in high light (Figure 6c).

The 1 μg ml^{-1} Tm-treated plants exhibited a clear tolerant phenotype to high light associated with lower levels of autoluminescence and HOTE compared with control plants (Figure 6a,b). We also investigated the expression of cell death marker genes in low Tm-treated plants exposed to high light stress compared with untreated plants. Those genes were selected according to their involvement in high light-induced cell death (*OXI1*), in oxidative stress-induced cell death (*MC8*) or in ER stress-induced NRP-mediated cell death (*NRP*s, γ*VPE*; He *et al.*, 2008; Reis *et al.*, 2016; Shumbe *et al.*, 2016). Under high light stress, all genes tested were repressed in plants pre-treated with 1 μg ml^{-1} (Figure 7a), in line with the higher phototolerance of the plants.

In plants treated with a high Tm concentration of 10 μg ml^{-1}, *bZIP60*, and *bZIP28* were significantly induced (Figure 6f), indicating ER stress, as expected. *BIP3* was only slightly upregulated whereas *ERdj3B* expression was not affected. High light further increased the expression of the *bZIP* genes, and this was particularly marked for *bZIP28*. Thus, high Tm concentration and high light had a synergistic effect on the ER stress. High light-induced increase in gene expression was moderate for *BIP3* and did not occur for *ERdj3B*. Moreover, the high Tm-treated plants exhibited increased photosensitivity compared with control plants. This was associated with higher intensity of autoluminescence and higher levels of HOTE (Figures 6d,e). The combination of enhanced ER stress levels with high light

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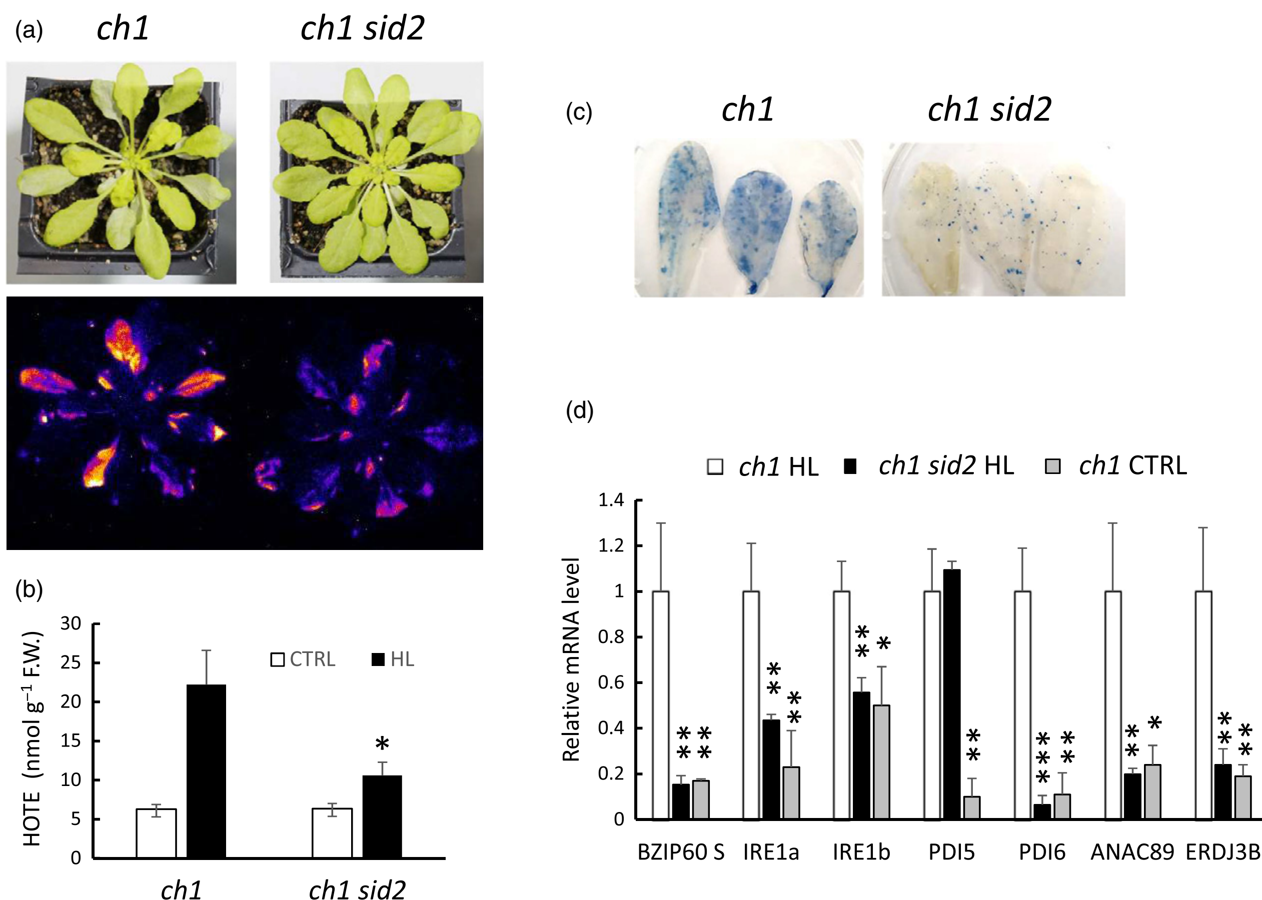

