Article type : Original Article

Arabidopsis E2Fc is required for the DNA damage response under UV-B radiation epistatically over the microRNA396 and independently of E2Fe

María Sol Gómez, Ma. Lorena Falcone Ferreyra, María Luján Sheridan, Paula Casati*

Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina.

*Correspondence: Paula Casati. Tel: +54-341-4371955. Email: casati@cefobi-conicet.gov.ar.

Running Title: E2Fc participates in the DNA damage response under UV-B

Keywords: UV-B, cell proliferation, DNA damage response, E2F transcription factor, miR396

SUMMARY

UV-B radiation inhibits plant growth, and this inhibition is, to a certain extent, regulated by miR396-mediated repression of Growth Regulating transcription Factors (GRFs). Moreover, E2Fe transcription factor also modulates Arabidopsis leaf growth. Here, we provide evidence that, at UV-B intensities that induce DNA damage, E2Fc participates in the inhibition of cell proliferation. We demonstrate that *E2Fc* deficient plants show a lower inhibition of leaf size under UV-B conditions that damage DNA, decreased cell death after exposure and altered *SOG1* and *ATR* expression. Interestingly, the previously reported participation of E2Fe in UV-B responses, which is a transcriptional target of E2Fc, is independent and different of that described for E2Fc. On the other hand, we here demonstrate that E2Fc has an epistatic role over the miR396 pathway under UV-B conditions. Finally, we show that inhibition of

cell proliferation by UV-B is independent of the regulation of class II TCP transcription factors. Together, our results demonstrate that E2Fc is required for miR396 activity on cell proliferation under UV-B, and that its role is independent of E2Fe, probably modulating DNA damage responses through the regulation of *SOG1* and *ATR* transcript levels.

INTRODUCTION

Plants are usually exposed to a changing environment, which impacts on their developmental programs. In particular, light affects different plant processes during development; and even though ultraviolet-B (UV-B) radiation (280–315 nm) represents only a small part of the solar radiation that reaches the Earth, it modifies plant morphology and physiology. One developmental response to UV-B in plants is the reduction of the leaf area (Wargent *et al.*, 2009a,b; Fierro *et al.*, 2015; Casadevall *et al.*, 2013; Yan *et al.*, 2012; Fina *et al.*, 2017a). This reduction can be a consequence of an inhibition of cell division and/or a decrease in cell expansion (Hectors *et al.*, 2010), which reflect differences in experimental conditions. For example, stress-inducing UV-B conditions that produce DNA damage can inhibit cell proliferation, while lower doses and/or chronic UV-B irradiation can result in both inhibition of cell proliferation and expansion (for a revision, see Dotto and Casati, 2018). Previously, we demonstrated that in Arabidopsis, at UV-B intensities that can produce accumulation of DNA damage, the reduction in leaf area was a result of the inhibition of cell proliferation mediated by miR396, a microRNA which downregulates the expression of *GROWTH REGULATING FACTORS* (*GRF*) transcription factors, among other targets (Casadevall *et al.*, 2013; Debernardi *et al.*, 2012). Arabidopsis GRFs are a family of transcription factors that regulate numerous developmental processes acting redundantly (Kim *et al.*, 2003). *GRF* genes are expressed in all seed plants examined (van der Knaap *et al.*, 2000; Kim *et al.*, 2003; Choi *et al.*; 2004; Zhang *et al.*, 2011). In leaf primordia, miR396 is expressed at low levels and increases during organ development, whereas *GRF*s are expressed in an opposite way (Rodriguez *et al.*, 2010; Debernardi *et al.*, 2012). Therefore, miR396 is expressed preferentially in the distal part of young developing leaves reducing cell proliferation through the down-regulation of GRF activity and other cell cycle genes (Rodriguez *et al.*, 2010). After UV-B exposure, while miRNA396 was upregulated in proliferating leaves, *GRF1*, *GRF2* and *GRF3* transcripts were decreased; and the induction of miR396 resulted in the inhibition of cell proliferation but not cell expansion (Casadevall *et al.*, 2013). Similar results were found using maize plants, where UV-B levels in solar radiation also inhibited leaf

growth as a consequence of a decrease in cell production in the meristems of the developing leaf; this decrease in the meristem size was also a consequence of the down-regulation of *GRF* transcription factors and of an increase in miR396 levels (Fina *et al.*, 2017a).

On the other hand, the decrease in leaf size can be the result of a reduction in the cell area, which can be due to changes in cell ploidy as a consequence of endoreduplication, an alternative cell cycle during which the cell increases its DNA content without cell division. The physiological role of endoreduplication is not clear, probably playing an important role during differentiation. One hypothesis is that mutations that are accumulated during plant sessile life can be protected by endoreduplication, as various copies of a gene in a genome would maintain a functional copy in case of damage (Gegas *et al.*, 2014). The switch from the mitotic cell cycle to the endocycle is regulated by the activity of transcriptional factors from the E2F family. Arabidopsis has 6 E2F transcription factors (E2Fa-f) and two DP interaction proteins (DPa and DPb). E2Fa, E2Fb and E2Fc require to heterodimerize with either DPa or DPb to function; while E2Fd/DEL2, E2Fe/DEL1 and E2Ff/DEL3 can act independently of DP proteins (Ramirez-Parra *et al.*, 2007). E2F/DP factors regulate the expression of a group of genes that are necessary for cell cycle progression, but also genes in other functional categories (Ramirez-Parra *et al.*, 2003; Vandepoele *et al.*, 2005; Ramirez-Parra *et al.*, 2007; Naouar *et al.*, 2009). In addition, the RETINOBLASTOMA-RELATED1 (RBR1) protein can bind and inhibit the E2F/DP transcription factor complex when bound to the promoter of genes, repressing their expression in the absence of a signal to enter a new cell cycle (Nakagami *et al.*, 1999 and 2002; Uemukai *et al.*, 2005; Zhao *et al.*, 2012). The *Arabidopsis thaliana* RBR1 binds the three typical E2F transcription factors, with E2Fa and E2Fb acting as activators and E2Fc as a repressor of cell proliferation (Inzé and De Veylder, 2006; Polyn *et al.*, 2015). RBR probably represses cell proliferation through E2Fb (Magyar *et al.*, 2012), while E2Fc acts as a transcriptional repressor and is required for the correct end of cell division and occurrence of endoreduplication (del Pozo *et al.*, 2006; de Jager *et al.*, 2009). Thus, both RBR1 and E2Fc probably act as negative regulators for cell division and are necessary for the exit from the mitotic cell cycle.

The change from a mitotic cell cycle into an endoreduplication cycle has been demonstrated to be controlled by E2Fe/DEL1, which represses the endocycle onset (Vlieghe *et al.*, 2005; Lammens *et al.*, 2008). Interestingly, E2Fe/DEL1 is also a transcriptional repressor of PHR1/UVR2, a cyclobutane pyrimidine dimer photolyase in Arabidopsis (Radziejwoski *et al.*, 2011). After UV-B exposure, *e2fe* mutants show higher DNA repair than wild type (WT) plants and also resume endoreduplication faster than control plants. As a

consequence, these plants show a lower inhibition of leaf growth by UV-B with larger cells showing higher ploidy levels (Radziejwoski et al., 2011). Besides, *E2Fe* is transcriptionally regulated by the typical E2Fb and E2Fc transcription factors; which antagonistically control *E2Fe* levels through the competition for a single E2F cis-acting binding site (Berckmans *et al.*, 2011). In this way, while E2Fb is an activator of *E2Fe* expression, E2Fc represses its expression.

In this work, to further expand our knowledge on the pathways that participate in the regulation of leaf growth under UV-B exposure in Arabidopsis and the participation of E2F transcription factors (TF) in this regulation, we investigated the role of the typical E2Fc TF, and its contribution, together with miR396 and GRFs, in the regulation of leaf size after UV-B exposure. We demonstrate that E2Fc regulates leaf size under UV-B without participating in DNA damage repair after UV-B. However, E2Fc probably participates in other DNA damage responses, as *E2Fc* deficient plants show decreased programmed cell death after UV-B exposure, and altered expression of *SOG1* and *ATR*, which regulate DNA damage responses after UV-B exposure in Arabidopsis. We also here provide evidence that *E2Fc* deficiency counteracts the effect of *MIM396*, suggesting that E2Fc may be a regulator of miR396 activity. Finally, we here demonstrate that UV-B effects that require the participation of miR396 are not regulated by class II TCPs transcription factors.

RESULTS

E2Fc regulates leaf growth under UV-B conditions

Previously, the transcription factor E2Fe/DEL1 was demonstrated to participate in the regulation of leaf growth under UV-B conditions, repressing the expression of the CPD photolyase *PHR1*/*UVR2* in Arabidopsis (Radziejwoski *et al.*, 2011). Because *E2Fe* is negatively regulated by E2Fc, we investigated if E2Fc also modulates the inhibition of cell proliferation after UV-B exposure in Arabidopsis. Thus, we analyzed the effect of UV-B radiation in growth inhibition and cell proliferation using Arabidopsis transgenic plants expressing an RNAi construct targeting the *E2Fc* gene (*E2FcRNAi*, del Pozo *et al.*, 2006). Transgenic *E2FcRNAi* plants show decreased levels of *E2Fc* (del Pozo *et al.*, 2007; Figure S1); and they are smaller than WT Col-0 plants, with curled rosette leaves, as already reported (del Pozo *et al.*, 2006 and 2007; Figure 1a-b). WT and transgenic plants were irradiated with a single UV-B light treatment during 4h at an intensity of 2W m^{-2} 12 days

after stratification (DAS). At this moment, leaf #5 is emerging and has proliferating cells (Casadevall *et al.*, 2013). Two days after the treatment, the rosette area of WT and *E2FcRNAi* plants is significantly smaller in UV-B treated plants than in plants that were kept in the absence of UV-B; this difference persists 8 days after the treatment (Figure 1a). However, UV-B irradiated *E2FcRNAi* plants have a similar rosette area as UV-B irradiated WT plants (Figure 1a-b). Thus, *E2FcRNAi* plant growth is less inhibited by UV-B than WT plants. To confirm that the phenotype observed was due to decreased *E2Fc* expression caused by the RNAi construct, we took advantage of the natural tendency of transgenes to be transcriptionally silenced. Silenced plants were identified by the presence of the transgene (determined by PCR amplification of the *35S* promoter, Figure S1e) combined with the analysis of *E2Fc* expression (Figure S1a). Silenced plants showed *35S* amplification, similar *E2Fc* transcript levels as WT plants, and looked like WT plants (Figure S1d). After a UV-B treatment, silenced *E2Fc* plants showed a similar decrease in rosette area as WT plants (Figure S1g), confirming that transgene expression is required to produce the observed phenotype.

The average area in fully expanded leaf #5 was also analyzed under control conditions and after UV-B exposure (Figure 1c); the results demonstrate that the decrease in rosette area by UV-B is due to a decrease in final leaf area in both lines. While leaf #5 area in *E2FcRNAi* plants is smaller than that of WT plants under control conditions; after UV-B exposure, both plants have leaves of similar sizes (Figure 1c-d). In *E2FcRNAi* control plants, average leaf #5 area is smaller because transgenic leaves have less palisade cells (Figure 1f) with smaller areas (Figure 1e). In UV-B irradiated plants, while leaf #5 area is significantly reduced in both plants; the average cell area is not affected by the treatment in any of the two lines analyzed (Figure 1e). However, the decrease in the number of cells already reported under our experimental conditions (Casadevall *et al.*, 2013) is significantly lower in *E2FcRNAi* than in WT leaves (Figure 1f). Therefore, *E2FcRNAi* leaf #5 have more cells than WT leaves after UV-B exposure, suggesting that *E2Fc* is important for the repression of cell proliferation in young leaves of Arabidopsis under UV-B.

