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The tomato DWD motif-containing protein DDI1 interacts with the CUL4–DDB1-based ubiquitin ligase and plays a pivotal role in abiotic stress responses

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ABSTRACT

CULLIN4(CUL4)–DAMAGED DNA BINDING PROTEIN1 (DDB1)-based ubiquitin ligase plays significant roles in multiple physiological processes via ubiquitination-mediated degradation of relevant target proteins. The DDB1–CUL4-associated factor (DCAF) acts as substrate receptor in the CUL4–DDB1 ubiquitin ligase complex and determines substrate specificity. In this study, we identified a tomato (*Solanum lycopersicum*) DDB1-interacting (DDI1) protein as a DCAF protein involved in response to abiotic stresses, including UV radiation, high salinity and osmotic stress. Co-immunoprecipitation and bimolecular fluorescence complementation assay indicated that DDI1 associates with CUL4–DDB1 in the nucleus. Quantitative RT-PCR analysis indicated the *DDI1* gene is induced by salt, mannitol and UV-C treatment. Moreover, transgenic tomato plants with overexpression or knockdown of the *DDI1* gene exhibited enhanced or attenuated tolerance to salt/mannitol/UV-C, respectively. Thus, our data suggest that DDI1 functions as a substrate receptor of the CUL4–DDB1 ubiquitin ligase, positively regulating abiotic stress response in tomato.

1. Introduction

Environmental stress, such as ultra-violet radiation, drought, high salinity, heavy metals and high/low temperatures, signify the most severe environmental pressure that significantly limits plant growth and productivity [1]. As sessile organisms, plants have evolved a number of adaptive strategies to overcome such unfavorable stresses, which is generally characterized by a high degree of homeostatic plasticity in response to environmental fluctuations, thereby optimizing their growth and development in a way that would maximize their opportunities for survival and reproduction [2].

One of the recently identified cullin proteins, CULLIN4 (CUL4), has been shown to play a pivotal role in many physiological signaling pathways, especially in regulation of homeostatic plasticity in response to biotic and/or abiotic stresses [3,4]. CUL4 assembles with DDB1 and another DDB1-interacting protein, termed DDB1–CUL4-associated factor (DCAF), to form the DDB1–CUL4-based ubiquitin ligase (CRL4) family, with the potential to assemble hundreds of distinct CRL4s [5]. In plants, CRL4 is assembled on CUL4, which provides the scaffold for two essential modules: a small RING-box domain protein (RBX1), which is responsible for binding to ubiquitin E2, and the CUL4-specific adaptor DDB1, which is associated with DCAF that specifically recognizes the corresponding target proteins [6].

As a highly conserved core component of the CRL4 ubiquitin ligase complex, DDB1 consists of three WD40 β-propeller domains (BPA, BPB, and BPC) and a C-terminal helical domain [7]. The BPB propeller mediates the interaction with CUL4, whereas the BPA and BPC propellers arrange themselves as a clam shape pocket, allowing for binding to DCAF, the substrate receptor for CRL4 ubiquitin ligase [5]. DCAF is also conserved in many eukaryotes and usually contains a 16-amino acid motif called DDB1-binding WD40 protein box (DWD) [8]. CRL4 ubiquitin ligases were originally identified as a regulator of DNA repairing, with a primary activity of DDB1 described as participating in the recognition of damaged DNA and initiation of nucleotide excision repair process [9]. Recently, CRL4 ligases have been demonstrated to play roles in multiple physiological processes, such as photomorphogenesis

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[10], cell cycling [11], plastid division [12] and stress responses [3], but the mechanistic basis by which the majority of these CRL4 ligases function still awaits elusive.

In the present study, we have identified a DWD-containing protein acting as a DCAF protein (termed DDI1) in tomato by yeast two-hybrid (Y2H) screening using the tomato DDB1 as bait. Using both loss-of-function and gain-of-function approaches, we have demonstrated that DDI1 is positively involved in regulating tolerance to multiple abiotic stresses, including ultraviolet radiation, high salinity and osmotic stresses.

2. Materials and methods

2.1. Yeast two-hybrid screening

The LexA-based Y2H screening was conducted to identify proteins interacting with DDB1. The LexA-DDB1 bait was transformed into the yeast EGY48 stain containing a report plasmid pSH18-34. The expression of the LexA-DDB1 fusion protein was verified and no autoactivation was detected. A tomato cDNA library generated in the prey vector pJG4-5 was further introduced to screen DDB1-interacting proteins. The protein–protein interaction was determined on yeast medium containing X-gal or without leucine supply. Primers used Y2H cloning are listed in Supplementary Table S1.

2.2. Tomato transformation

The full length *DDI1* (AK320463.1) and relevant RNA-interference fragment were PCR-amplified and cloned into pBI121 vector at the *Xba*I and *Sac*I sites to generate *DDI1*-overexpression or *DDI1-RNAi* construct, both of which were under the control of the *Cauliflower mosaic virus* (CaMV) 35S promoter. Transgenic tomato plants were generated by *Agrobacterium tumefaciens*-mediated transformation according to the method described by Fillatti [13]. Primers used in the cloning and PCR verification are listed in Supplementary Table S1.