Figure 3. Effects of salicylate on photooxidative damage and UPR response in the *ch1* mutant. The *ch1* single mutant and the salicylate-deficient double mutant *ch1 sid2* were exposed to high light stress for 28 h. (a) Photograph of the plants (top) and autoluminescence image of lipid peroxidation (bottom) after high light stress. (b) Hydroxy octadecatrienoid acid (HOTE) levels before and after high light stress. Data are mean values of three experiments + SD. *, different from *ch1* at $P < 0.05$ (Student's *t*-test). (c) Extent of cell death as measured by trypan blue staining. (d) RT-qPCR of *UPR* gene expression. Data are expressed relative to the *ch1* HL values and are mean values of three separate experiments + SD. *, ** and ***, different from *ch1* HL at $P < 0.05$, 0.01 and 0.001, respectively (Student's *t*-test). S, spliced.

exposure led to extensive damage, indicating synergic effects of Tm and high light on PCD induction. Cell death marker genes were induced by high light in plants pre-treated with 10 μg ml^{-1} Tm (Figure 7b), consistently with the damage shown in Figure 6. Thus, elevated ER stress at a high Tm concentration increased the sensitivity to high light and was associated with induction of PCD while a low Tm concentration preserved plants from cell death. Moreover, this experiment with two Tm concentrations shows a correlation between *bZIP28* expression and plant photosensitivity/tolerance. Low Tm concentration that enhanced phototolerance was associated with a downregulation of this gene. Conversely, increased photosensitivity at high Tm concentration was associated with an upregulation of *bZIP28*. In contrast, *bZIP60* and *BIP3* responded almost similarly at either low or high Tm concentrations.

bzip60 bzip28 double mutant is more tolerant to high light

Single mutations in *bZIP* genes of the plant UPR signalling pathway do not lead to prominent phenotypes (Deng *et al.*, 2013). Since PCD was associated with simultaneous induction of the two arms of the UPR (Figures 2 and 6), we analyzed the high light stress response of the double mutant *bzip60 bzip28* affected in both arms of the UPR (Figure 4c,d). The double mutant was found to be substantially more resistant to high light than WT, with less leaves exhibiting high autoluminescence (Figure 4a) and with lowered HOTE levels (Figure 4b). Thus, the concomitant loss of *bZIP60* and *bZIP28* appears to preclude the development of cell death, indicating the involvement of the two UPR pathways in PCD. This is at variance with Tm-induced ER stress which was shown to have more effects on the *bzip60 bzip28* double mutant compared with WT (Ruberti *et al.*, 2018), indicating that the mechanisms underlying Tm-induced PCD and high light-induced PCD do not fully overlap.

DISCUSSION

Singlet oxygen triggers ER stress and the UPR response

Gene expression analyses of the *ch1* mutant under different light conditions presented here show that $^1O_2$ induces

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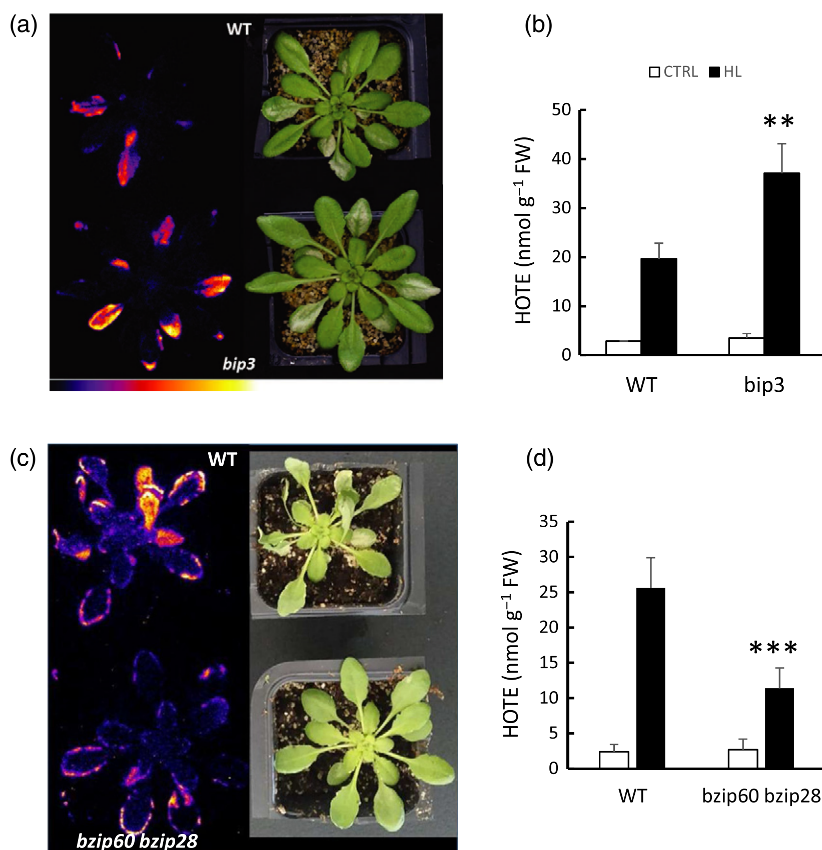