Average leaf area in fully expanded leaves #1/2 and #7 was also analyzed, both under control conditions and after UV-B exposure. Leaf #1/2, at the moment of the UV-B treatment, has only expanding cells that have finished division; while leaf #7 is emerging (Casadevall *et al.*, 2013). As shown in Figure S2, whereas both leaves #1/2 and #7 showed a decrease in average area after UV-B exposure in WT and *E2FcRNAi* plants, the decrease in leaf #1/2 was similar for both genotypes while significantly different in leaf #7. Leaf #7 from

E2FcRNAi plants, similarly as leaf #5, showed a lower inhibition of growth due to a lower inhibition of cell proliferation by UV-B. Similarly as shown for leaf #5, in leaves where cells are dividing at the moment of the irradiation, E2Fc participates in the control of cell proliferation. In contrast, growth of leaf #1/2 from WT and *E2FcRNAi* plants was similarly decreased by UV-B as a consequence of a similar decrease in cell expansion (Figure S2). Therefore, E2Fc affects cell division but it does not participate in the inhibition of cell expansion by UV-B.

Previously, we demonstrated that UV-B inhibition of cell proliferation was mediated by the action of miR396 and GRF transcription factors (Casadevall *et al.*, 2013; Fina *et al.*, 2017a). To investigate if the decrease in cell proliferation by UV-B in *E2Fc* deficient plants is through the regulation of the expression of miR396 and GRFs, we analyzed the expression of *GRF2* and *GRF3*. Both under control conditions and after UV-B exposure, *GRF2* and *3* were similarly expressed in WT Col-0 and in *E2FcRNAi* leaf #5 (Figure 2a-b). However, although miR396 was up-regulated by UV-B in leaves of both plants, this up-regulation was significantly lower in *E2FcRNAi* leaf #5 (Figure 2c). Thus, E2Fc may regulate miR396 levels under UV-B conditions, without affecting *GRFs* expression.

Furthermore, transcript levels encoding other E2F and DP proteins were measured. *E2Fa* and *E2Fe* (Figure 2d-e) but not *E2Fb, E2Fd, E2Ff* or *DPb* (Figure S3) were increased in *E2Fc* deficient plants, both under control conditions and after exposure. Although *DPa* levels were similar under control conditions in WT and *E2Fc* deficient plants, the up-regulation by UV-B was lower in the transgenic plants than WT plants (Figure 2f). Previously, del Pozo *et al.* (2006) demonstrated that E2Fc regulates the expression of cell cycle genes. In our experiments, under control conditions in the absence of UV-B, *KNOLLE* and *H4* transcripts levels were slightly but significantly increased in *E2FcRNAi* leaf #5. Interestingly, after UV-B exposure, these transcripts, and also the *CYCLIN-DEPENDENT KINASE B1,2* (*CDKB1;2*), showed increased levels compared to those in WT leaves (Figure 2g-i). A time course analysis of selected transcripts shows that after 4h of UV-B exposure, changes in gene expression of cell cycle genes and levels of miRNA are more evident than those measured after shorter exposure times, or under recovery conditions in the absence of UV-B (Figure S4). Together, the results demonstrate that decreased *E2Fc* levels affect expression of proteins in the E2F regulation pathway and cell cycle genes, which in turn may affect cell proliferation under UV-B.

Similarly, transcripts encoding proteins that participate in UV-B signaling and response through the UVR8 photoreceptor were studied (Brown *et al.*, 2005). *UVR8* levels were similar in WT and *E2FcRNAi* plants, after UV-B exposure and under control conditions (Figure 3a). *HY5* (encoding a transcription factor that regulates UV-B responses in the UVR8 pathway), *CHS* and *F3H* (encoding chalcone synthase and flavanone 3-hydroxylase, respectively, which participate in the biosynthesis of UV-B absorbing pigments) were significantly and similarly increased after UV-B exposure in WT and *E2FcRNAi* plants (Figure 3b-d). Nevertheless, UV-B absorbing pigments and anthocyanins levels were higher in *E2FcRNAi* plants (Figure 3e and g); both under control and UV-B conditions (Figure 3f and h). Despite this, both WT and *E2FcRNAi* plants showed similar accumulation of superoxide anion, a reactive oxygen species, by nitroblue tetrazolium staining after UV-B exposure, suggesting that differences in UV-B absorbing pigments do not modulate the oxidative state of *E2FcRNAi* plants under UV-B conditions (Figure 3i). Thus, the inhibition of plant growth by UV-B mediated by E2Fc seems to be independent of the activation of the UV-B signaling pathway mediated by UVR8 and HY5, pigment accumulation and oxidative stress status.

Experiments were also done using plants overexpressing a truncated form of E2Fc that lack the N-terminal region that is involved in regulating protein stability but retains functional activities *in vivo* (*E2FcOE*; del Pozo *et al.*, 2002; Figure S1b). As previously reported, plants looked similar as WT plants (Figure S5; del Pozo *et al.*, 2002). Interestingly, while *E2FcOE* leaf#5 had a similar size as the same leaf from WT plants (Figure S5c-d), it had less cells (Figure S5i) with bigger areas (Figure S5f-g); both under control conditions and after UV-B exposure. While leaf#5 area decreased after UV-B exposure in both plants, this decrease was similar without showing significant differences between genotypes (Figure S5e). UV-B radiation did not affect cell area in leaf #5 of neither plant (Figure S5h), while cell number decreased to a similar extent in leaves from both plants (Figure S5j). It is interesting to note that *E2FcOE* leaf #5 has less cells with bigger areas, demonstrating that the overexpression of the DP dimerization partners is not required for the phenotype observed (Figure S5f, g and i). Similar results were obtained when leaves#1/2 and #7 were analyzed (Figure S2). In this way, *E2Fc* overexpression does not affect plant growth under UV-B conditions, suggesting that a threshold level of this transcription factor is enough for its function in UV-B responses.

E2Fc regulates root growth and have a role in the induction of root stem-cell death by UV-B regulating *ATR* and *SOG1* expression, but it does not participate in DNA damage repair

UV-B sensitivity in *E2FcRNAi* plants was also investigated by inhibition of primary root elongation assays (Tong *et al.*, 2008; Fina *et al.*, 2017b). Under control conditions in the absence of UV-B, *E2FcRNAi* plants had shorter primary roots than WT plants. One day after the end of the UV-B treatment, while WT plants showed a significant decrease in primary root elongation compared with untreated plants, *E2FcRNAi* plants showed a lower inhibition. This difference persisted 4 days after the treatment, so transgenic plants showed a lower inhibition of primary root elongation than WT plants after UV-B exposure (Figure 4a,b). Inhibition of primary root elongation after UV-B was also similar in WT and *E2FcOE* plants (Figure S6), again suggesting that a threshold E2Fc level seems to be enough for its function in root elongation under UV-B.

To investigate if E2Fc participates in the induction of programmed cell death (PCD) in response to UV-B, we evaluated cell death in root tips (Furukawa *et al.*, 2010; González Besteiro and Ulm, 2013; Falcone Ferreyra *et al.*, 2016). Neither line showed dead cells when they were kept under control conditions in the absence of UV-B. *E2FcRNAi* root tips showed significantly fewer dead cells compared to WT roots (Figure 4c-d), suggesting that E2Fc has a role in PCD after UV-B exposure. *E2Fc* overexpression did not change PCD responses after exposure (Figure S6e), suggesting that E2Fc levels in the cell are sufficient to elicit the UV-B response observed.

It was previously demonstrated that PCD induced by UV-B radiation requires Suppressor of Gamma Response 1 (SOG1; Furukawa *et al.*, 2010). SOG1 is a transcription factor that regulates many responses to genotoxic agents in Arabidopsis. UV-B-induced PCD also requires the activation of the DNA-damage protein kinases Ataxia Telangiectasia Mutated (ATM), and ATM and Rad3-related (ATR; Furukawa *et al.*, 2010). Therefore, we analyzed if *E2FcRNAi* plants showed altered *SOG1*, *ATM* and *ATR* expression. Figure 4e,f shows that *E2FcRNAi* plants have constitutive higher *SOG1* and *ATR* mRNA levels than WT plants, and transcripts levels in the transgenic plants are not regulated by UV-B radiation as WT plants. In contrast, *ATM* levels are similar in both plants (Figure 4g). Thus, E2Fc may regulate PCD after UV-B exposure by regulating the expression of *SOG1* and *ATR* genes in Arabidopsis.

Absorption of UV-B by DNA induces the formation of pyrimidine dimers (CPDs) in the DNA, and, to a lesser extent, pyrimidine (6-4) pyrimidone photoproducts (Friedberg *et al.*, 1995). *e2fe* mutants accumulate lower CPD levels after UV-B exposure because they overexpress *PHR1/UVR2*, which encodes the type-II cyclobutane pyrimidine dimer photolyase (Radziejwoski *et al.*, 2011). Therefore, we also examined CPD accumulation after UV-B exposure in *E2FcRNAi* and *E2FcOE* plants, to analyze if the DNA damage phenotype in *e2fe* mutants is regulated by E2Fc. After a 4-h-UV-B treatment, similar levels of unrepaired lesions were measured in WT, *E2FcRNAi* and *E2FcOE* transgenic plants (Figure S6b and f). Moreover, WT and *E2FcRNAi* lines showed similar expression of the DNA repair enzymes *UVR2* and *UVR3* (encoding a 6-4 photoproduct photolyase), both under control conditions and after a UV-B treatment (Figure S6c-d), in contrast to what was previously reported in *E2Fe* deficient plants (Radziejwoski *et al.*, 2011).

In contrast to what was previously reported using *e2fe* knockout plants (Radziejwoski *et al.*, 2011), UV-B radiation did not affect ploidy levels in *E2FcRNAi* plants (Figure S7a). While transgenic plants had a lower calculated endoreduplication index than WT Col-0 plants under control conditions, this index was not affected by the treatment (Figure S7b). Together, changes in plant growth by UV-B in *E2Fc* deficient plants are independent on DNA damage repair mechanisms and ploidy changes, contrary to what was previously reported using *E2Fe* deficient plants, suggesting that both transcription factors participate in different UV-B responses pathways, affecting plant growth in different ways. Therefore, UV-B effects mediated by E2Fe are not through its regulation by E2Fc.

E2Fc is required for miR396 activity

Previously, we demonstrated that *MIM396* plants, expressing an artificial target mimic directed against miR396 and showing a decrease in the endogenous microRNA activity, were less sensitive to the inhibition of leaf growth by UV-B (Casadevall *et al.*, 2013). These results are similar to those presented in Figure 1 using *E2FcRNAi* plants. Moreover, our results show that in *E2FcRNAi* proliferating leaves, UV-B up-regulation of miR396 is decreased (Figure 2c). Thus, E2Fc may affect miR396 activity, or viceversa.

To test these hypotheses, we crossed *MIM396* with *E2FcRNAi* transgenic plants to generate *MIM396xE2FcRNAi* double transgenic plants, expressing the target mimicry (Figure S1c) and showing a similar decrease in *E2Fc* expression as *E2FcRNAi* plants (Figure S1a). *MIM396xE2FcRNAi* plants (as well as the reciprocal cross, *E2FcRNAixMIM396*) were

smaller than WT and *MIM396* plants when they were grown in the absence of UV-B, but looked similar to *E2FcRNAi* plants (Figure 5a,b; Figure S8a). Similarly as *E2FcRNAi* plants, the double transgenic plants had smaller leaves (Figure 5c,d; Figure S8b) with smaller and lower number of cells (Figure 5e,f; Figure S8c,d) than WT and *MIM396* plants. After UV-B exposure, the double transgenic plants also looked similar to *E2FcRNAi* plants and different from WT and *MIM396* plants (Figure 5; Figure S8), suggesting the E2Fc is probably epistatic over miR396.

