Tomato UV-B receptor SlUVR8 mediates plant acclimation to UV-B radiation and enhances fruit chloroplast development via regulating SlGLK2

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Plants utilize energy from sunlight to perform photosynthesis in chloroplast, an organelle that could be damaged by solar UV radiation. The ultraviolet-B (UV-B) photoreceptor UVR8 is required for UV-B perception and signal transduction. However, little is known about how UVR8 influence chloroplast development under UV-B radiation. Here, we characterized tomato UVR8 gene (SlUVR8) and our results indicated that SlUVR8 facilitate plant acclimation to UV-B stress by orchestrating expression of the UVB-responsive genes (HY5 and CHS) and accumulating UV-absorptive compounds. In addition, we also discovered that SlUVR8 promotes fruit chloroplast development through enhancing accumulation of transcription factor GOLDEN2-LIKE2 (SlGLK2) which determines chloroplast and chlorophyll levels. Furthermore, UV-B radiation could increase expression of SlGLK2 and its target genes in fruits and leaves. SlUVR8 is required for UVB-induced SlGLK2 expression. Together, our work not only identified the conserved functions of SlUVR8 gene in response to UV-B stress, but also uncovered a novel role that SlUVR8 could boost chloroplast development by accumulating SlGLK2 proteins.

Sunlight provides the energy of photosynthesis in sessile plants and also plays an essential role in regulation of their entire life cycle. However, ultraviolet-B (UV-B) light, as an indispensable component of sunlight, can retard plant growth by causing DNA damage, generating reactive oxygen species, and inhibiting photosynthesis. To survive in sunlight, plants have to evolve the specific mechanisms perceiving and responding to the UV-B radiation.

Recent studies revealed that UV RESISTANCE LOCUS8 (UVR8) protein was responsible for UV-B perception and signal transduction in Arabidopsis. The UVR8 gene was first identified in a mutation screen for UV-sensitive plants and the uvr8 mutant was hypersensitive to UV-B radiation. The abolished UV acclimation of uvr8 mutant is caused by failure of UV-induced expression of defense genes involved in UV damage repairment and UV protection, such as chalcone synthase (CHS) gene which is the committing enzyme for UV-absorptive flavonoid and anthocyanin biosynthesis. Further investigations revealed that the transcription factor ELONGATED HYPOCHOTYL5 (HY5) was a fundamental factor of UV signal pathway and UVR8 regulated HY5 expression through physical association with chromatin in its promoter region. Besides, UVR8-mediated
signal facilitates HY5 and its homolog HYH binding to a T/G-box cis-acting element in the promoters of the UV-responsive genes.[2]

UVR8 forms homodimers in cytoplasm and their instant monomerization, which requires two tryptophan residues serving as the UV-B chromophore, can be activated by UV-B radiation.[3–7]. The monomerized UVR8 proteins are translocated from cytoplasm to the nucleus for fulfilling its function and signal transduction.[8–10]. UVR8 protein interacts with multifunctional E3 ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) protein, a key regulator of light signaling, which is also involved in response to UV-B.[11–13]. COP1 interacts with DAMAGED DNA BINDING PROTEIN 1 (DDB1) and CULLIN4 (CUL4) to form the super complex of CUL4-DDB1-COP1-SPA E3 ligase which inhibits the photomorphogenesis by targeting HY5 for degradation. Under UV-B radiation, COP1-SPA complex dissociates from CUL4-DDB1 and interacts with monomerized UVR8[14] to form UVR8-COP1-SPA complex which plays a positive role in stabilizing HY5 protein and its activity.[15]. It appears that UVR8 modulates plant response to UV-B through regulating key transcription factor HY5 at both transcriptional and posttranslational level.

DDB1 was first demonstrated to be involved in damaged DNA repair, since it binds to the UV-induced DNA lesions and mediates nucleotide excision repair processes.[16]. In Arabidopsis, DDB1 is associated with CUL4 and additional substrate receptor proteins including COP1 to form CUL4-RING ubiquitin ligase (CRL4) which is required for many cellular processes.[17–21]. In tomato (Solanum lycopersicum), CUL4-DDB1 complex was proved to participate in plastid development and secondary metabolism.[22–24], epigenetic regulation,[25] and stress response.[26–28]. The mutant high pigment -1 (hp1), which is caused by a point mutation in tomato DDB1 (SiddB1) gene, displays the enhanced fruit nutrient contents resulting from the increased plastid (chloroplast) numbers and sizes in fruit cells.[29]. Genetic suppression of tomato CUL4 (Sicula4) and SIDD1 genes resulted in the phenotype of the hp1 mutant.[30]. These studies suggested that CUL-DDB1 complex plays a crucial role in plastid development in tomato. A transcription factor GOLD2-LIKE (SlGKL2), which determines plastid and chlorophyll levels by enhancing photosynthesis gene expression and chloroplast development.[31–33], is a target of CUL4 ubiquitin E3 ligase.[34]. The degradation of SGLK2 protein is impaired in the hp1 mutant and Sicula4 silencing plants.[35].