Figure 4. Response of the *bip3* mutant and the *bzip60 bzip28* double mutant to high light stress. WT and single/double mutant plants were exposed to high light stress (1 500 μmol m^{-2} sec^{-1} at 7°C) for 28 h. (a, c) Photographs of the plants and autoluminescence images of lipid peroxidation after high light stress. (b, d) HOTE levels before and after high light stress. **, Different from WT at $P < 0.01$; ***, different from WT at $P < 0.001$; $n = 3$).

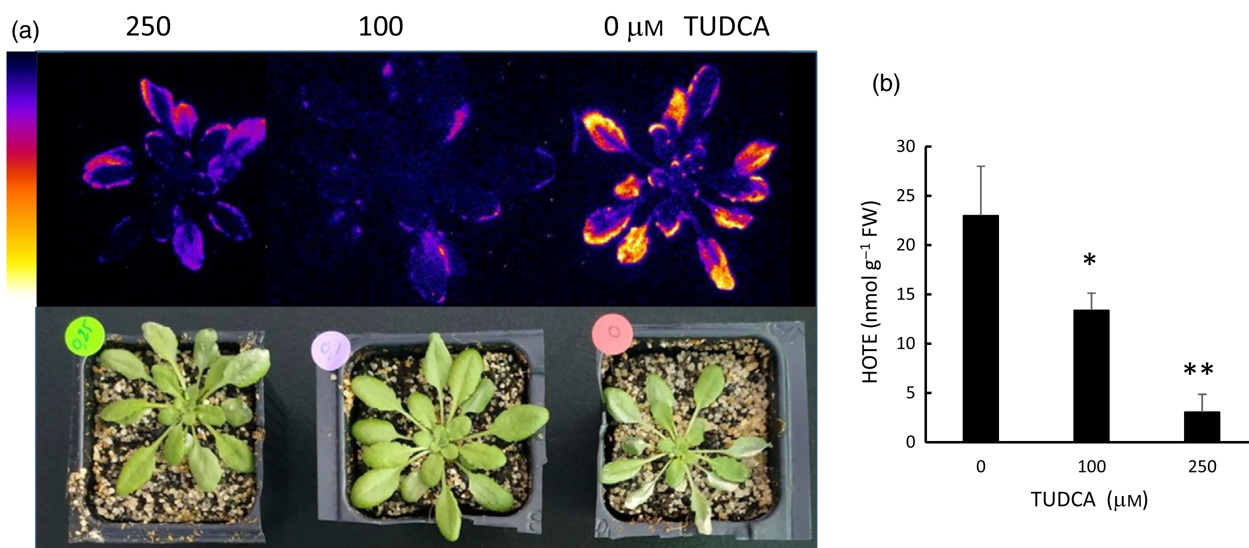

Figure 5. Effect of TUDCA on the tolerance of Arabidopsis to high light stress. WT plants, sprayed with 0.1 and 0.25 mM TUDCA, were let to dry for 1 h before exposure to high light stress (1 500 μmol m^{-2} sec^{-1}). (a) Photograph of the plants after high light stress (bottom); autoluminescence imaging of lipid peroxidation (top). (b) HOTE levels *, different from WT at $P < 0.05$; **, different from WT at $P < 0.01$; $n = 3$).

ER-mediated UPR in Arabidopsis leaves. Chloroplast-to-ER interorganellar signalling was previously considered in the Arabidopsis *ceh1* mutant (Xiao *et al.*, 2012) and in Arabidopsis plants treated with DCMU (Ozgur *et al.*, 2015). However, in contrast with our results, high doses of DCMU were found to downregulate UPR genes (Ozgur *et al.*,