2.3. Real-time PCR analyses

Total RNAs were isolated using Trizol reagent (Invitrogen) and treated with DNaseI. Reverse transcription was conducted using the First Strand cDNA Synthesis kit (Toyobo). Real-time PCR analysis was performed on the Applied Biosystems StepOne Real-Time PCR System (Applied Biosystems) using SsoFast EvaGreen Supermix (Bio-Rad) reagent, with the tomato *UBI3* gene (X58253) as an internal reference. Primers used in real-time PCR are listed in Table S1.

2.4. Co-immunoprecipitation and bimolecular fluorescence complementation (BiFC) analysis

The C-terminal HA-tagged and FLAG-tagged constructs were generated by PCR amplification of tomato *DDI1*, *DDB1* or *CUL4* cDNA and consequently cloned into the binary vector pBTEX. For generation of the BiFC constructs, the appropriate genes were cloned into the vectors pBSPYNE or pBSPYCE, which contain the N-terminal 1–155 amino acids or C-terminal 156–239 amino acids of yellow fluorescence protein (YFP), respectively. The resulting CaMV 35S promoter-driven constructs were introduced into the *A. tumefaciens* GV2260 for the *Agrobacterium*-mediated transient expression as described previously [14]. The agrobacterial infiltration into *Nicotiana benthamiana* leaves was carried out at an inoculum of OD_{600} = 0.5. *N. benthamiana* leaf tissues (≈3cm^2 discs) were collected at 36 h after infiltration and used for co-immunoprecipitation assay as described previously [15]. The BiFC assay was conducted by DAPI-staining of the epidermal cell layers, followed by confocal microscopy to simultaneously capture DAPI and YFP signals at 48 h after agrobacterial infiltration. Primers used for generation of the BiFC constructs are listed in Table S1.

2.5. Root sensitivity to UV-C, salt and mannitol

The WT and T_3 transgenic seeds were germinated on 1/2 MS medium for 4–5 days. 6–8 seeds with similarly protruded radicle were transferred to 1/2 MS medium with or without 100 mM NaCl or Mannitol for vertical growth for another 3–4 days. For the UV-C sensitivity assay, after growing another 2–3 days vertically on 1/2 MS medium, seedlings were irradiated at 20 kJ/m^2 by UV-C lamps (TUV 11W T5, Philips), followed by a 12-h recovery process in dark for 4 consecutive days. The relative root growth rate (root length of UV-treated seedlings/root length of untreated seedlings × 100%) was measured at 12 h after the last irradiation.

3. Results

3.1. The tomato DDI1 interacts with DDB1 and CUL4 in vivo

In an attempt to identify potential regulatory factors associated with the tomato DDB1 protein, which acts as the CUL4-adaptor in the CRL4 ligase, we carried out a Y2H screening using the functional DDB1 isoform DDB1^F as bait [11]. Several DDB1-interacting proteins (DDIs) were identified and one of them (termed DDI1) contains five putative WD40 repeats and one DWD motif (Fig. 1A, B and S1A), suggesting DDI1 is a potential substrate receptor of the CUL4-DDB1-based CRL4 ubiquitin ligase in tomato.

All CRL4 ubiquitin ligases share a common architectural feature and the components exist in cell as a complex, in which CUL4 acts as the backbone of the protein scaffold and DDB1 binds to other adaptor(s) linking substrates [16]. We next determined the association of DDI1 with DDB1–CUL4 complex in plant cells by co-immunoprecipitation (co-IP) assay. HA- or FLAG-tagged DDI1, DDB1 or CUL4 were transiently co-expressed in *N. benthamiana* leaves via *Agrobacterium*-mediated transient expression, with three different co-expression combinations: CUL4-HA/DDI1-FLAG, DDB1-HA/DDI1-FLAG and CUL4-HA/DDB1-FLAG. Two days after agrobacterial infiltration, proteins were extracted from leaf tissues and immunoprecipitated with the anti-HA antibody matrix. The protein identity in the immunoprecipitated complexes was verified by the anti-FLAG antibody to determine the *in vivo* association among these three proteins. As shown in Fig. 1C, DDI1-FLAG was co-immunoprecipitated with CUL4-HA or DDB1-HA, but not with the control empty vector. In addition, CUL4 was detected in the DDB1-immunocomplexes as expected. Taken together, our co-IP assay results suggest these three proteins exist as a CUL4–DDB1–DDI1 complex in plant cells.

3.2. CUL4, DDB1 and DDI1 co-localize in the nucleus

We next sought to determine the cellular compartment where DDI1–DDB1, DDI1–CUL4 and DDB1–CUL4 interactions occur inside the living plant cells. To this end, we adopted the bimolecular fluorescence complementation (BiFC) approach that combines molecular and cell biology technique to validate protein interactions in living cells and identify the subcellular location of interactions as well [14]. DDB1, DDI1 and CUL4 were cloned into the split YFP binary vectors under the control of the CaMV 35S promoter. The resulting constructs were co-expressed in *N. benthamiana* leaves as follows: DDI1-NYFP with DDB1-CYFP, DDI1-NYFP with CUL4-CYFP, and CUL4-NYFP with DDB1-CYFP. In addition, the