We also analyzed CPD accumulation and PCD after UV-B exposure. Although all single and double transgenic plants showed lower and similar root meristematic death cells after UV-B exposure than WT plants (Figure 6a-b; Figure S9a); while *MIM396* transgenic plants accumulated lower CPDs after a UV-B treatment, *MIM396xE2FcRNAi* plants showed similar DNA damage levels as WT and *E2FcRNAi* plants (Figure 6c; Figure S9b). Thus, higher DNA repair after UV-B damage in *MIM396* plants requires E2Fc activity.

To further validate the hypothesis that E2Fc acts epistatically over miR396, we analyzed *GRF1*, *2* and *3* expression in *MIM396xE2FcRNAi* plants. While *GRF1*, *2* and *3* were highly expressed and were not repressed by UV-B radiation in *MIM396* plants as previously reported (Debernardi *et al.*, 2012; Casadevall *et al.*, 2013); these 3 TFs were similarly expressed in WT Col-0, *E2FcRNAi* and *MIM396xE2FcRNAi* plants, both under control conditions and after UV-B exposure (Figure 7a-c). Therefore, postranscriptional regulation of GRFs by miR396 is overcome by decreased levels of E2Fc.

Finally, primary root elongation after UV-B exposure was analyzed. As shown in Figures S9c and S10, *MIM396* plants have a shorter primary root than WT plants, but they show a lower inhibition of primary root elongation after UV-B exposure. Interestingly, all transgenic plants analyzed exhibited a lower inhibition of primary root length by UV-B than WT plants (Figures S9c and S10b), so this UV-B response is similar for all genotypes studied.

E2Fc expression and UV-B regulation is independent of the UVR8 photoreceptor and the MPK3 pathway

To analyze if the UVR8 photoreceptor modulates E2Fc-mediated inhibition of plant growth by UV-B regulating its expression and that of other transcription factors from this family, we analyzed E2F expression in *uvr8* mutants under control conditions and after UV-B exposure. Interestingly, *E2Fc* expression was similar in *uvr8* and in WT plants, both under control

conditions and after UV-B exposure (Figure 7d). In both plants, *E2Fc* was significantly upregulated by UV-B, as previously reported (Fina *et al.*, 2017b). Similar expression and UV-B-regulation in WT plants and *uvr8* mutants were also measured for the other Arabidopsis *E2F* TFs (Figure 7d).

Other molecular pathways also participate in plant responses to UV-B, for example the MPK3 regulated pathway (Jenkins, 2017). Nevertheless, in *mpk3* mutants, transcript levels of *E2Fc* and all *E2F* TFs are similar to those in WT plants, both under control conditions and after UV-B (Figure 7e), demonstrating that their regulation is independent of the MPK3 pathway, in contrast to what was previously reported on the UV-B regulation of *GRF*s (Casadevall *et al.*, 2013). Therefore, *E2Fc* expression may be mediated by alternative UV-B regulated pathways, such as DNA damage signaling; MPK6 kinase activity, reactive oxygen species, or hormone signaling (Jenkins, 2017).

TCP transcription factors are not required for the UV-B responses mediated by miR396

The *miR396* precursor is encoded by two genes, *MIR396A* and *MIR396B*; *MIR396B* is transcriptionally regulated by class II TCPs TFs (Schommer *et al.*, 2014). Thus, we finally investigated if the UV-B modulated growth responses affected in *MIM396* plants were also regulated by this class of TFs. Figure S11 shows that *tcp2x3x4x10* quadruple mutant, deficient in the expression of 4 class II TCPs (Bresso *et al.*, 2018), has a smaller rosette area under control conditions in the absence of UV-B than WT Col-0 plants (Figure S11a,b), which is decreased after UV-B exposure. However, the decrease in rosette area after UV-B was similar in both plants (Figure S11b, inset). Similarly, primary root length of the *tcp2x3x4x10* mutant was shorter than that of WT plants, both under control conditions and after UV-B exposure (Figure S11c), but the decrease observed did not show statistically significant differences after exposure between both lines (Figure S11d). Moreover, the quadruple mutant did not show significant differences in DNA damage accumulation (Figure S11e) nor in PCD (Figure S11f) after a UV-B treatment from WT plants. Therefore, miR396 and E2Fc UV-B induced responses are independent of the regulation of class II TCPs.

DISCUSSION

We previously demonstrated that Arabidopsis and maize plants irradiated with UV-B at intensities similar as those present in natural sunlight show an inhibition of leaf growth, and this occurs because UV-B impairs cell proliferation in developing organs (Casadevall *et al.*, 2013; Fina *et al.*, 2017a). This inhibition is, at least in part, regulated by miR396-mediated repression of transcription factors of the GRF family. Interestingly, Radziejwoski *et al.* (2011) also showed that E2Fe/DEL1 modulates Arabidopsis leaf growth under UV-B conditions; E2Fe controls the basal expression of a CPD photolyase and it is also a repressor of the endocycle onset (Vlieghe *et al.*, 2005). These authors suggested that when DNA is damaged, plants endoreduplicate slower than control plants in the absence of UV-B. Thus, coordinated transcriptional induction of the photolyase with the endoreduplication onset by E2Fe could help plants to adapt to increased UV-B (Radziejwoski *et al.*, 2011). In this work, we analyzed if the role of E2Fe in UV-B responses is regulated by the action of E2Fc, as *E2Fe* is a transcriptional target of E2Fc (Berckmans *et al.*, 2011). We here demonstrate that E2Fc modulates plant growth under UV-B conditions, but in a different way as E2Fe. Both *E2Fc* and *E2Fe* deficient plants show a lower decrease in leaf growth than WT plants after UV-B (Radziejwoski *et al.*, 2011). However, while *e2fe* mutant leaves after irradiation are bigger mostly because they have bigger cells correlating with increased endoreduplication (Radziejwoski *et al.*, 2011); *E2FcRNAi* plants have leaves with more cells than WT plants; without showing differences in cell ploidy after exposure. Also, *e2fe* mutants accumulate lower levels of CPDs after UV-B irradiation, whereas *E2FcRNAi* plants show similar DNA damage as WT plants (Figure S6, Radziejwoski *et al.*, 2011). Despite *E2FcRNAi* plants show increased levels of *E2Fe* transcripts, E2Fc and E2Fe probably regulate UV-B responses in distinct ways. Besides E2Fe, E2Fc regulates, directly or indirectly, other targets, such as *SOG1* and *ATR*, which may control cell proliferation; while E2Fe, not through E2Fc regulation, coordinates DNA repair by regulating photolyase expression and endoreduplication onset. As both E2Fb and E2Fc antagonistically control *E2Fe* expression under light conditions, and *E2Fe* levels are regulated through the balance between *E2Fb* and *E2Fc* expression (Berckmans *et al.*, 2011); it is possible that under UV-B conditions, photolyase levels and the endoreduplication onset may be established through E2Fe mostly regulated by E2Fb, or by other TFs.

RBR1 can bind to the three typical E2F transcription factors E2Fa-c, and inhibit the E2F/DP transcription factor complex when bound to the promoter of genes, repressing their expression in the absence of a signal to enter a new cell cycle (Nakagami *et al.*, 1999 and 2002; Uemukai *et al.*, 2005; Zhao *et al.*, 2012). Interestingly, RBR1 has a role protecting genome integrity in Arabidopsis. When DNA damage occurs, RBR1 and E2Fa are recruited to damaged DNA (Horvath *et al.*, 2017). RBR1 interacts with the DNA repair protein AtBRCA1, which can also co-localize with RBR1 foci, suggesting that they could function together maintaining genome integrity. RBR1 also leads to cell death, especially after exposure to genotoxic agents such as aluminium and *rbr1* mutant cells show increased levels of DNA lesions (Biedermann *et al.*, 2017). As shown in Figure 7, UV-B increases *E2Fc* and *E2Fa* levels; moreover, *E2Fa* expression is further increased in *E2FcRNAi* plants (Figure 2). Since RBR regulates cell death through E2Fa (Horvath *et al.*, 2017), the interconnection between these E2Fs might play regulatory role in the control of organ growth and cell death.

In our experiments, despite E2Fc does not directly participate in DNA repair, it has a role in the DNA damage response after UV-B irradiation, as after exposure, there are less dead root meristematic cells in *E2FcRNAi* compared to those in WT roots. Plants employ PCD when they suffer from severe DNA damage, particularly in stem cells. We here show that E2Fc regulates the expression of *SOG1* and *ATR.* Arabidopsis SOG1 is a plant-specific transcription factor that plays a central role in the DNA damage response, regulating the expression of genes that participate in DNA repair, cell proliferation and cell death. In response to a genotoxic treatment, SOG1 is phosphorylated in an ATM-dependent and ATR-independent manner (Yoshiyama *et al.*, 2013). ATR and ATM are involved in different responses to DNA damage, while ATR mostly recognizes ssDNA damage or replicative stress, ATM recognizes double strand breaks; when these damages occur, either or both kinases activate PCD induction (Culligan *et al.*, 2006, Yoshiyama *et al.*, 2014). Thus, DNA damage by UV-B usually produces ATR-activating structures. Interestingly, in UVB-irradiated *atr* and *atm* mutants, there is elevated PCD, suggesting that ATR and/or ATM may both activate pathways that regulate PCD after exposure (Furukawa *et al.*, 2010). In contrast, *sog1-1* mutant plants are deficient of PCD after UV-B exposure, indicating that SOG1 also has a role in the stem cell death (Furukawa *et al.*, 2010). Interestingly, the *sog1-1* mutation impairs transcriptional repression of the cell cycle related genes *CDKB2;1* and *KNOLLE* after DNA damage (Yoshiyama *et al.*, 2009), suggesting that SOG1 is involved in DNA damage-induced cell cycle arrest, and consequently, altered expression of cell cycle genes in *E2FcRNAi* plants may be mediated by SOG1 regulation. Noteworthy, both *SOG1* and *ATR*

have putative E2F consensus binding sites in their promoters (Figure S12; Vandepoele *et al.*, 2005). Although the direct binding of E2F TF to both promoters needs to be validated, it is possible that these genes may be direct targets of E2Fc. Together, E2Fc may modulate plant growth and DNA damage response after UV-B by regulating *SOG1* and *ATR* expression. In this way, E2Fc may regulate PCD, without having a direct participation in DNA repair.

Besides repressing cell proliferation in developing leaves, E2Fc also regulates root elongation under UV-B conditions. The results presented here demonstrate that that E2Fc not only can act as a repressor, but it can also be an activator during normal development. This was previously shown in de Jager *et al.* (2009), where they demonstrated that E2Fc can also activate the expression of a number of genes; therefore its *in vivo* role seems not only as a repressor but also as an activator of gene expression. Changes in the expression of cell cycle genes in del Pozo *et al.* (2006) are similar as those described now in this work; and despite that there does not seem to be correlation between cell cycle gene expression and the cell phenotypes shown here, both analysis demonstrate that E2Fc is required for proper expression of these genes, and that their altered expression could be related to the observed leaf cell phenotypes in control leaves and after UV-B exposure. On the other hand, we here show leaf and root growth regulation is independent of the expression of UV-B responsive genes of the UVR8 pathway, MPK3 activity, pigment accumulation or oxidative stress responses. On the other hand, *E2Fc* overexpression does not modify the UV-B responses analyzed. Thus, regulation of target genes by E2Fc probably only requires basal expression levels normally occurring in the cells. Alternatively, overexpression of a truncated E2Fc might function in a different way, eg. competing with related E2Fs for DP proteins.