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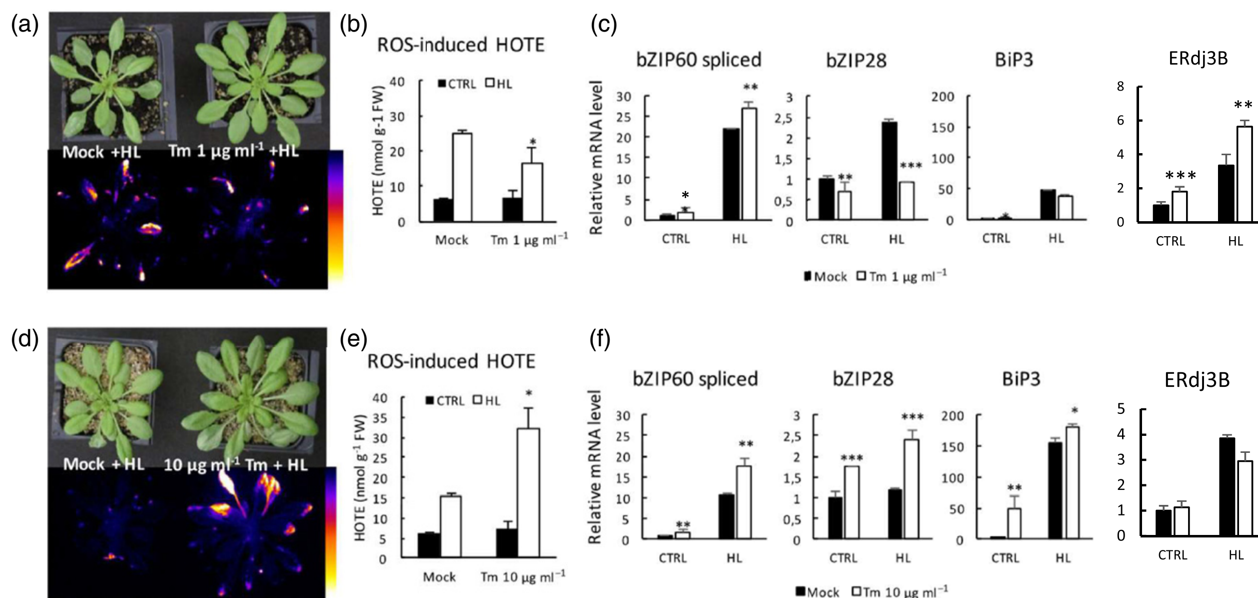

Figure 6. Induction of cell death or acclimation by Tm. Arabidopsis plants were sprayed with two different concentrations of Tm: 1 μg ml^{-1} (panels a–c) and 10 μg ml^{-1} (panels d–f). High light stress (1500 μmol photons m^{-2} sec^{-1}) was imposed 5 h after the Tm pre-treatment. (a) Phenotype and autoluminescence imaging of Tm-treated or Mock-treated plants after 30 h of high light exposure. (b) HOTE levels before (CTRL) and after high light stress (HL) in Tm-treated and Mock-pre-treated plants (Student's *t*-test: *, different from WT at $P < 0.05$; $n = 3$). (c) RT-qPCR analysis of *bZIP60* (spliced version), *bZIP28*, *BIP3* and *ERdj3B* before and after high light stress in Tm-treated and Mock-treated plants (Student's *t*-test: *, different from WT at $P < 0.05$; **, different from WT at $P < 0.01$; ***, different from WT at $P < 0.001$; $n = 3$). (d) Phenotype and autoluminescence imaging of Tm-treated or Mock-treated plants after 26 h of high light exposure. (e) HOTE levels before (CTRL) and after high light stress (HL) in Tm- and Mock-pre-treated plants (Student's *t*-test: **, different from WT at $P < 0.05$; $n = 3$). (f) RT-qPCR analysis of *bZIP60* (spliced version), *bZIP28*, *BIP3* and *ERdj3B* before and after high light stress in Tm-treated and Mock-treated plants (Student's *t*-test: *, different from WT at $P < 0.05$; **, different from WT at $P < 0.01$; ***, different from WT at $P < 0.001$; $n = 3$).

2015). DCMU blocks the binding of the primary quinone electron acceptor (Q_A) to PSII, hence inhibiting photosynthetic electron transport and increasing $^1O_2$ production by PSII (Fufezan *et al.*, 2002). Anyway, the treatment used by Ozgur *et al.* (2015) induced a severe oxidative stress in the chloroplasts, which is not comparable with the effects of $^1O_2$ production in *ch1* in our study (Figure 2). Only when *ch1* was exposed to high light stress conditions for a prolonged period of time (28 h, instead of 8 h as in Figure 2), photooxidative damage occurred in leaves (see Figure 3), and this condition also led to a marked repression of UPR genes (Figure S2). So, the downregulation of the UPR, previously found with high DCMU doses, could be the consequence of extensive damage to leaf cells rather than a direct effect of DCMU-induced $^1O_2$ formation on ER stress-related genes.