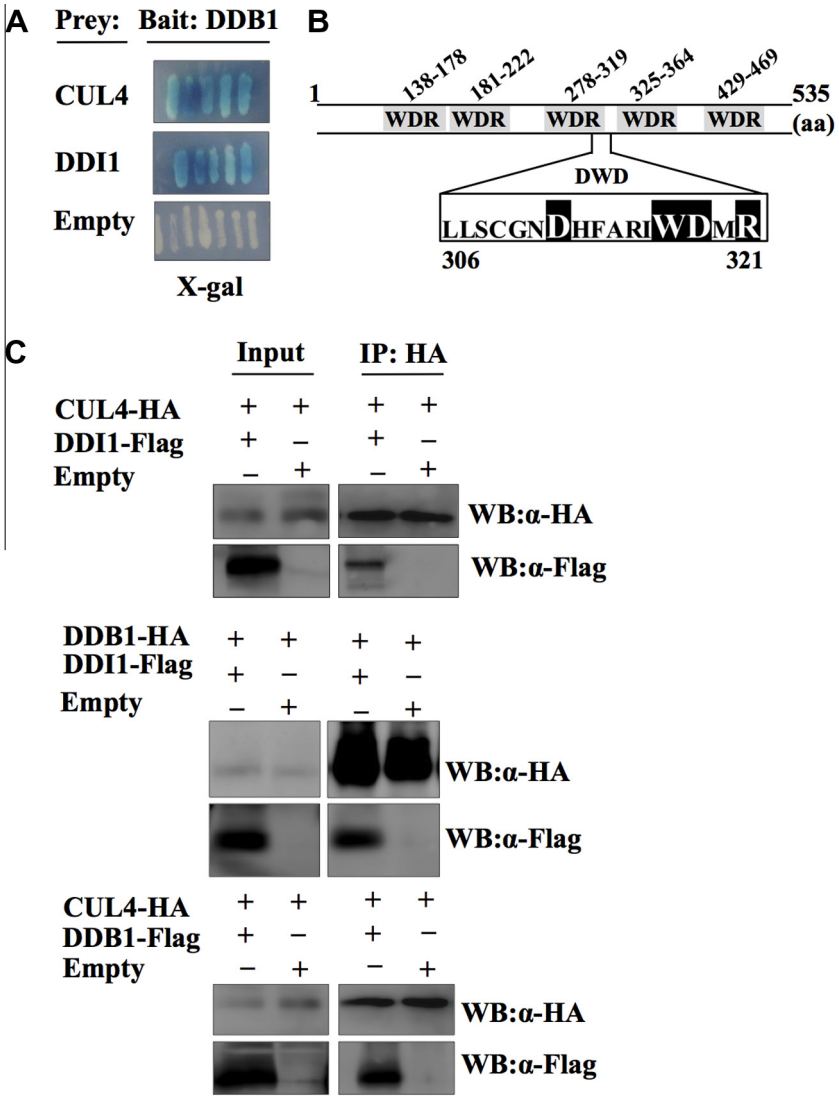

Fig. 1. DDI1 interacts with DDB1–CUL4 complex *in vivo*. (A) DDI1 interacts with DDB1 in yeast. The tomato DDB1, which did not exhibit self-activation in Y2H, was expressed as the bait and DDI1 was expressed as prey. Blue colorization of yeast colonies grown on the X-Gal-containing medium indicates the interaction between DDB1 and DDI1. Note that the interaction between DDB1 and CUL4 serves as the positive control. (B) A schematic diagram of DDI1 protein. The predicted WD repeat region is highlighted in gray and the conserved residues in DWD motif are shaded in black. (C) *In vivo* interaction of DDI1 with the DDB1–CUL4 complex determined by co-immunoprecipitation. *A. tumefaciens* GV2260 strains containing the CaMV 35S promoter-driven epitope-tagged DDI1 (DDI1-FLAG), DDB1 (DDB1-HA), or CUL4 (CUL4-HA) construct were syringe-infiltrated into *N. benthamiana* leaves in the appropriate combination at the agrobacterial inoculum of OD_{600} = 0.4. Protein extracts isolated from *N. benthamiana* leaf tissues were incubated with anti-HA antibody-conjugated agarose. The immunoprecipitated complex (IP) and the total extracts (Input) were subjected to Western blotting analysis using anti-FLAG antibody. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

empty CYFP vector was co-expressed with DDI1-NYFP or CUL4-NYFP to serve as control. As shown in Fig. 2, The YFP signal was observed in *N. benthamiana* epidermal cells where DDI1-NYFP and DDB1-CYFP, DDI1-NYFP and CUL4-CYFP, or CUL4-NYFP and DDB1-CYFP were co-expressed, whereas no YFP signal was detected in cells expressing the control constructs. In addition, the YFP signal was limited in the nucleus, which was verified by DAPI staining, indicating the interactions occur in the nucleus. Together with the co-IP results, our BiFC data demonstrate that CUL4, DDB1, and DDI1 interact with each other in the nucleus as a complex, in which DDI1 likely serves as a substrate receptor participating in the CRL4 ubiquitin ligase machinery.