We here also provide evidence that the regulation of cell proliferation under UV-B conditions mediated by the miR396 pathway requires E2Fc activity. In Arabidopsis plants, UV-B radiation induces the accumulation of miR396 in proliferating cells, and this increase parallels a decrease in the expression of *GRF1*, *GRF2*, and *GRF3* (Casadevall *et al.*, 2013). In this work, we show that miR396 levels are increased by UV-B in *E2FcRNAi* plants, but to a lower degree than that in WT plants. *MIM396* plants that were crossed with *E2FcRNAi* plants look similar to *E2FcRNAi* plants, both under control conditions and after UV-B exposure. Moreover, while *MIM396* accumulates lower DNA damage after UV-B exposure, *E2FcRNAi*, *MIM396xE2FcRNAi* and *E2FcRNAixMIM396* plants have similar CPD levels as WT plants. Moreover, in *MIM396xE2FcRNAi* plants, *GRF1*, *2* and *3* are similarly expressed as in WT and *E2FcRNAi* plants under control conditions and they are repressed by UV-B, while in *MIM396* plants they are highly expressed and are not down-regulated by UV-B

radiation as previously reported (Figure 7, Debernardi *et al.*, 2012; Casadevall *et al.*, 2013). We previously demonstrated that post-transcriptional regulation of GRFs by miR396 was important for the regulation of leaf growth inhibition by UV-B in maize and Arabidopsis (Casadevall *et al.*, 2013; Fina *et al.*, 2017a). Therefore, postranscriptional regulation of *GRF*s by miR396 is overcome by decreased levels of E2Fc. Together, E2Fc seems to act epistatically over miR396. As miR396 levels after UV-B exposure are lower in *E2FcRNAi* plants, the expression of miR396 may require E2Fc activity; or alternatively, proteins that process the miR396 precursor may be regulated by E2Fc. Furthermore, our results show that under control conditions in the absence of UV-B, genes related to cell cycle regulation and DNA damage responses are significantly increased in the *E2FcRNAi* line (Figure 2 and Figure 4), suggesting that either E2Fc and/or (a) repressor(s) regulated by E2Fc independently of UV-B may modulate plant growth under control conditions, and this could be a reason why the phenotype in *E2FcRNAi* plants is dominant over that of *MIM396* plants. Moreover, under UV-B conditions, a different growth regulator may be expressed, that may affect the expression of miR396, *DPa*, the cell cycle genes and/or *SOG1*, which, may also further regulate the decrease leaf area and cell number measured after exposure.

Finally, we provide evidence that the regulation of cell proliferation under UV-B in Arabidopsis is independent of the regulation of class II TCP transcription factors. In Arabidopsis, five class II TCPs (*TCP2*, *TCP3*, *TCP4*, *TCP10*, and *TCP24*) are regulated by the microRNA miR319. miR319-regulated TCPs have been demonstrated to control leaf development and cell proliferation in *A. thaliana* leaves, by activating the expression of *MIR396b* (Palatnik *et al.*, 2003; Schommer *et al.*, 2014). The *tcp2x3x4x10* quadruple mutant, deficient in the expression of 4 class II TCPs (Bresso *et al.*, 2018), has similar UV-B related phenotypes as WT plants. Consequently, miR396 and E2Fc UV-B induced responses are independent of the regulation of class II TCPs.

In conclusion, we here demonstrate that, at UV-B intensities that induce DNA damage, inhibition of cell proliferation is regulated by the E2Fc, which regulates the expression of *SOG1* and *ATR*. E2Fc has also an epistatic role over the miR396 pathway. The inhibition of cell proliferation is independent on the regulation of class II TCP transcription factors. In addition, the previously reported participation of E2Fe in UV-B responses, which is a transcriptional target of E2Fc, is independent and different of that described for E2Fc.

EXPERIMENTAL PROCEDURES

Plant material, growth conditions and irradiation protocols

A. thaliana ecotype Columbia (Col-0) was used for all experiments. The *E2FcRNAi* and *E2FcOE* seed stocks were provided by Dr. Crisanto Gutierrez (Centro de Biología Molecular Severo Ochoa, Spain), the *MIM396* and *tcp 2x3x4x10* lines were provided by Dr. Javier Palatnik (IBR, Rosario, Argentina); and the *uvr8* and *mpk3* mutants were provided by Dr. Roman Ulm (University of Geneva, Switzerland). Plants were grown and UV-B irradiated as described in Casadevall *et al.* (2013).

Primary root elongation assays were done as describe in Fina *et al.* (2017b).

Quantitative RT-PCR

Total RNA purification and qRT-PCR were done as described in Casadevall *et al.* (2013). Primers for each transcript under study were designed using the PRIMER3 software (Rozen and Skaletsky, 2000). Transcript levels were normalized to those of the *A. thaliana* calcium dependent protein kinase3 that has been previously demonstrated not to be UV-B regulated (Ulm *et al.*, 2004; *CPK3*, Table S1). miR396 levels were determined by stem-loop qRT-PCR, as previously described (Chen *et al.*, 2005). Each qRT-PCR was repeated at least three times on three different biological replicates.

Identification of the artificial target mimic directed against miR396

The identification of the artificial target mimic in *MIM396, E2FcRNAixMIM396* and *MIM396xE2FcRNAi* lines was done using a RT-PCR-based approach. The cDNA was synthesized as described in Casadevall *et al.* (2013), and PCR analysis was carried out using primers which hybridize to a specific sequence in the mimic construction (Table S1). Primers that amplify *CBP20* were used as a positive control.

DNA damage analysis, root length measurements and rosette area quantification

Cyclobutane pyrimidine dimers (CPD) accumulation, primary root elongation and rosette area were analyzed as described in Fina *et al.* (2017b).

Microscopic observations and programmed cell death analysis

Microscopic observations of leaves and programmed cell death analysis of meristematic root cells were done as described in Casadevall *et al.* (2013) and Falcone Ferreyra *et al.* (2016).

Flow cytometric analysis of leaf #5

Flow cytometric analysis of leaf #5 was done as described in Fina *et al.* (2017b). The endoreduplication index (EI) was calculated from the percentage values of each ploidy class with the formula: EI=[(0x%2C)+(1x%4C)+(2x%8C)+ (3x%16C)+(4x%32C)]/100 (Barrow and Meister, 2003). This experiment was done in triplicate, each time using 10 plants corresponding to each treatment/genotype. In every experiment, for each treatment/genotype, at least 5,000 nuclei were analyzed.

Extraction of total UV-B absorbing compounds

UV-B absorbing compounds extraction was performed as previously described (Falcone Ferreyra *et al.*, 2016). UV-B absorbing compounds were quantified by absorbance at 330 nm, and anthocyanins at 540 nm.

Superoxide staining

For superoxide detection, plants were vacumm-infiltred with 0.35 mg mL^{-1} nitroblue tetrazolium in 25 mM Hepes buffer pH 7.4. Plants were transferred to 80% (v/v) ethanol and boiled for 2 min. Experiments were repeated three times using at least three different plants.

Analysis of promoter sequences

The analysis of promoter sequences was done using the PlantPAN2.0 software (http://plantpan2.itps.ncku.edu.tw/).

Statistical analysis

Data presented were analyzed using ANOVA models (a = 0.05) using the Sigma Plot package. When comparing two data sets, Student's t test was used ($p < 0.05$), and significant differences are indicated with different letters.

Accession number

E2Fa (AT2G36010), *E2Fb* (AT5G22220), *E2Fc* (AT1G47870), *E2Fd/DEL2* (AT5G14960), *E2Fe/DEL1* (AT3G48160), *E2Ff/DEL3* (AT3G01330), *DPa* (AT5G02470), *DPb* (AT5G03415), *UVR2* (AT1G12370), *UVR3* (AT3G15620), *GRF1* (AT2G22840), *GRF2* (AT4G37740), *GRF3* (AT2G36400), *HY5* (AT5G11260), *F3H* (AT3G51240), *CHS* (AT5G13930), *UVR8* (AT5G63860), *ATM* (AT3G48190), *ATR* (AT5G40820), *SOG1* (AT1G25580),, *KNOLLE* (AT1G08560), *CDKB1;2* (AT3G54180), *H4* (At2g28740), *miR396A* (AT2G10606); *miR396B* (AT5G35407).

ACKNOWLEDGMENTS

This research was supported by FONCyT grants PICT 2016-141 and 2015-157 to P.C.. M.L.F.F and P.C. are members of the Researcher Career of CONICET and are Professors at UNR. M.S.G is a doctoral fellow from CONICET, and M.S. is a doctoral fellow from FONCYT. We thank Javier Palatnik for discussions and *MIM396* and *tcp2x3x4x10* seeds, Crisanto Gutierrez for *E2Fc RNAi* and *E2FcOE* lines and Roman Ulm for *uvr8* and *mpk3* seeds. We thanks María José Maymó for care in cultivating Arabidopsis plants.

The authors declare no conflicts of interest.

SHORT SUPPLEMENTAL INFORMATION LEGENDS:

Figure S1. Relative expression levels of *E2Fc* and *MIM396* in WT Col-0, *E2FcRNAi*, silenced *E2FcRNAi*, *MIM396xE2FcRNAi* and *E2FcRNAixMIM396* plants; and phenotypes of silenced *E2FcRNAi* plants.

Figure S2. UV-B affects cell expansion in leaves with non-proliferating cells, and cell proliferation in leaves with diving cells of Col-0, *E2FcRNAi* and *E2FcOE A. thaliana* plants.

Figure S3. UV-B effect on expression levels of *E2F* and *DP* proteins in Col-0 and *E2FcRNAi* leaf #5.

Figure S4. UV-B effect on expression levels of miR396, *E2Fa* and *KNOLLE* in Col-0 and *E2FcRNAi* leaf #5 after different times of UV-B exposure.

Figure S5. UV-B effects on leaf growth in proliferating leaves of Col-0 and *E2FcOE* plants.

Figure S6. Primary root inhibition assays, number of stem cells that are dead and relative CPD levels in Col-0 and *E2FcOE* plants after UV-B exposure. CPD levels, *UVR2* and *UVR3* expression in Col-0 and *E2FcRNAi* plants after UV-B exposure.

Figure S7. Analysis of DNA ploidy in leaf #5 of Col-0 and *E2FcRNAi* plants.

Figure S8. Rosette area, average leaf area, average cell area, and cell number of fully expanded leaf #5 of control and UV-B treated Col-0, *E2FcRNAi*, *MIM396* and *E2FcRNAixMIM396* plants.

Figure S9. Number of stem cells that are dead, relative CPD levels and primary root inhibition assays in Col-0, *E2FcRNAi*, *MIM396* and *E2FcRNAixMIM396* plants after UV-B exposure.

Figure S10. Primary root inhibition assays in Col-0, *E2FcRNAi*, *MIM396* and *MIM396xE2FcRNAi* plants after UV-B exposure.

Figure S11. UV-B effect on cell proliferation is independent of class II TCP transcription factors.

Figure S12. Schemes of the *ATR* and *SOG1* promoters showing the positions and sequences of putative E2F-binding sites.

Table S1. Primers used for RT-qPCR and RT-PCR.

REFERENCES

Barrow, M., and Meister, A. (2003) Endopolyploidy in seed plants is differently correlated to systematic, organ, life strategy and genome size. *Plant Cell Environ.* **26,** 571-584.

Berckmans, B., Lammens, T., Van Den Daele, H., Magyar, Z., Bögre, L., and De Veylder, L. (2011) Light-Dependent Regulation of *DEL1* Is Determined by the Antagonistic Action of E2Fb and E2Fc. *Plant Physiol.* **157,** 1440–1451.