The involvement of ER in a chloroplast retrograde signalling process has been proposed to take place in the *ceh1* mutant that accumulates MEcPP (Walley *et al.*, 2015). MEcPP-mediated signalling was shown to induce formation of specific ER body structures (Wang *et al.*, 2017). Those ER bodies have also been observed in seedlings and in rosette leaves in response to stresses and methyl jasmonate treatment (Matsushima, 2002). It is possible that such bodies are formed in the ER to reduce ROS production and oxidative stress. For instance, water-soluble chlorophyll-binding proteins (WSCPs) co-localize with ER bodies (Takahashi *et al.*, 2012). Those WSCP are known to bind chlorophyll and to act as scavenging systems when chloroplasts are injured, preventing photodynamic $^1O_2$ formation. However, we do not know if $^1O_2$ induces formation of such ER bodies and, in the transcriptome of *ch1* (Ramel *et al.*, 2013a), we did not find induction by high light of the genes required for ER bodies (*PYK10/BGLU23, NAI1, NAI2*). Moreover, the induction of UPR genes in the *ceh1* mutant affected indiscriminately a large array of ER-associated genes, while the UPR induction by $^1O_2$ was found to be specific to a classical response to ER stress, such as the response triggered by Tm. Consequently, the signalling pathways induced by the *ceh1* mutation and by high $^1O_2$ levels appear to be different phenomena. This is not unexpected as *ceh1* plants are not known to be $^1O_2$ overproducers.

Low $^1O_2$ production in *ch1* leaves under normal growth conditions led to a moderate activation of the bZIP60-dependent UPR branch, suggesting that *ch1* undergoes a chronic ER stress. Hence, chronic ER stress and UPR activation can be considered as an acclimation mechanism to $^1O_2$ production. The participation of ER in a $^1O_2$ signalling pathway could be related to the existence of contact sites between ER and other organelles (Wu *et al.*, 2018). Chloroplasts can generate membrane extensions called

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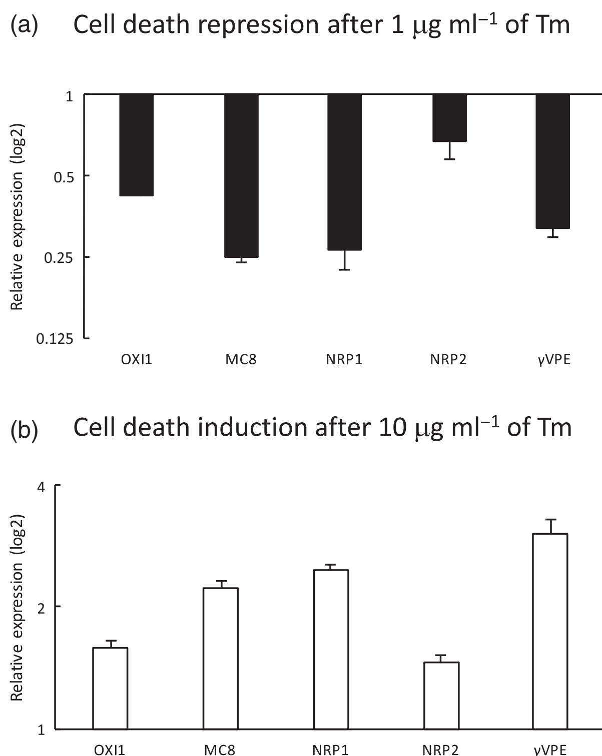

Figure 7. Effect of high light stress on the expression of cell death gene markers in Arabidopsis plants pre-treated with Tm. Gene expression was measured by RT-qPCR in plants exposed to high light stress after a pre-treatment with (a) 1 μg ml^{-1} Tm; or (b) 10 μg ml^{-1} Tm. Data, normalized to the response of plants pre-treated with Mock, are expressed as $\log_2$. (n = 3). Conditions of light stress are given in the legend of Figure 6.

stromules, and different assumptions have been made about their functions: they may recycle plastid content, remove toxic compounds or be a path for interorganellar communication (Hanson and Hines, 2018). Even if their exact role is still unclear, stromule formation has been shown to increase upon ROS production (Brunkard *et al.*, 2015), leading to the idea that stromules could play a role in chloroplast retrograde signalling. ER also forms membrane extensions enabling contacts with chloroplasts (Andersson *et al.*, 2007). They seem to play a role in lipid trafficking during biosynthesis of membranes (Block and Jouhet, 2015). However, involvement of those structures in stress responses is poorly documented. Mehrshahi *et al.* (2013) showed that complementation of the tocopherol-deficient *vte1* mutant of Arabidopsis with an ER-targeted tocopherol cyclase enzyme VTE1 partially restored tocopherol production. The authors proposed that an exchange of non-polar compounds could occur through membrane contact sites. We can then imagine that those contact sites could play a role in $^1O_2$ signalling by forming a route for signal molecules allowing interorganellar communication. Several metabolites have been identified in the $^1O_2$ signalling pathway leading to stress acclimation (Estavillo *et al.*, 2013), which constitute potential candidates for this signal transfer from the chloroplasts to the ER. It is clear that the monitoring of interorganellar migration of those $^1O_2$ signalling metabolites will constitute a major challenge for future works.