3.3. The DDI1gene is induced by UV-C treatment, high salinity and osmotic stress

We next investigated the temporal and spatial expression pattern of the *DDI1* gene in tomato. Real-time PCR analysis was performed to determine the expression of the *DDI1* gene in various tissues (root, stem, leaf, flower and fruit). We found *DDI1* is highly expressed in flowers and leaves, while only basal level of *DDI1* transcript was detected in other tested tissues (Fig. 3A). Sequence analysis of the *DDI1* promoter by the PLACE WEB Signal Scan (http://www.dna.affrc.go.jp/PLACE/signalscan.html) indicated it contains several cis-regulatory motifs critical for the UV-dependent gene expression (Fig. S1B), suggesting the expression of *DDI1* may be regulated by UV treatment. To test this notion, we carried out UV sensitivity assay by exposing the two-week-old tomato seedlings to UV-C at a dose of 5 kJ/m^2. It has been demonstrated that, unlike mammals, plants possess the specific and efficient DNA repair machinery, in which direct DNA repair is mediated by photolyases that reverse DNA lesions in the presence of visible light [17]. To avoid the confounding effect of photoreactivation and to better assess the possible role of *DDI1* in the dark repair pathway, tomato seedlings were maintained in dark for recovery after exposure to UV-C light (Fig. 3B). As shown in

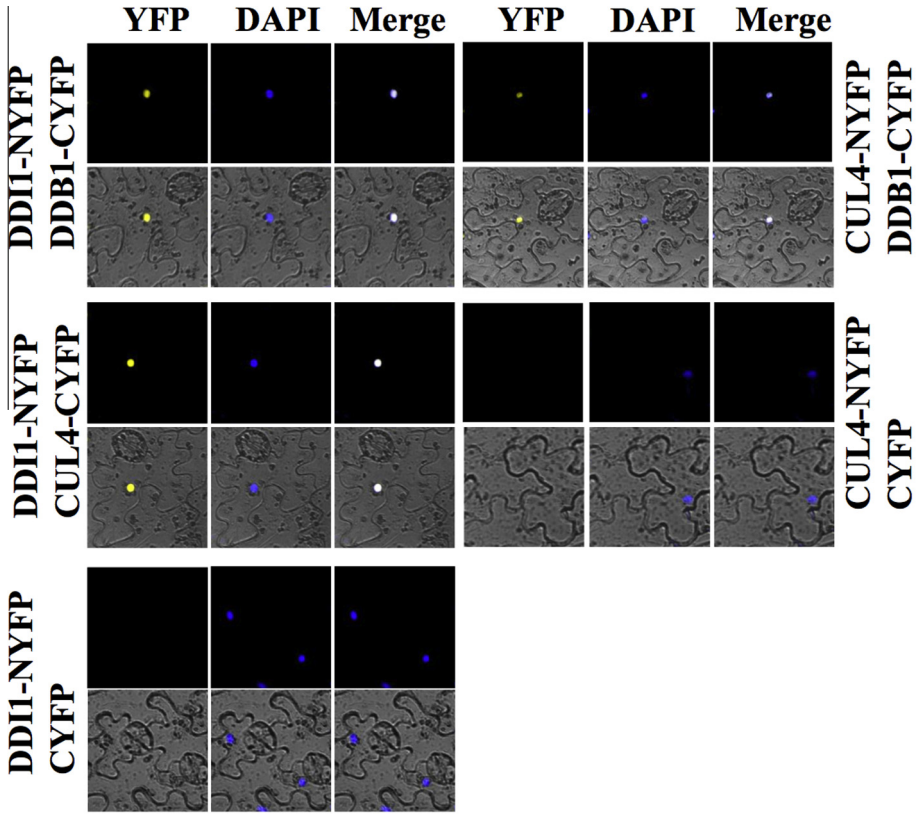

Fig. 2. DDI1 interacts with CUL4 and DDB1 in the nucleus. The appropriate CaMV 35S promoter-driven BiFC constructs were co-expressed in the *N. benthamiana* leaves via *Agrobacterium*-mediated transient expression. After DAPI staining of the leaf tissue to locate the nucleus, the epidermal cell layers were examined by confocal microscopy to capture the YFP signal due to interaction between DDB1, DDI1 and CUL4. Co-expression of CYFP with CUL4-NYFP or DDI1-NYFP served as the negative controls, in which no restored YFP signal was observed. DIC images of the same view are merged and aligned underneath the YFP signal images.

Fig. 3C, the *DDI1* transcript level rapidly increased upon UV-C treatment and reached to a peak of 3.5-fold induction at 45 min after treatment. Significantly, the *DDI1* transcripts returned to normal level after 1.5-h dark recovery.

We also examined whether *DDI1* gene can be induced by other abiotic stresses, in particular high salinity and osmotic stress. To this end, we monitored *DDI1* transcript levels in tomato seedlings in response to 100 mM NaCl or 100 mM mannitol treatment. The Real-time PCR analysis showed *DDI1* was induced upon exposure to NaCl or mannitol (Fig. 3D), suggesting *DDI1* plays a role in salt and osmotic stress response in tomato.