Biedermann, S., Harashima, H., Chen, P., Heese, M., Bouyer, D., Sofroni, K., and Schnittger, A. (2017) The retinoblastoma homolog RBR1 mediates localization of the repair protein RAD51 to DNA lesions in Arabidopsis. *EMBO J.* **36,** 1279–1297.

Bresso, E.G., Chorostecki, U., Rodriguez, R.E., Palatnik, J.F., and Schommer, C. (2018) Spatial control of gene expression by miR319-regulated TCP transcription factors in leaf development. *Plant Physiol.* **176,** 1694-1708.

Brown, B.A., Cloix, C., Jiang, G.H., Kaiserli, E., Herzyk, P., Kliebenstein, D.J., and Jenkins, G. I. (2005) A UV-B-specific signaling component orchestrates plant UV protection. *Proc. Natl. Acad. Sci. USA* **102,** 18225–18230.

Casadevall, R., Rodriguez, R.E., Debernardi, J.M., Palatnik, J.F., and Casati, P. (2013) Repression of growth regulating factors by the microRNA396 inhibits cell proliferation by UV-B radiation in Arabidopsis leaves. *Plant Cell* **25,** 3570–3583.

Chen, C., Chen, C., Ridzon, D.A., Broomer, A.J., Zhou, Z., Lee, D.H., Nguyen, J.T., Barbisin, M., Xu, N.L., Mahuvakar, V.R., Andersen, M.R., Lao, K.Q., Livak, K.J., and Guegler, K.J. (2005) Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res.* **33,** e179.

Choi, D., Kim, J.H., and Kende, H. (2004) Whole genome analysis of the OsGRF gene family encoding plant-specific putative transcription activators in rice (Oryza sativa L.). *Plant Cell Physiol.* **45,** 897–904.

Culligan, K.M., Robertson, C.E., Foreman, J., Doerner, P., and Britt, A.B. (2006) ATR and ATM play both distinct and additive roles in response to ionizing radiation. *Plant J.* **48,** 947-961.

de Jager, S.M., Scofield, S., Huntley, R.P., Robinson, A.S., den Boer, B.G.W., Murray, J.A.H. (2009) Dissecting regulatory pathways of G1/S control in *Arabidopsis*: common and distinct targets of CYCD3;1, E2Fa and E2Fc. *Plant Mol. Biol.* **71,** 345–365.

Debernardi, J.M., Rodriguez, R.E., Mecchia, M.A., and Palatnik, J.F. (2012) Functional specialization of the plant miR396 regulatory network through distinct microRNA-target interactions. *PLoS Genet.* **8,** e1002419.

De Veylder, L., Beeckman, T., Beemster, G.T.S., de Almeida Engler, J., Ormenese, S., Maes, S., Naudts, M., Van Der Schueren, E., Jacqmard, A., Engler, G., and Inze´, D. (2002) Control of proliferation, endoreduplication and differentiation by the Arabidopsis E2Fa-DPa transcription factor. *EMBO J.* **21,** 1360–1368.

del Pozo, J.C., Boniotti, M.B., and Gutierrez, C. (2002) Arabidopsis E2Fc functions in cell division and is degraded by the ubiquitin- SCF(SKP2) pathway in response to light. *Plant Cell* **14,** 3057–3071.

del Pozo, J.C., Diaz-Trivino, S., Cisneros, N., and Gutierrez, C. (2006) The balance between cell division and endoreplication depends on E2FCDPB, transcription factors regulated by the ubiquitin- SCFSKP2A pathway in Arabidopsis. *Plant Cell* **18,** 2224-2235.

del Pozo, J.C., Diaz-Trivino, S., Cisneros, N., and Gutierrez, C. (2007) The E2FC-DPB Transcription Factor Controls Cell Division, Endoreplication and Lateral Root Formation in a SCFSKP2A-Dependent Manner. *Plant Signal. Behav.* **2,** 273-274.

Dotto, M., and Casati, P. (2017) Developmental reprogramming by UV-B radiation in plants. *Plant Sci.* **267,** 96-101.

Falcone Ferreyra, M.L., Casadevall, R., D'Andrea, L., AbdElgawad, H., Beemster, G.T.S., and Casati, P. (2016) AtPDCD5 Plays a Role in Programmed Cell Death after UV-B Exposure in Arabidopsis". *Plant Physiol.* **170,** 2444-2460.

Fierro, A.C., Leroux, O., De Coninck, B., Cammue, B.P.A., Marchal, K., Prinsen, E., Van Der Straeten, D., and Vandenbussche, F. (2015) Ultraviolet-B radiation stimulates downward leaf curling in *Arabidopsis thaliana*. *Plant Physiol. Biochem.* **93,** 9–17.

Fina, J.P., Casadevall, R., AbdElgawad, H., Prinsen, E., Markakis, M., Beemster, G., and Casati P. (2017a) UV-B inhibits leaf growth through changes in Growth Regulating Factors and gibberellin levels. *Plant Physiol*. **174,** 1110-1126.

Fina, J.P., Masotti, F., Rius, S.P., Crevacuore, F., and Casati P. (2017b) HAC1 and HAF1 Histone Acetyltransferases have different roles in UV-B Responses in Arabidopsis. *Frontiers Plant Sci*. **8,** 1179.

Furukawa, T., Curtis, M.J., Tominey, C.M., Duong, Y.H., Wilcox, B.W.L., Aggoune, D., Hays, J.B., and Britt, A.B. (2010) A shared DNA-damage-response pathway for induction of stem-cell death by UVB and by gamma irradiation. *DNA Repair (Amst)* **9,** 940–948.

Galbraith, D.W., Harkins, K.R., and Knapp, S. (1991) Systemic endopolyploidy in *Arabidopsis thaliana*. *Plant Physiol.* **96,** 985-989.

Gegas, V.C., Wargent, J.J., Pesquet, E., Granqvist, E., Paul, N.D., and Doonan, J.H. (2014) Endopolyploidy as a potential alternative adaptive strategy for Arabidopsis leaf size variation in response to UV-B. *J. Exp. Bot.* **65,** 2757-2766.

González Besteiro, M.A., and Ulm, R. (2013) ATR and MKP1 play distinct roles in response to UV-B stress in Arabidopsis. *Plant J.* **73,** 1034–1043.

Hectors, K., Jacques, E., Prinsen, E., Guisez, Y., Verbelen, J.P., Jansen, M.A.K., and Vissenberg, K. (2010) UV radiation reduces epidermal cell expansion in leaves of Arabidopsis thaliana. *J. Exp. Bot.* **61,** 4339–4349.

Heyman, J., Van den Daele, H., De Wit, K., Boudolf, V., Berckmans, B., Verkest, A., Lessa, A. K. C., De Jaeger, G., Koncz, C., and De Veylder, L. (2011) The Arabidopsis Ultraviolet-B-Insensitive4 maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. *Plant Cell* **23,** 4394-4410.

Horiguchi, G., Kim, G.T., and Tsukaya, H. (2005) The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *Plant J.* **43,** 68-78.

Horvath, B.M., Kourova, H., Nagy, S., Nemeth, E., Magyar, Z., Papdi, C., Ahmad, Z., Sanchez-Perez, G.F., Perilli, S., Blilou, I., Pettkó-Szandtner, A., Darula, Z., Meszaros, T., Binarova, P., Bogre, L., and Scheres, B. (2017) Arabidopsis RETINOBLASTOMA RELATED directly regulates DNA damage responses through functions beyond cell cycle control. *EMBO J.* **36,** 1261–1278.

Inzé, D., and De Veylder, L. (2006) Cell Cycle Regulation in Plant Development. *Annual Rev. Genetics* **40,** 77–105.

Jenkins, GI. (2017) Photomorphogenic responses to ultraviolet-B light. *Plant Cell Environ.* **40,** 2544–2557.

Jiang, L., Wang, Y., Björn, L.O., and Li S. (2011) Does cell cycle arrest occur in plant under solar UV-B radiation? *Plant Signal. Behav.* **6,** 892–894.

Kim, J.H., Choi, D., and Kende, H. (2003) The AtGRF family of putative transcription factors is involved in leaf and cotyledon growth in Arabidopsis. *Plant J.* **36,** 94–104.

Lammens, T., Boudolf, V., Kheibarshekan, L., Zalmas, L.P., Gaamouche, T., Maes, S., Vanstraelen, M., Kondorosi, E., La Thangue, N.B., Govaerts, W., Inzé, D., and De Veylder, L. (2008) Atypical E2F activity restrains APC/$C^{CCS52A2}$ function obligatory for endocycle onset. *Proc. Nat. Acad. Sci. USA* **105,** 14721–14726.

Magyar, Z., De Veylder, L., Atanassova, A., Bako´, L., Inze´, D., and Bögre, L. (2005) The role of the Arabidopsis E2FB transcription factor in regulating auxin-dependent cell division. *Plant Cell* **17,** 2527–2541.

Magyar, Z., Horváth, B., Khan, S., Mohammed, B., Henriques, R., De Veylder, L., Bako´, L., Scheres, B., Bögre, L. (2012) Arabidopsis E2FA stimulates proliferation and endocycle separately through RBR-bound and RBR-free complexes. *EMBO J.* **31,** 1480-1493.

Nakagami, H., Kawamura, K., Sugisaka, K., Sekine, M., and Shinmyo, A. (2002) Phosphorylation of retinoblastoma-related protein by the cyclin D/cyclin-dependent kinase complex is activated at the G1/S-phase transition in tobacco. *Plant Cell* **14,** 1847-1857.

Nakagami, H., Sekine, M., Murakami, H., and Shinmyo, A. (1999) Tobacco retinoblastoma-related protein phosphorylated by a distinct cyclin-dependent kinase complex with Cdc2/cyclin D in vitro. *Plant J.* **18,** 243-252.

Naouar, N., Vandepoele, K., Lammens, T., Casneuf, T., Zeller, G., Van Hummelen, P., Weigel, D., Rätsch, G., Inzé, D., Kuiper, M., De Veylder, L., and Vuylsteke, M. (2009) Quantitative RNA expression analysis with Affymetrix Tiling 1.0R arrays identifies new E2F target genes. *Plant J.* **57,** 184-194.

Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C., and Weigel, D. (2003) Control of leaf morphogenesis by microRNAs. *Nature* **425,** 257–263.

Polyn, S., Willems, A., and De Veylder, L. (2015) Cell cycle entry, maintenance, and exit during plant development. *Curr. Opin. Plant Biol.* **23,** 1–7.

Radziejwoski, A., Vlieghe, K., Lammens, T., Berckmans, B., Maes, S., Jansen, M.A.K., Knappe, C., Albert, A., Seidlitz, H.K., Bahnweg, G., Inze´, D., and De Veylder, L. (2011) Atypical E2F activity coordinates PHR1 photolyase gene transcription with endoreduplication onset. *EMBO J.* **30,** 355–363.

Ramirez-Parra, E., Frundt, C., and Gutierrez, C. (2003) A genome-wide identification of E2F regulated genes in Arabidopsis. *Plant J.* **33,** 801–811.

Ramirez-Parra, E., and Gutierrez, C. (2007) The many faces of chromatin assembly factor 1, *Trends Plant Sci.* **12,** 570–576.

Rodriguez, R.E., Mecchia, M.A., Debernardi, J.M., Schommer, C., Weigel, D., and Palatnik, J.F. (2010) Control of cell proliferation in Arabidopsis thaliana by microRNA miR396. *Development* **137,** 103– 112.

Rozen, S., and Skaletsky, H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Edited by Krawetz, S.A. and Misener S. pp. 365-386. Totowa NJ: Humana Press.

Schommer, C., Debernardi, J.M., Bresso, E.G., Rodriguez, R.E., and Palatnik, J.F. (2014) Repression of cell proliferation by miR319-regulated TCP4. *Mol. Plant* **7,** 1533-1544.