The *ch1* mutant is known to grow slower than WT plants, and this was previously attributed to $^1O_2$ signalling and associated hormonal changes (Ramel *et al.*, 2013b). Interestingly, chronic ER stress has also been reported to inhibit plant growth (Guo *et al.*, 2018). ER stress induces downregulation of auxin receptors and auxin efflux transporters of the PIN family are involved in UPR signalling (Wang *et al.*, 2007; Chen *et al.*, 2014). We can therefore speculate that UPR-induced growth inhibition could be one component of the growth impairment of *ch1*.

Light acclimation of the *ch1* mutant can mitigate ER stress through activation of the bZIP28 arm of the UPR

We have shown that genetic inhibition of the UPR affects the responses of Arabidopsis plants to photooxidative stress in different manners and, in particular, that the double mutant of bZIP60 and bZIP28 is more tolerant to excessive light while the mutant of BIP3 is more sensitive. Furthermore, depending on the intensity of the ER stress and on the components of the UPR that are induced, we obtained opposite results with a low UPR induction resulting in a protective response and a strong ER stress leading to a sensitive phenotype and activation of PCD in strong light. Altogether those results highlight the role of ER in light stress tolerance.

UPR genes are chronically expressed in *ch1*, and this expression is further enhanced in high light stress that ultimately leads to PCD. Conversely, light acclimation of the *ch1* mutant put a limitation in the induction of UPR genes in high light, except for *bZIP28* and *BIP3*, which were further induced. Particularly, *BIP3* was strongly upregulated when acclimated plants were exposed to high light compared with unacclimated plants. Thus, light acclimation conditions seem to favour the bZIP28 branch of the UPR relative to the bZIP60-dependent branch. This finding is consistent with Ruberti *et al.* (2018) who showed that recovery from ER stress is associated with bZIP28-dependent UPR, triggering a proadaptive signal. This is also in line with the results of Guo *et al.* (2018) who showed that inhibition of the bZIP60/IRE1 branch of the UPR is beneficial to growth under moderate ER stress.

Increased photosensitivity of the *bip3* mutant confirms the involvement of BIP3-dependent UPR in the acclimation to high light. The role of chaperones in high light stress was also confirmed by the protective action of TUDCA, a chemical chaperone known to be able to mitigate ER stress. Interestingly, knocking-out *BIP3* in potato promoted an increased sensitivity to drought stress (Carvalho *et al.*, 2014). Inversely, *BIP3* overexpression in soybean and

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Arabidopsis led to the opposite phenotype under osmotic and drought stresses, showing the importance of this chaperone in stress resistance (Reis *et al.*, 2011).

As *BIP3* expression is modulated by *bZIP60* and *bZIP28* (Ruberti *et al.*, 2018), *BIP3* expression levels should be low in the *bzip60 bzip28* double mutant. However, high light-induced cell death was markedly reduced in *bzip60 bzip28*, indicating that the protective role of BIP3 comes into play under conditions when bZIP-dependent PCD is triggered.

Chemical induction of ER stress can lead to acclimation or to accelerated cell death under high light stress, depending on ER stress levels

Tm is widely used to induce ER stress and to characterize molecular mechanisms of the UPR in young seedlings (Chen and Brandizzi, 2013). However, Tm treatments on adult plants have not yet been addressed in the literature. Using a relatively low Tm concentration (1 μg ml^{-1}), we were able to induce acclimation of well-developed plants to high light exposure. This was associated with a downregulation of *OXI1*, *MC8* and genes involved in ER stress-induced NRP-mediated PCD (namely *NRP1*, *NRP2* and γ*VPE*; Silva *et al.*, 2015; Reis *et al.*, 2016). Rather surprisingly, this Tm-induced acclimation was associated with a downregulation of *bZIP28* under high light stress, in apparent contradiction with the behaviour of this branch during *ch1* photoacclimation. However, the regulation of the UPR branches is known to be kinetically complex. Indeed, it has been reported that 72 h after a 1 μg ml^{-1} Tm pulse in Arabidopsis plants, UPR genes expression tends to return to a basal level (Guo *et al.*, 2018). Then, it is possible that, once plants are acclimated, cellular protein folding is stabilized and UPR signalling is not required anymore, hence explaining the low *bZIP28* expression levels in plants exposed to acclimatory light conditions for 2 days. This would also suggest that the changes in expression of *bZIP28* and *bZIP60* have different kinetics. In this context, it should also be noted that *ERdj3B*, an UPR marker gene controlled by *bZIP28* (Ruberti *et al.*, 2018), was induced in plants pre-treated with low Tm concentrations and exposed to high light. This finding supports the involvement of the bZIP28 arm of the UPR in the acclimation of Arabidopsis to light.