3.4. DDI1 is involved in response to UV-C radiation

To further determine the role of *DDI1* in DNA damage repair, which is critical for tolerance to UV radiation, transgenic tomato lines with overexpression or knockdown of *DDI1* were generated by introducing the CaMV 35S promoter-driven overexpression construct (DDI1-OE) or RNA-interference construct (DDI1-Ri) into the wild type (WT) tomato plants. Real-time PCR analysis of the T_3 homozygous transgenic plants verified significant increase or decrease of *DDI1* transcripts in *DDI1*-overexpression or -knockdown lines (DDI1-OE-1∼3, DDI1-Ri-1∼3, respectively) (Fig. S1C). All transgenic plants did not exhibit any aberrant developmental phenotype when grown in normal conditions.

The T_3 homozygous DDI1-OE and DDI1-Ri seedlings were selected to determine the role of *DDI1* in regulation of UV sensitivity by root growth inhibition assay. The reason we used roots to examine the defect in UV tolerance is that roots have the lowest photolyase activity [18]. The T_3 transgenic and WT seedlings grown vertically on the agar surface were irradiated with 20 kJ/m^2 UV-C, followed by 12-h recovery in dark for four consecutive days, to determine the root growth inhibition. As show in Fig. 4A, the root growth in all seedlings was impaired to different extents when exposed to UV-C light. In particular, the root growth is more severely inhibited in the DDI1-Ri seedlings than the WT seedlings, whereas the DDI1-OE seedlings exhibited significant tolerance to UV-C light (Fig. 4A and B), again suggesting *DDI1* plays a significant role in UV stress tolerance in tomato.

3.5. DDI1 plays a significant role in salt and osmotic stress in tomato

To evaluate the role of *DDI1* in response to abiotic stress, in particular the high salinity and osmotic stress, WT and T_3 transgenic tomato lines with overexpression or knockdown of *DDI1* were examined by growing the seedlings on 1/2 MS plates containing 100 mM NaCl or mannitol. When grown under 100 mM NaCl, the growth of WT and DDI1-Ri seedlings was arrested. In the contrast, the DDI1-OE seedlings exhibited significant tolerance to such high salinity (Fig. 4C). Similar results were observed in the osmotic stress tolerance assay. When grown on medium supplemented with 100 mM mannitol, growth was inhibited at different extents of severity in three types of seedlings, as manifested by the growth of roots was reduced in an order of severity of DDI1-OE < WT < DDI1-Ri (Fig. 4C and D). Thus, together with the observation that *DDI1* expression was induced by salt and mannitol (Fig. 3D), these results suggest that the *DDI1* acts as a positive regulator in salt and osmotic stress response.

4. Discussion

In this study, we identified a tomato DWD motif-containing protein DDI1 as a DDB1-interacting protein by Y2H screening.