Sozzani, R., Maggio, C., Varotto, S., Canova, S., Bergounioux, C., Albani, D., and Cella, R. (2006) Interplay between Arabidopsis activating factors E2Fb and E2Fa in cell cycle progression and development. *Plant Physiol.* **140,** 1355–1366.

Tong, H., Leasure, C.D., Hou, X., Yuen, G., Briggs, W., and He, Z.H. (2008) Role of root UV-B sensing in Arabidopsis early seedling development. *Proc. Nat. Acad. Sci. USA* **105,** 21039–21044.

Uemukai, K., Iwakawa, H., Kosugi, S., de Jager, S., Kato, K., Kondorosi, E., Murray, J.A.H., Ito, M., Shinmyo, A., and Sekine, M. (2005) Transcriptional activation of tobacco E2F is repressed by cotransfection with the retinoblastoma-related protein: cyclin D expression overcomes this repressor activity. *Plant Mol. Biol.* **57,** 83-100.

Ulm, R., Baumann, A., Oravecz, A., Máté, Z., Adám, E., Oakeley, E.J., Schäfer, E., and Nagy F. (2004) Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. *Proc. Nat. Acad. Sci. USA* **101,** 1397–1402.

van der Knaap, E., Kim, J.H., and Kende, H. (2000) A novel gibberellin-induced gene from rice and its potential regulatory role in stem growth. *Plant Physiol.* **122,** 695–704.

Vandepoele, K., Vlieghe, K., Florquin, K., Hennig, L., Beemster, G.T., Gruissem, W., Van de Peer, Y., Inzé, D., and De Veylder, L. (2005) Genome-wide identification of potential plant E2F target genes. *Plant Physiol.* **139,** 316–328.

Vlieghe, K., Boudolf, V., Beemster, G.T.S., Maes, S., Magyar, Z., Atanassova, A., de Almeida Engler, J., De Groodt, R., Inzé, D., and De Veylder, L. (2005) The DP-E2F-like gene DEL1 controls the endocycle in Arabidopsis thaliana. *Current Biol.* **15,** 59-63.

Wargent, J.J., Gegas, V.C., Jenkins, G.I., Doonan, J.H., and Paul, N.D. (2009a) UVR8 in Arabidopsis thaliana regulates multiple aspects of cellular differentiation during leaf development in response to ultraviolet B radiation. *New Phytol.* **183,** 315–326.

Wargent, J.J., Moore, J.P., Roland Ennos, A., and Paul, N.D. (2009b) Ultraviolet radiation as a limiting factor in leaf expansion and development. *Photochem. Photobiol.* **85,** 279–286.

Yan, A., Pan, J., An, L., Gan, Y., and Feng, H. (2012) The responses of trichome mutants to enhanced ultraviolet-B radiation in *Arabidopsis thaliana*. *J. Photochem. Photobiol. B Biol.* **113,** 29–35.

Yoshiyama, K. , Conklin, P.A. , Huefner, N.D., Britt, A.B. (2009) Suppressor of gamma response 1 (SOG1) encodes a putative transcription factor governing multiple responses to DNA damage. *Proc. Natl. Acad. Sci. USA* **106,** 12843-12848

Yoshiyama, K.O., Kobayashi, J., Ogita, N., Ueda, M., Kimura, S., Maki, H., and Umeda, M. (2013) ATM-mediated phosphorylation of SOG1 is essential for the DNA damage response in *Arabidopsis*. *EMBO Rep.* **14,** 817–822.

Yoshiyama, K.O., Kimura, S., Maki, H., Britt, A.B., and Umeda, M. (2014) The role of SOG1, a plant-specific transcriptional regulator, in the DNA damage response. *Plant Signal. Behav.* **9,** e28889.

Zhang, H., Jin, J.P., Tang, L., Zhao, Y., Gu, X.C., Gao, G., and Luo, J.C. (2011) PlantTFDB 2.0: Update and improvement of the comprehensive plant transcription factor database. *Nucleic Acids Res.* **39 (Database issue),** D1114–D1117.

Zhao, X., Harashima, H., Dissmeyer, N., Pusch, S., Weimer, A.K., Bramsiepe, J., Bouyer, D., Rademacher, S., Nowack. M.K., Novak, B., Sprunck, S., and Schnittger A. (2012) A general G1/S-phase cell-cycle control module in the flowering plant *Arabidopsis thaliana*. *PLoS Genet.* **8,** e1002847.

FIGURE LEGENDS:

Figure 1. UV-B affects cell proliferation but not cell expansion in proliferating leaves of Col-0 and *E2FcRNAi A. thaliana* plants.

(a) Col-0 and *E2FcRNAi* plants that were treated with UV-B for 4 h or were kept in the absence of UV-B at 12 DAS, 15 days after the end of the treatment.

(b) Rosette area of control and UV-B treated Col-0 and *E2FcRNAi* plants measured every 2 days from germination until 20 DAS. Plants were UV-B treated at 12 DAS.

(c) Fully expanded leaf #5 from UV-B treated or control Col-0 and *E2FcRNAi* plants. Average leaf area (d), average cell area (e) and cell number (f) of fully expanded leaf #5 from UV-B treated or control plants. Results represent the average ± S.E.M. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$).

Figure 2. UV-B effect on expression of cell cycle, proliferation-related genes, and *E2F* transcription factors in Col-0 and *E2FcRNAi* leaf #5.

Relative expression levels of *GRF2* (a), *GRF3* (b), miR396 (c), *E2Fa* (d), *E2Fe* (e), *DPa* (f), *KNOLLE* (g), *CDKB1;2* (h) and *H4* (i) by RT-qPCR in Col-0 and *E2FcRNAi* leaf #5 under control conditions (C), or immediately after a 4 h-UV-B treatment (UV-B). Results represent the average ± S.E.M. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$). Data represent 3 biological replicate experiments. Each qRT-PCR was repeated at least three times on each biological replicate.

Figure 3. Expression analysis of UV-B responsive genes and UV-B absorbing pigment content in Col-0 and *E2FcRNAi* leaf #5 after UV-B exposure.

(a-d) Relative expression levels of *UVR8* (a), *HY5* (b), *CHS* (c) and *F3H* (d) by RT-qPCR in Col-0 and *E2FcRNAi* leaf #5 under control conditions (C) or after a 4 h-UV-B treatment (UV-B).

(e-h) UV-B absorbing pigments (e-f) and anthocyanins (g-h) in Col-0 and *E2FcRNAi* leaves under control conditions (C), or after a 4 h-UV-B treatment (UV-B). (f and h) Ratios of absorbances from UV-B-irradiated relative to control leaves are shown.

Results represent the average ± S.E.M. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$). Data represent 3 biological replicate experiments. Each experiment was repeated at least three times on each biological replicate.

(i) NBT staining for superoxide in WT Col-0 and *E2FcRNAi* transgenic plants under control condition (C) and after UV-B exposure (UV-B).

Figure 4. Primary root inhibition, stem cell death and expression of DNA damage response genes in Col-0 and *E2FcRNAi* plants after UV-B.

(a and b) Average root lengths in Col-0 and *E2FcRNAi* plants (a), and representative pictures of one experiment (b). Results represent the average of 30 biological replicates ± S.E.M.

(c) Representative images of stem cells and adjacent daughter cells from WT Col-0 and *E2FcRNAi* plants that were scored for intense PI staining to count dead stem cells per root after UV-B. (d) Number of stem cells that are dead after UV-B exposure.

(e-g) UV-B effect on expression levels of *SOG1* (e), *ATR* (f) and *ATM* (g) in Col-0 and *E2FcRNAi* leaf #5. Relative expression levels were determined by RT-qPCR under control conditions (C), or after a 4 h-UV-B treatment (UV-B). Results represent the average ± S.E.M. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$). Data represent 3 biological replicate experiments. Each qRT-PCR was repeated at least three times on each biological replicate.

Figure 5. UV-B effect in cell proliferation mediated by miR396 requires E2Fc.

(a) Col-0, *E2FcRNAi*, *MIM396* and *MIM396xE2FcRNAi* plants that were treated with UV-B radiation for 4 h or were kept under conditions at 12 DAS, fifteen days after the end of the treatment.

(b) Rosette area of control and UV-B treated Col-0, *E2FcRNAi*, *MIM396* and *MIM396xE2Fc* plants measured every 2 days from germination until 20 DAS. Plants were UV-B treated at 12 DAS.

(c) Fully expanded leaf #5 from UV-B treated or control Col-0, *E2FcRNAi*, *MIM396* and *MIM396xE2Fc* plants.

Average leaf area (d), average cell area (e), and cell number (f) of fully expanded leaf #5 from UV-B treated or control plants. Results represent the average ± S.E.M. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$).

Figure 6. Lower DNA damage after UV-B in *MIM396* plants requires E2Fc.

(a) Representative images of stem cells and adjacent daughter cells from WT Col-0, *E2FcRNAi*, *MIM396* and *MIM396xE2FcRNAi* plants that were scored for intense PI staining to count dead stem cells per root after UV-B.

(b) Number of stem cells that are dead after UV-B exposure.

(c) Relative CPD levels in the DNA of WT Col-0 *E2FcRNAi*, *MIM396* and *MIM396xE2FcRNAi* plants immediately after a 4-h UV-B treatment. Results represent averages ± S.E.M. of six independent biological replicates. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$).

Figure 7. Effect of UV-B radiation on *GRF* and *E2F* expression in leaf #5 from different lines.

(a-c) Relative expression levels of *GRF1* (a), *GRF2* (b) and *GRF3* (c) in Col-0, *E2FcRNAi*, *MIM396* and *MIM396xE2FcRNAi* leaf #5.

(d-e) Relative expression levels of *E2Fa-f* in Col-0, *uvr8* (d) and *mpk3* (e) leaf #5.

Transcript levels were determined by RT-qPCR under control conditions (C), or after a 4 h-UV-B treatment (UV-B). Results represent the average ± S.E.M. Different letters indicate statistical significant differences applying ANOVA test ($P < 0.05$). Data represent 3 biological replicate experiments. Each qRT-PCR was repeated at least three times on each biological replicate.