Application of a high Tm concentration (10 μg ml^{-1}) led to increased high light sensitivity associated with activation of both bZIP60 and bZIP28 UPR arms, as observed in *ch1* exposed to high light stress. In parallel, marker genes of PCD were induced by high light in Tm-treated plants. It has been reported that, under high light, $^1O_2$ enhances salicylate levels in leaves (Ramel *et al.*, 2013a). The role of the salicylate-induced cell death in plant–pathogen interactions and in the response to (a)biotic stresses has been extensively documented (Durrant and Dong, 2004; Khan *et al.*, 2015). In fact, during pathogen infection, salicylic acid triggers the setup of systemic acquired resistance (Vernooij *et al.*, 1994), a defence response that is accompanied by localized PCD around the site of infection during the hypersensitive response (Alvarez, 2000; Nimchuk *et al.*, 2003). Chandran *et al.* (2009) reported that pathogen infection triggers UPR gene expression in a salicylate-dependent manner. Salicylic acid is also instrumental in high light-induced cell death (Beaugelin *et al.*, 2019), and we have shown here that $^1O_2$-induced cell death in *ch1* is a salicylate-dependent mechanism. Thus, high light-induced $^1O_2$ production seems to induce a response resembling the response to biotic stresses. Accordingly, it has been reported that there is a greater overlap of the genes induced in the $^1O_2$-overproducing *flu* mutant and during biotic infection than the overlap between *flu* and abiotic stresses (Mor *et al.*, 2014). It has been shown that ER stress-induced NRP-mediated PCD was common to many stress signalling pathways and was salicylate dependent (Reis and Fontes, 2012). Similarly, induction of ER stress genes in *ch1* under high light stress was noticeably inhibited when salicylate biosynthesis was blocked by the *sid2* mutation. Moreover, exogenous application of salicylate has been reported to activate both arms of the UPR (Nagashima *et al.*, 2014). We can then assume that, under the combination of high light and Tm, salicylate is also responsible for the double activation of UPR arms.

Similar to high Tm concentrations, both arms of the UPR response were induced in the *ch1* mutant exposed to high light stress conditions leading to PCD (Figure 2). Moreover, concomitant inactivation of *BZIP60* and *BZIP28* impaired the development of high light-induced PCD. In fact, the role of ER in $^1O_2$-induced PCD is also supported by our recent finding that photooxidative damage and cell death are modulated by DAD1 (Beaugelin *et al.*, 2019). Arabidopsis DAD1 is a RE-located protein (Danon *et al.*, 2004) and is inducible by Tm (Perez-Martin *et al.*, 2014). In mammalian cells, DAD1 is a subunit of oligosaccharide transfer protein and plays a role in *N*-linked glycosylation in the ER (Makashima *et al.*, 1997). *dad1* loss-of-function mutations in Arabidopsis result in accelerated cell death in response to ER stress elicited by Tm (Yan *et al.*, 2019) and enhanced the extent of cell death under high light stress (Beaugelin *et al.*, 2019).

In conclusion, we have shown that ER and the associated UPR process are part of the high light and $^1O_2$ signalling pathways. $^1O_2$ signalling triggers UPR activation, with different UPR levels inducing different responses to high light stress. We were able to chemically induce light acclimation with the application of a low concentration of Tm that induced low levels of ER stress. Light acclimation changed the balance between the two UPR arms by preferentially upregulating the bZIP28-dependent branch compared with the bZIP60/IRE1 branch. Finally, we have also shown that PCD is synergistically induced by high light

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and a high Tm concentration that triggered both UPR arms. This study thus confirms the dual action of the ER stress, previously reported under other stress conditions leading either to cell death induction or to death avoidance (Howell, 2013): a moderate ER stress is part of the acclimatory response of plants to excess light energy while a strong induction of the UPR by high light and $^1O_2$ is associated with the induction of cell death.

EXPERIMENTAL PROCEDURES

Growth and stress conditions

Arabidopsis plants (*Arabidopsis thaliana*) were grown in phytotrons under controlled conditions of air temperature (20/18°C, day/night), light (130 μmol photons m^{-2} sec^{-1}, 8h day/16 h night) and relative air humidity (65%). The experiments were performed on 5-week-old plants of WT Arabidopsis (ecotype Col-0), the *bip3* mutant (GK075D06_011890) (Srivastava *et al.*, 2013), the double mutant *bzip60 bzip28* (Deng *et al.*, 2013), and the salicylic acid-deficient mutant *sid2-2* (Wildermuth *et al.*, 2001). The $^1O_2$-overproducing *ch1* mutant and the double mutant *ch1 sid2* were grown for 8 weeks before experiments (Ramel *et al.*, 2013a).

High light stress was imposed by exposing plants to 1500 μmol photons m^{-2} sec^{-1} photon flux density (PFD) at 7/18°C (day/night) and 380 ppm CO_2 in a growth chamber. Because of its high photosensibility, *ch1* plants were exposed to milder stress conditions: 1000 μmol photons m^{-2} sec^{-1} at 10/18°C (day/night). Light acclimation of *ch1* was carried out by applying an intermediate PFD of 450 μmol photons m^{-2} sec^{-1} at 18°C for 2 days, as previously described (Ramel *et al.*, 2013a).