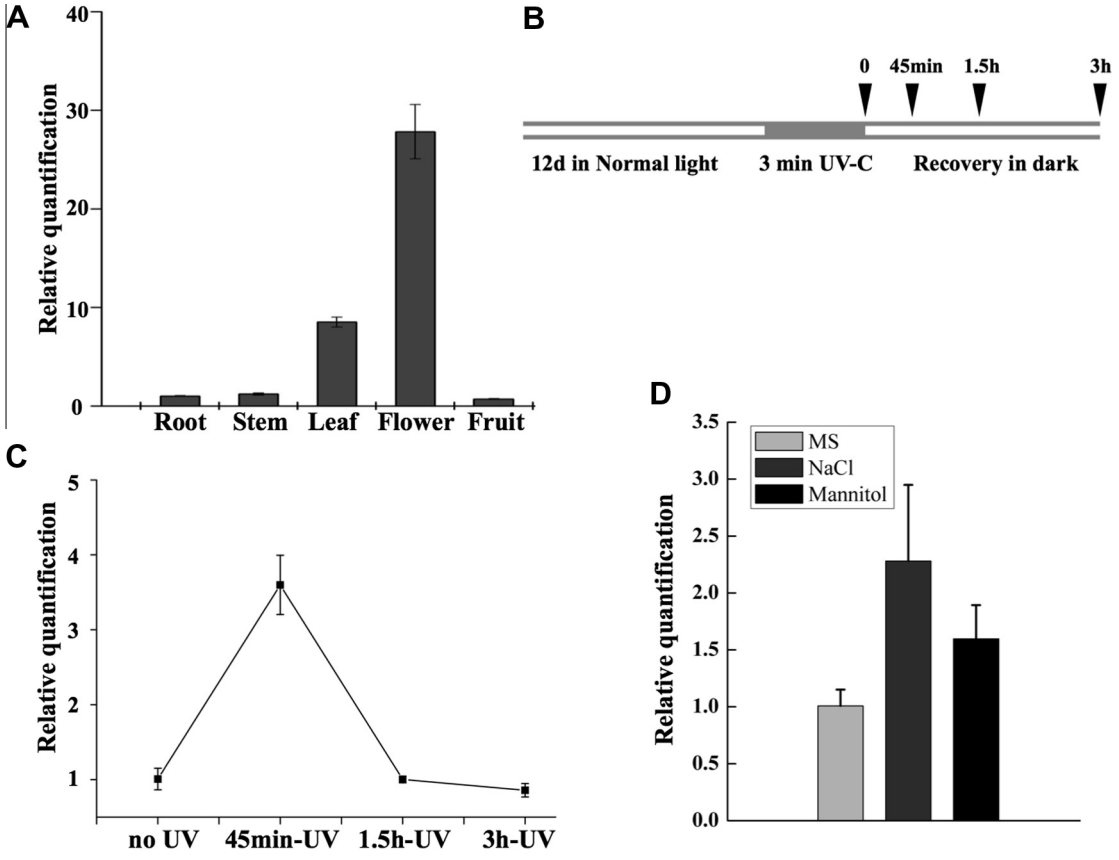

Fig. 3. Induction of *DDI1* gene by abiotic stresses. (A) The *DDI1* expression level in various tissues was quantified by quantitative RT-PCR with *UBI3* as the internal control. (B) A schematic diagram for the UV-C treatment assay. Seedlings were grown for 12 days under the standard growth condition before treated with UV-C for 3 min, followed by recovery in dark. RNAs were obtained before or after UV treatment at the indicated time points. (C) qRT-PCR analysis of the relative *DDI1* expression levels after UV treatment. (D) qRT-PCR analysis of *DDI1* expression in tomato seedlings grown on medium with or without 100 mM NaCl or 100 mM mannitol for 4 days. Values are means ± SD of three replicates.

Significantly, we demonstrated the *in vivo* association of DDB1 with DDI1, CUL4 with DDI1, and DDB1 with CUL4, which implicates construction of a CUL4-DDB1^{DDI1} ubiquitin ligase complex in tomato. Our BiFC assay results further suggest this tomato CUL4–DDB1^{DDI1} complex exists in nucleus, which resembles the case of DDB1–CUL4^{DCAF} complex in Arabidopsis [19] and suggests the CRL4 ubiquitin ligase is highly conserved in many plant species.

The *DDI1* gene was induced by multiple abiotic stresses including UV-C, salt and mannitol. It is generally thought that stress-promoted rapid induction of genes reflects a role of these genes in stress response. In addition, *DDI1*-knockdown tomato plants exhibited enhanced sensitivity to UV-C, high salinity and mannitol, whereas the *DDI1*-overexpression tomato plants displayed tolerance to these stresses. It is notable that, upon UV-C treatment, the Ri-3 line showed most severe root shortening despite there was no difference of *DDI1* repression among Ri-1/2/3 lines. In addition, the OE-3 line displayed slightly more severe root shortening in response to NaCl treatment despite more *DDI1* transcripts were detected in the OE-3 line. These results implicate a complexity of regulation in abiotic stress responses in tomato. Since the CUL4–DDB1^{DDI1} ubiquitin ligase acts as an enzyme complex, only overexpression or repression of the substrate adaptor DDI1 may not necessarily results in proportionally phenotypical alternations. Nevertheless, all these results point a similar trend of change of abiotic stress responses, which is over-expression of DDI1 results in significant tolerance, whereas repression of DDI1 leads to enhanced susceptibility, to abiotic stresses, including UV-C, high salinity and mannitol. Given the fact that DDI1 functions as the substrate bridge in the CRL4 ubiquitin ligase complex and DDI1 associates with both DDB1 and CUL4 in the nucleus, our data implicate that CUL4–DDB1^{DDI1} ligase complex is important for the nucleus-dependent regulation of signaling for DNA damage repair, salt and osmotic stress response in tomato.

Although components of several CRL4 ligases have been shown to play a role in stress response, the mechanistic basis of CRL4-dependent ubiquitination-mediated degradation of substrates is largely unknown, particularly how DCAF interacts and recruits stress-relevant CRL4 substrates. Studies by Lee and colleagues have shown that the nucleus-localized PRL1 (Pleiotropic regulatory locus 1) functions as an integrator of stress, glucose, and hormonal responses in Arabidopsis. PRL1 possesses a conserved DWD motif and interacts with CUL4–DDB1 *in vivo*, thereby acting as substrate receptor of CUL4–DDB1-based ligase. Genetic and biochemical evidences suggest PRL1, at least in part, regulates the SNF1-related protein kinase (SnRK1) AKIN10 by targeting it to the CUL4–DDB1-based ligase for degradation [20], which is consistent with the previous finding that SnRK1 kinases are central integrators of stress, carbon, and energy signaling in plants [21].

In Arabidopsis, the CUL4–DDB1-based ubiquitin ligase plays a role in repairing UV-induced DNA lesions together with a DWD-containing protein DDB2 [22]. Genetic impairing of *CUL4*, *DDB1*, or *DDB2* leads to similar hypersensitivity to UV-C [23]. Further research revealed that, in addition to DDB2, another DWD-containing protein CSA1 (Cockayne syndrome factor A) is also involved in repairing UV-damaged DNA in conjunction with CUL4 [24]. We found significantly altered responses to UV-radiation in transgenic tomato lines with overexpression or knockdown of *DDI1* gene,