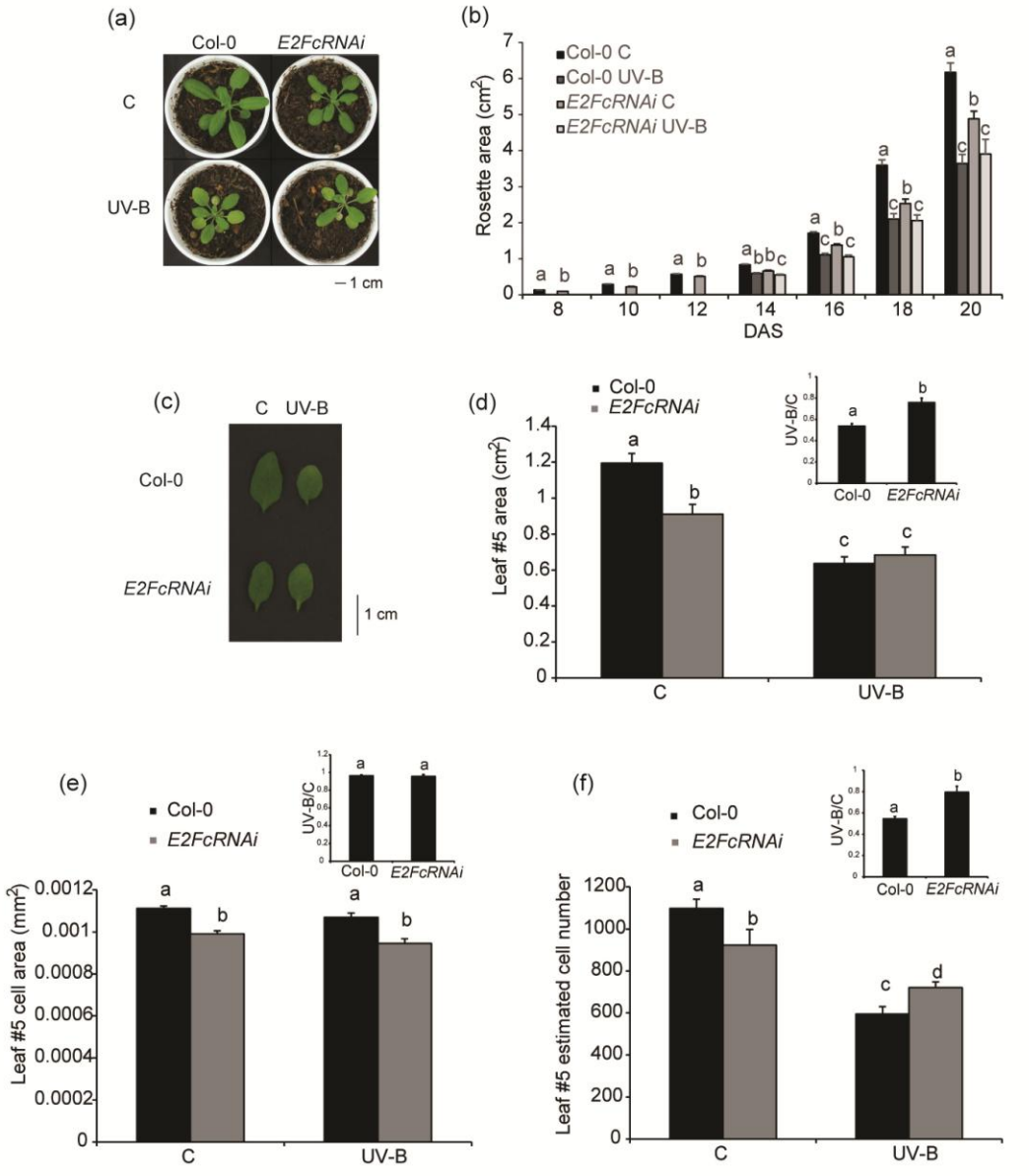

Figure 1

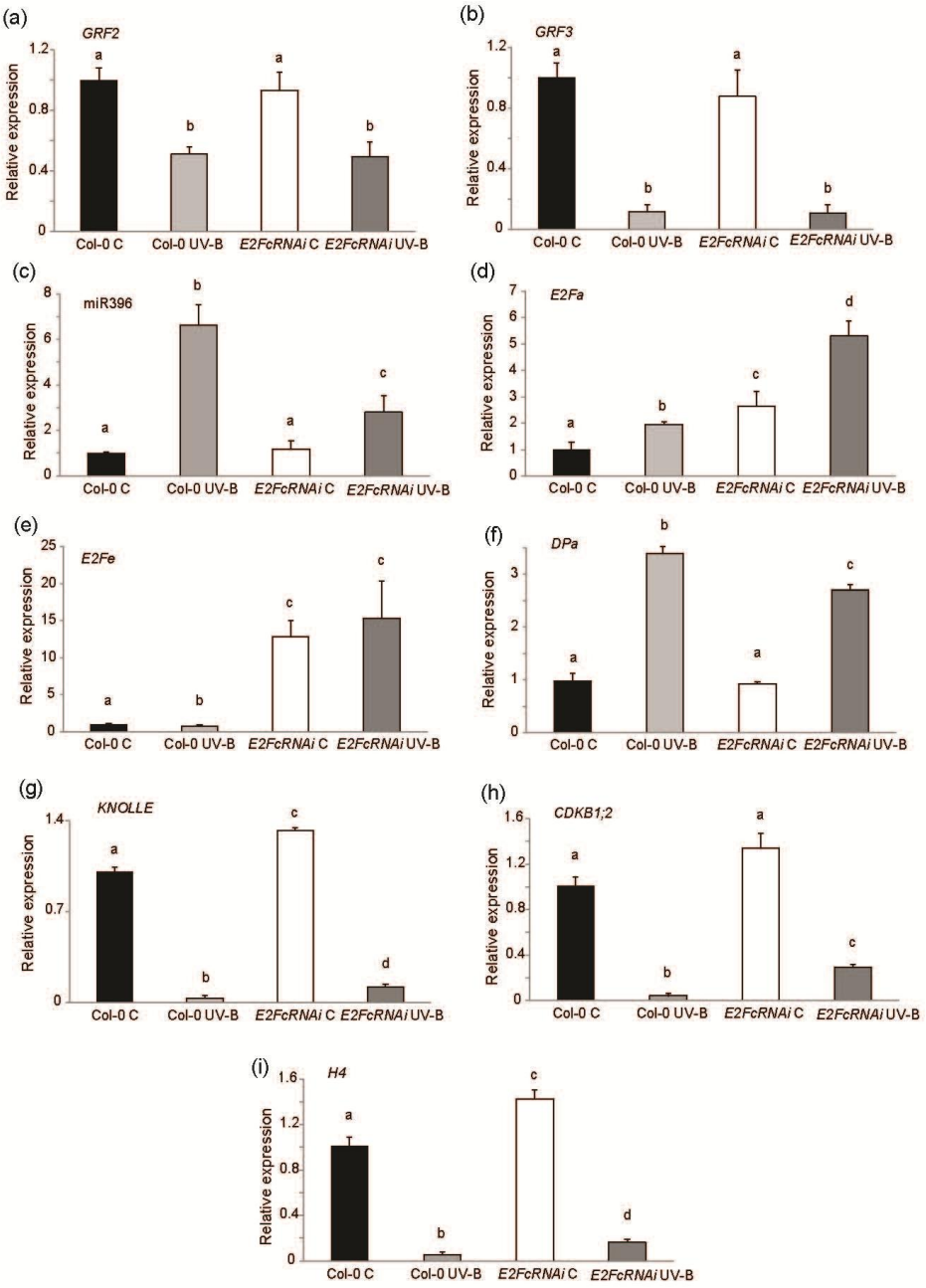

Figure 2.

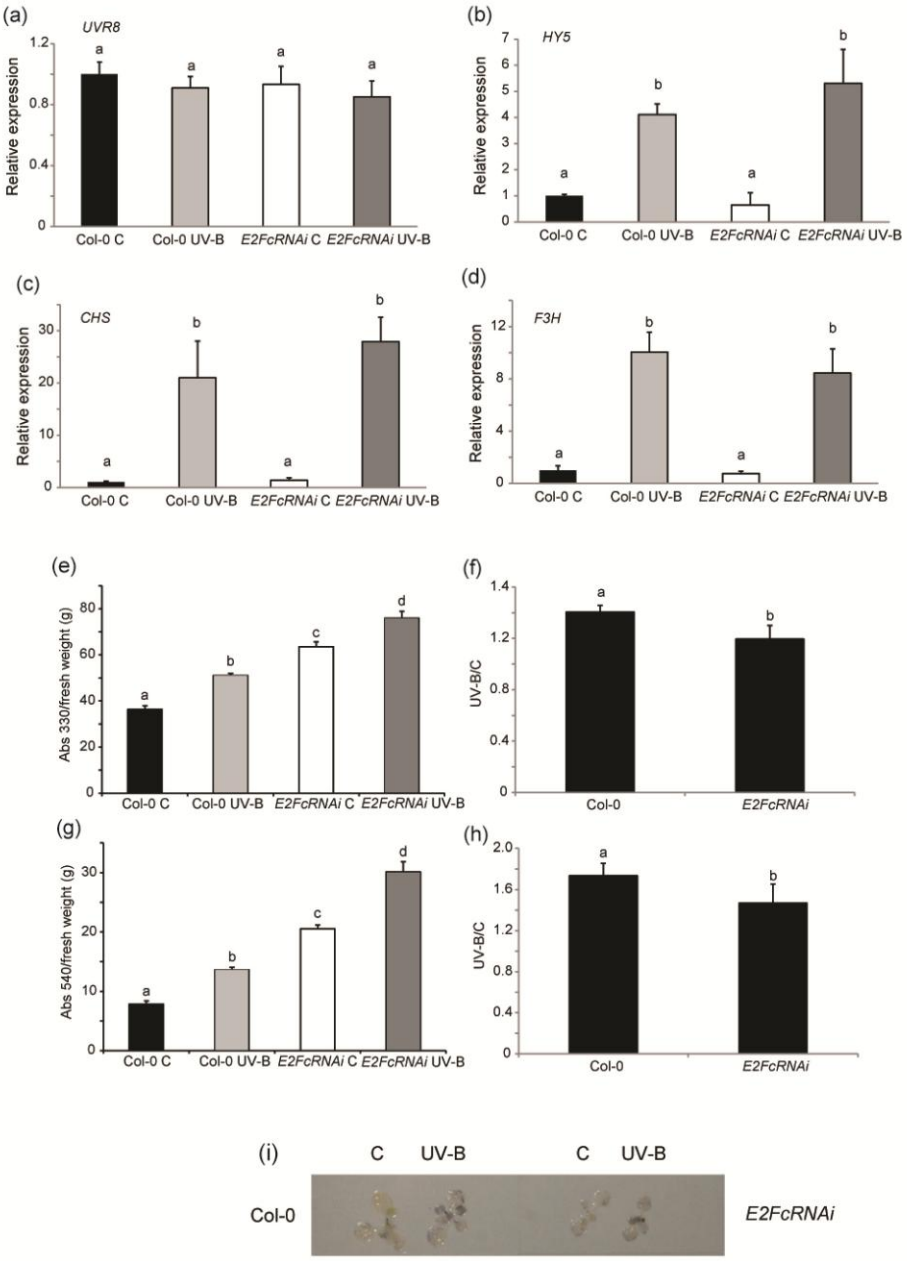

Figure 3.