Tunicamycin (Sigma Aldrich) was sprayed on 5-week-old plants at two different concentrations (1 and 10 μg ml^{-1}, 6 h before high light stress exposure. Plants were sprayed with 100 and 250 μM tauroursodeoxycholic acid (TUDCA; Selleckchem), 1.5 h before the high light stress.

Functional category enrichment analysis

Gene Ontology (GO) term enrichment was carried out using Virtual Plant 1.3 software (Mi *et al.*, 2013). GO terms with a *P*-value less than 0.05 were considered enriched.

RNA isolation and qRT-PCR

Total RNA was isolated from 200 mg of leaves by adding 500 μl TRI-reagent and 200 μl chloroform, the addition of absolute EtOH (v/v) to the supernatant allows transferring to violet column of the Nucleospin RNA plant kit (Macherey-Nagel). The concentration was measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific). First-strand cDNA was synthesized from 3 μg of total RNA using the PrimeScript reverse transcriptase kit (TaKaRa). qRT-PCR was performed on a real-time PCR instrument (Roche LightCycler 480 system). Here, 6 μl of a reaction mixture comprising SYBR Green I Master (Roche), 10 μM forward and reverse primers (5:1) were added to 4 μl of 10-fold diluted cDNA sample in a 384-well plate. The PCR program used was as follows: 95°C for 10 min, then 45 cycles of 95°C for 15 sec, 60°C for 15 sec, and 72°C for 15 sec. Primers (Table S2) were designed using Universal Probe Library (Roche). The experiments were done in triplicate (three different plants), taking two or three leaves per plant. Each sample was analyzed in triplicate (technical replicates). *PROFILIN1* (At2g19760) was used as the reference gene for the calculation of the gene expression levels.

Lipid peroxidation quantification and imaging

Lipids were extracted from about 0.5 g of leaves frozen in liquid nitrogen. The leaves were ground in an equivolume of methanol/chloroform solution containing 5 mM triphenyl phosphine, 1 mM 2,6-tert-butyl-*p*-cresol (5 ml g^{-1} fresh weight), and citric acid (2.5 ml g^{-1} fresh weight), using an Ultra-Turrax blender. Internal standard 15-HEDE was added to a final concentration 100 nmol g^{-1} fresh weight and mixed properly. After centrifugation at 1000 ***g*** and 4°C for 5 min, the lower organic phase was carefully taken out with the help of a glass syringe into a 15-ml glass tube. The syringe was rinsed with 2.5 ml of chloroform and transferred back into the tube. The process was repeated, and the lower layer was again collected and pooled to the first collection. The solvent was evaporated under N_2 gas at 40°C. The residues were recovered in 1.25 ml of absolute ethanol and 1.25 ml of 3.5 N NaOH and hydrolyzed at 80°C for 30 min. The ethanol was evaporated under N_2 gas at 40°C for 10 min. After cooling to 25°C, pH was adjusted between 4 and 5 with 2.1 ml of citric acid. Hydroxy fatty acids were extracted with hexane/ether (v/v). The organic phase was analyzed by straight phase HPLC-UV, as previously described (Montillet *et al.*, 2004). HOTE isomers (9-, 12-, 13-, and 16-HOTE derived from the oxidation of the main fatty acid in Arabidopsis leaves, linolenic acid) were quantified based on the 15-HEDE internal standard.

Lipid peroxidation was also visualized in whole plants by autoluminescence imaging. Plants were dark adapted for 2 h, and the autoluminescence signal, originating from the spontaneous decomposition of lipid peroxides (Birtic *et al.*, 2011), was captured using a highly sensitive liquid N_2-cooled CCD camera (Roper VersArray) using binning 2 × 2 and acquisition time of 20 min. Images were treated using ImageJ software (National Institutes of Health, USA).

Trypan blue staining

Cell death was visualized by staining detached leaves with trypan blue following the protocol described by Fernandez-Bautista *et al.* (2016). Leaves were incubated for 40 min in 1 mg ml^{-1} trypan blue. The staining solution was prepared by dissolving 40 mg of trypan blue in a solution containing 10 ml lactic acid (85% w/w), 10 ml phenol (buffer equilibrated, pH 7.5–8.0), 10 ml glycerol and 10 ml distilled water. Leaves were then rinsed with ethanol and left in ethanol overnight.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

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DATA AVAILABILITY STATEMENT

All data generated and used in this study are available upon request from the corresponding author, Michel Havaux (michel.havaux@cea.fr). Figure 1 is based on published results; the original data can be found in the articles quoted in the text and the figure legend.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Expression of UPR genes in WT plants treated with 1 μg ml^{-1} Tm for 5 h.

Figure S2. Expression of UPR genes in *ch1* leaves exposed to high light stress for 8 h or 28 h.

Table S1 Comparative transcriptomic analysis of ER stress-related genes induced by $^1O_2$ in the Arabidopsis mutants, *ch1* and *flu*

Table S2 List of primers for qRT-PCR

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