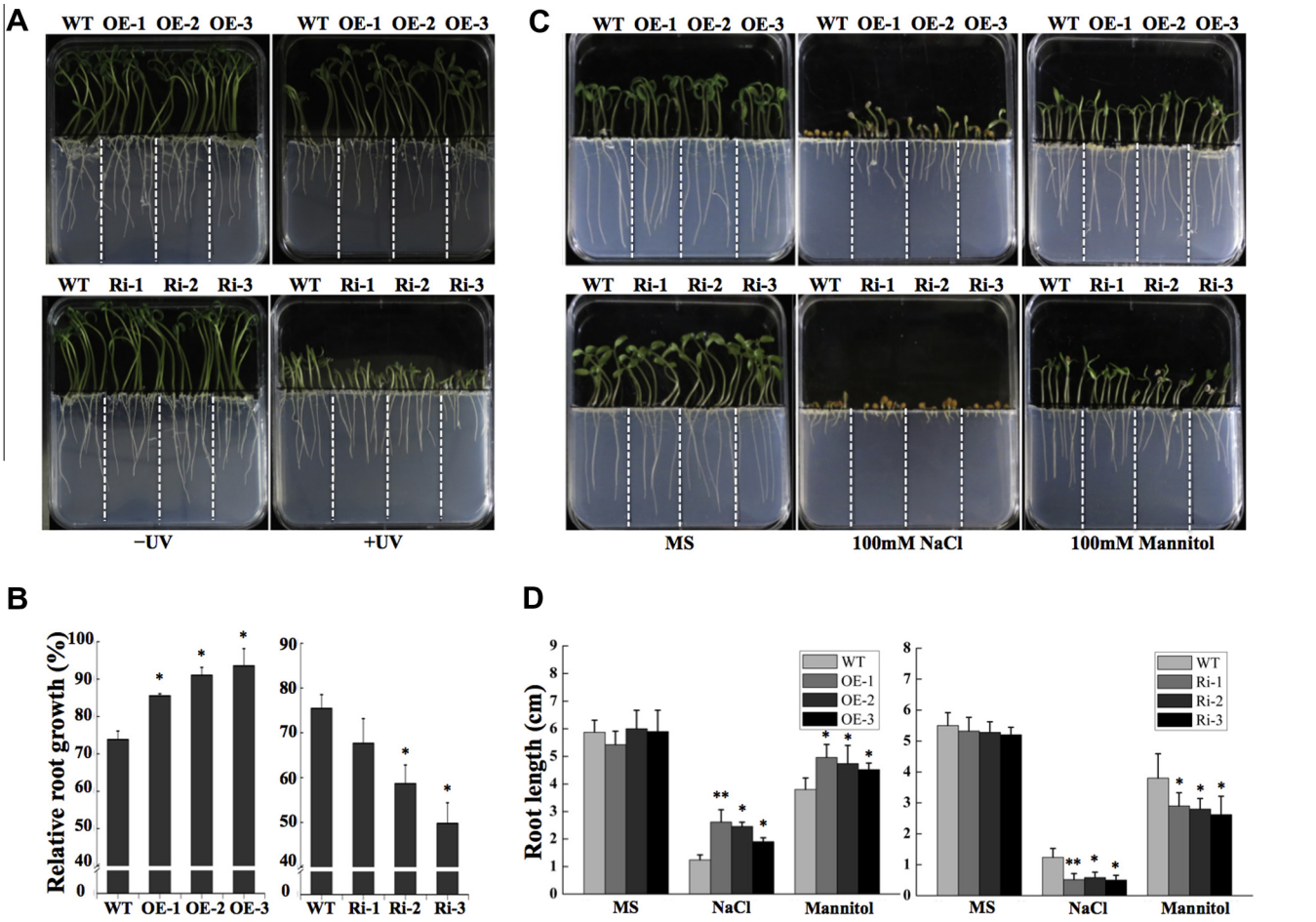

Fig. 4. Role of *DDI1* in abiotic stress responses. (A) and (B) Altered responses to UV-C treatment in the WT or transgenic tomato seedlings. Photographs were taken at 4 days after normal growth without UV (−UV, left panel) or with continuous UV-C treatment (+UV, right panel). The root length of seedlings shown in (A) was measured at 12 h after the last UV irradiation in the 4th day of treatment. (C) WT or transgenic tomato seedlings vertically grown for 4 days on 1/2 MS plates supplemented with 100 mM NaCl or 100 mM mannitol. (D) Quantification of the root length of seedlings shown in (C). Values shown in (B) and (D) are means ± SD (n = 18–21). Error bars indicate standard deviation. Significant difference was determined by Student's t-test ($P < 0.05$).

suggesting the existence in tomato of a CUL4–DDB1^{DDI1} complex involved in repairing UV-induced DNA damage. It appears that DDI1 is a tomato ortholog of DDB2 or CSA1, or it is a third DWD-containing protein residing in the CUL4–DDB1 ubiquitin ligase complex responsible for DNA repair, again presumably acting as a bridge for target proteins involved in specific signaling pathway.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.011.