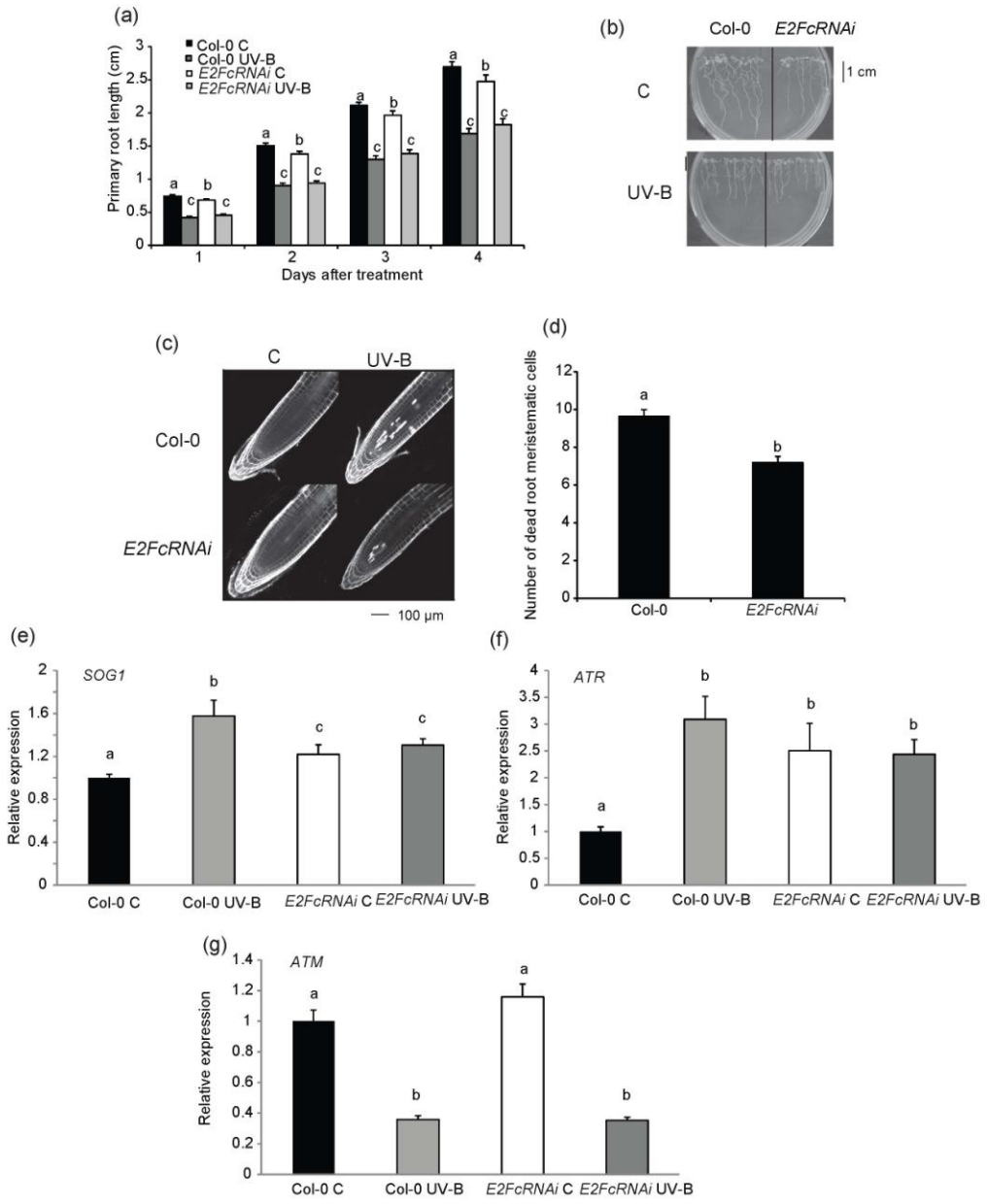

Figure 4

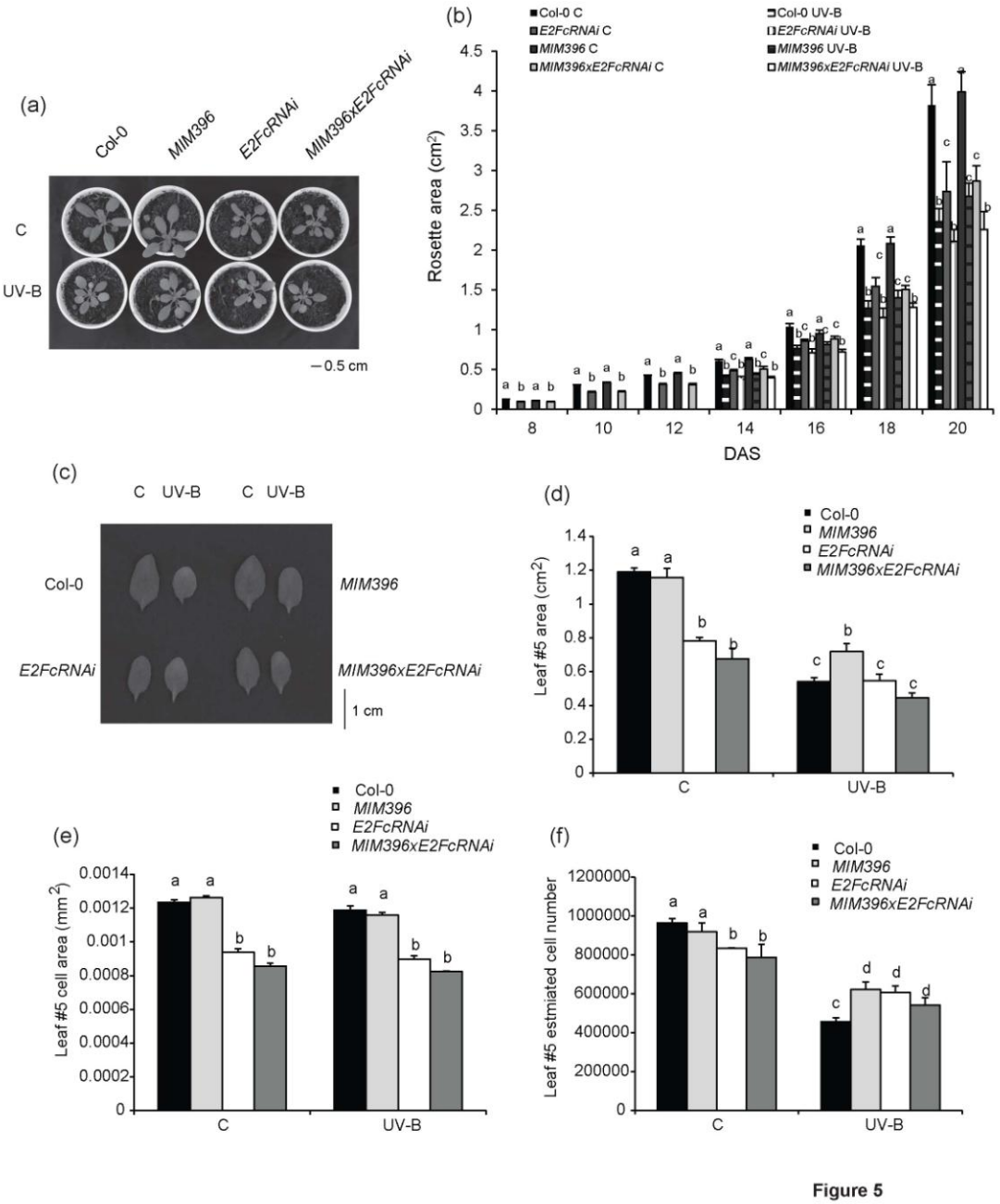

Figure 5

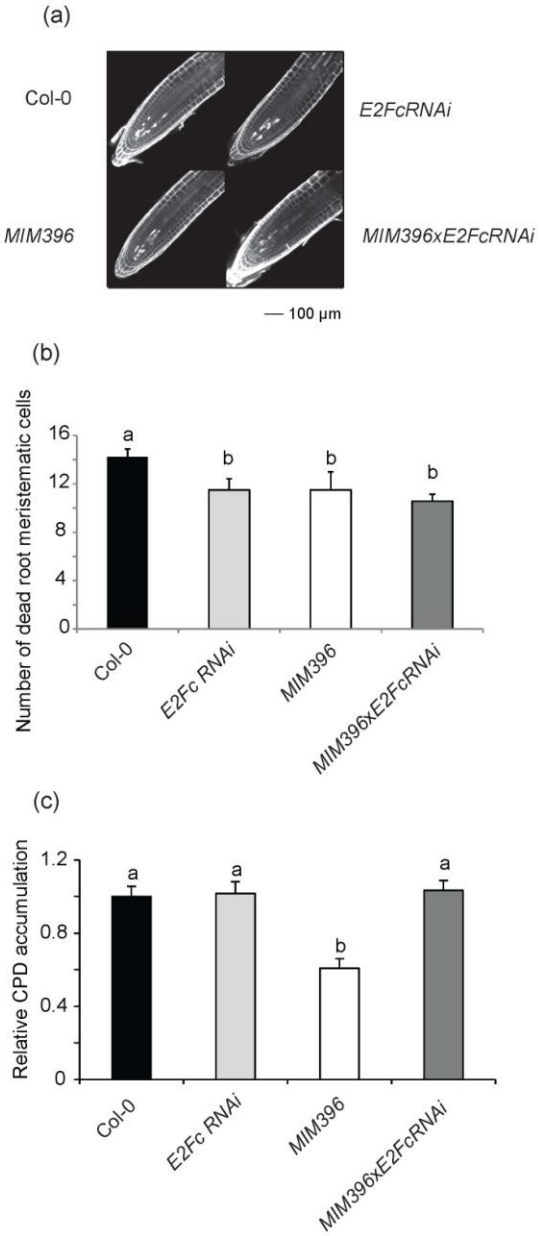

Figure 6

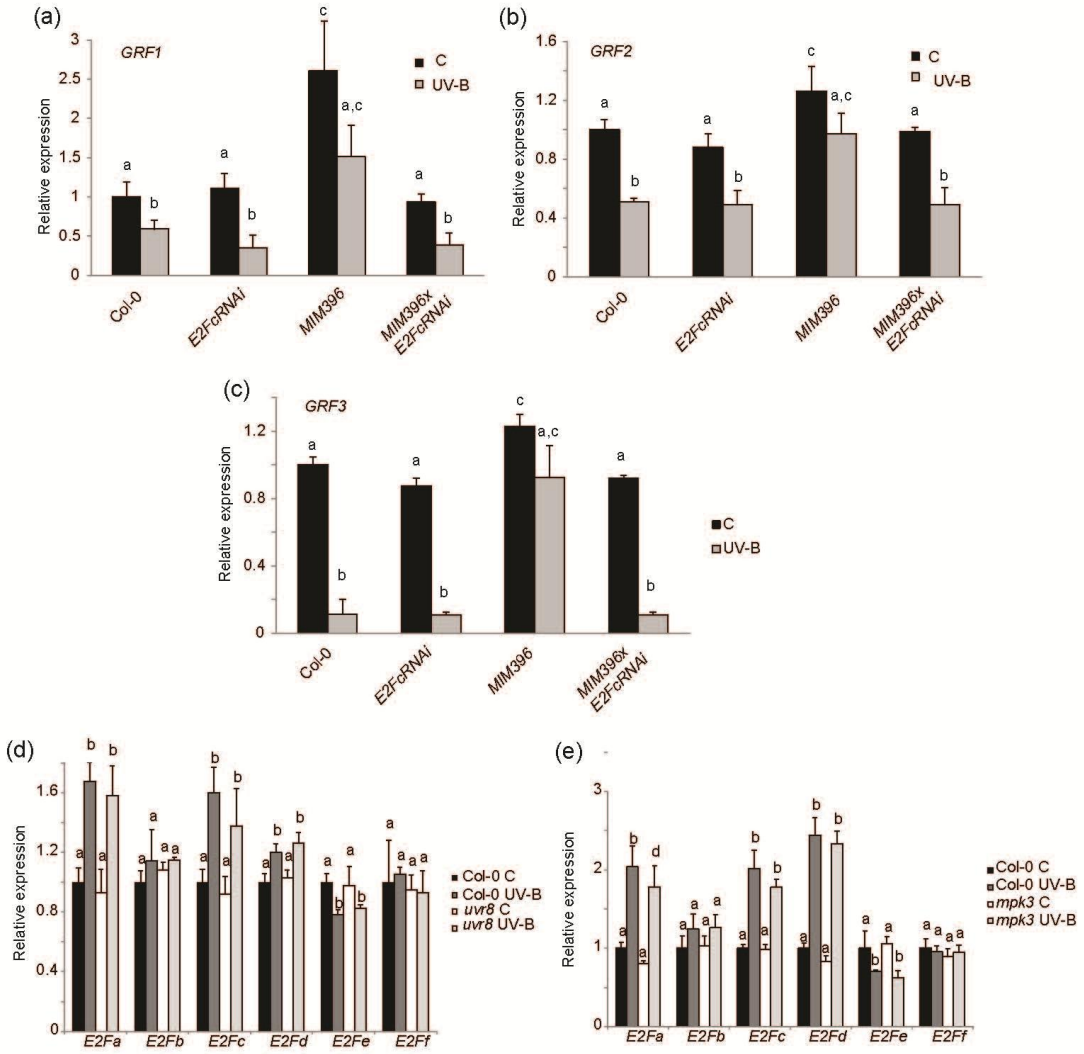

Figure 7.