References

[1] J.S. Boycer, Plant productivity and environment, Science 218 (1982) 443–448.
[2] B. Vinocur, A. Altman, Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations, Curr. Opin. Biotechnol. 16 (2005) 123–132.
[3] J. Liu, H. Li, M. Miao, X. Tang, J. Giovannoni, F. Xiao, Y. Liu, The tomato UV-damaged DNA-binding protein-1 (DDB1) is implicated in pathogenesis-related (*PR*) gene expression and resistance to *Agrobacterium tumefaciens*, Mol. Plant Pathol. 13 (2012) 123–134.
[4] J. Lee, W.T. Kim, Regulation of abiotic stress signal transduction by E3 ubiquitin ligases in arabidopsis, Mol. Cell 31 (2010) 201–208.
[5] Z. Hua, R.D. Vierstra, The cullin-RING ubiquitin-protein ligases, Annu. Rev. Plant Biol. 62 (2011) 299–334.
[6] J. Lee, P. Zhou, DCAFs, the missing link of the CUL4–DDB1 ubiquitin ligase, Mol. Cell 26 (2007) 775–780.
[7] S. Angers, T. Li, X. Yi, Molecular architecture and assembly of the DDB1–CUL4A ubiquitin ligase machinery, Nature 443 (2006) 590–593.
[8] Y.J. He, C.M. McCall, J. Hu, DDB1 functions as a linker to recruit receptor WD40 proteins to CUL4-ROC1 ubiquitin ligases, Genes Dev. 20 (2006) 2949–2954.
[9] Y. Fujiwara, C. Masutani, T. Mizukoshi, J. Kondo, F. Hanaoka, S. Iwai, Characterization of DNA recognition by the human UV-damaged DNA-binding protein, J. Biol. Chem. 274 (1999) 20027–20033.
[10] I. Jang, R. Henriques, H.S. Seo, A. Nagatani, N. Chua, Arabidopsis phytochrome interacting factor proteins promote phytochrome b polyubiquitination by COP1 E3 ligase in the nucleus, Plant Cell. 22 (2010) 2370–2383.
[11] J. Liu, X. Tang, L. Gao, Y. Gao, Y. Li, S. Huang, X. Sun, M. Miao, H. Zeng, X. Tian, X. Niu, L. Zheng, J. Giovannoni, F. Xiao, Y. Liu, A role of tomato UV-damaged DNA binding protein 1 (DDB1) in organ size control via an epigenetic manner, PLoS One 7 (2012) e42621.
[12] S. Wang, J. Liu, Y. Feng, X. Niu, J. Giovannoni, Y. Liu, Altered plastid levels and potential for improved fruit nutrient content by downregulation of the tomato DDB1-interating protein CUL4, Plant J. 55 (2008) 89–103.
[13] J.J. Fillatti, J. Kiser, R. Rose, L. Comai, Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector, Biotechnology 5 (1987) 726–730.
[14] M. Walter, C. Chaban, K. Schutze, O. Batistic, K. Weckermann, C. Näke, D. Blazevic, C. Grefen, K. Schumacher, C. Oecking, K. Harter, J. Kudla, Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation, Plant J. 40 (2004) 428–438.

[15] F. Xiao, P. He, R.B. Abramovitch, J.E. Dawson, L.K. Nicholson, J. Sheen, G.B. Martin, The N-terminal region of Pseudomonas type III effector AvrPtoB elicits Pto-dependent immunity and has two distinct virulence determinants, Plant J. 52 (2007) 595–614.
[16] A. Scrima, R. Konícková, B.K. Czyzewski, Y. Kawasaki, P.D. Jeffrey, R. Groisman, Y. Nakatani, S. Iwai, N.P. Pavletich, N.H. Thomä, Structural basis of UV DNA-damage recognition by the DDB1–DDB2 complex, Cell 135 (2008) 1213–1223.
[17] A.B. Britt, Molecular genetics of DNA repair in higher plants, Trends Plant Sci. 4 (1999) 20–25.
[18] W.M. Waterworth, Q. Jiang, C.E. West, M. Nikaido, C.M. Bray, Characterization of *Arabidopsis* photolyase enzymes and analysis of their role in protection from ultraviolet-B radiation, J. Exp. Bot. 53 (2002) 1005–1015.
[19] Y. Zhang, S. Feng, F. Chen, H. Chen, J. Wang, C. McCall, Y. Xiong, X.W. Deng, Arabidopsis DDB1–CUL4 Associated Factor1 forms a nuclear E3 ubiquitin ligase with DDB1 and CUL4 that is involved in multiple plant developmental processes, Plant Cell 20 (2008) 1437–1455.
[20] J. Lee, W. Terzaghi, G. Gusmaroli, J. Charron, H. Yoon, H. Chen, Y. He, Y. Xiong, X. Deng, Characterization of *Arabidopsis* and rice DWD proteins and their roles as substrate receptors for CUL4-RING E3 ubiquitin ligases, Plant Cell 20 (2008) 152–167.
[21] C. Polge, M. Thomas, SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control?, Trends Plant Sci 12 (2007) 20–28.
[22] J. Molinier, E. Lechner, E. Dumbliauskas, P. Genschik, Regulation and role of *Arabidopsis* CUL4-DDB1A-DDB2 in maintaining genome integrity upon UV stress, PLoS Genet. 4 (2008) e1000093.
[23] A. Koga, T. Ishibashi, S. Kimura, Y. Uchiyama, K. Sakaguchi, Characterization of T-DNA insertion mutants and RNAi silenced plants of Arabidopsis thaliana UV-damaged DNA binding protein 2 (AtUV-DDB2), Plant Mol. Biol. 61 (2006) 227–240.
[24] C. Zhang, H. Guo, J. Zhang, G. Guo, K.S. Schumaker, Y. Guo, Arabidopsis cockayne syndrome A-like proteins 1A and 1B form a complex with CULLIN4 and damage DNA binding protein 1A and regulate the response to UV irradiation, Plant Cell 22 (2010) 2353–2369.