Although the homologous genes of Arabidopsis UVR8 were cloned in several plant species including Arabidopsis, Populus euphratica[36], apple[37], grape berry[38], grapevine[39], radish sprouts[40], and Chlamydomonas reinhardtii[41], little is known about characterization of tomato UVR8 (SiuVR8). In this study, we cloned SiUVR8 gene and confirmed its conserved role in response to UV-B radiation. In addition, our results also revealed that SiUVR8 could mediate fruit plastid development under UV-B radiation, possibly through regulating the accumulation of transcription factor SGLK2.

Results

Cloning of tomato UVR8 gene. We BLAST in tomato (Solanum lycopersicum) genome sequence database (https://sgn.cornell.edu/organism/Solanum_lycopersicum/genome) with Arabidopsis UVR8 protein sequence and got only one positive hit (Soly05g018620), termed as SiUVR8, which suggested that the tomato genome contained only one homologous gene of UVR8. We cloned the gene by RT-PCR and the encoding protein shared 79% identities with Arabidopsis UVR8 (Supplemental Fig. 1). SiUVR8 also contains multiple repeated RCC1 domains, similar to UVR8 protein in Arabidopsis. The phylogenetic analysis indicated that SiUVR8 shared the best amino acid identity with Solanum tuberosum UVR8 (StUVR8) (Supplemental Fig. 2).

The SiUVR8 gene was expressed constitutively in all the organs of tomato plants tested. The expression levels in the leaves and flowers were apparently higher than that in other tissues, as indicated by quantitative RT-PCR (qRT-PCR) analysis (Fig. 1A). To check the sub-cellular localization of SiUVR8 protein, the gene of Green Fluorescent Protein (GFP) was fused with SiUVR8 gene and GFP-SiUVR8 construct was transformed in protoplasts of tobacco leaves. The transformed protoplasts were observed by confocal microscope. As shown in Fig. 1B, GFP-SiUVR8 proteins were localized in the nucleus and cytoplasm simultaneously, similar to the sub-cellular localization of Arabidopsis UVR8[4,15]. Western-blot analysis indicated the both GFP and GFP-SiUVR8 were really expressed in the transformed protoplasts (Fig. 1C).

SiUVR8 is required for tomato acclimation to UV-B radiation. To study the function of SiUVR8-encoded protein and to explore its physiological role in UV-B response, two different kinds of transgenic tomato lines were generated by Agrobacterium tumefaciens-mediated transformation. One was the repression lines by using RNA interference (SiUVR8Ri), expressing SiUVR8-derived inverted-repeat sequences under the direction of the CaMV 35S promoter. The other was over-expression lines of SiUVR8 (SiUVR8OE), also driven by 35S promoter. After screening the T0 generation plants with the quantitative RT-PCR (qRT-PCR) assays of SiUVR8 expression, three independent lines were chosen for SiUVR8Ri (-1, -2, and -3) and SiUVR8OE (-1, -2, and -3), respectively. The homologous plants for each line were obtained in T2 generation. As validated by qRT-PCR, and -3), respectively. The homolozygous plants for each line were obtained in T2 generation. As validated by qRT-PCR, three independent lines were chosen for SlUVR8Ri (-1, -2, and -3) and SlUVR8OE (-1, -2, and -3) gene, displays the enhanced fruit nutrient contents resulting from the increased plastid (chloroplast) numbers and sizes in fruit cells.[29]. Genetic suppression of tomato CUL4 (Sicula4) and SIDD1 genes resulted in the phenotype of the hp1 mutant.[30]. These studies suggested that CUL-DDB1 complex plays a crucial role in plastid development in tomato. A transcription factor GOLD2-LIKE (SlGKL2), which determines plastid and chlorophyll levels by enhancing photosynthesis gene expression and chloroplast development.[31–33], is a target of CUL4 ubiquitin E3 ligase.[34]. The degradation of SGLK2 protein is impaired in the hp1 mutant and Sicula4 silencing plants.[35].

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The growth and development of transgenic plants were indistinguishable from WT plants in absence of UV-B light (Fig. 2C). After additional treatment with UV-B radiation for 1 week, however, SiUVR8Ri plants showed hypersensitive phenotypes including retarded growth, curly and bleached leaves, and even premature cell death (Fig. 2C) while SiUVR8OE plants displayed much less bleached leaves than WT plants (Fig. 2C and D).

In addition, anthocyanin and chlorophyll accumulation were significantly reduced in the leaves of SiUVR8Ri plants after UV-B treatment (Fig. 2A and B). However, SiUVR8OE plants enhanced accumulation of anthocyanin and chlorophyll in leaves (Fig. 3A and B). Moreover, qRT-PCR assays indicated that expression of tomato HY5 (SiHY5), as well as CHS (SiCHS) gene, was down-regulated in SiUVR8Ri plants and up-regulated SiUVR8OE plants (Fig. 3C) compared with WT. Together, these results demonstrated that SiUVR8 was required for tomato
Figure 1. The expression pattern and sub-cellular localization of SIUVR8. (A) Constitutive expression of tomato UVR8 in various tissues. The mRNA levels for tomato UVR8 was analyzed by quantitative RT-PCR. Total RNAs were extracted from roots, stems, leaves, flowers and fruit pericarps at various developmental stages (10, and 20 days post-anthesis, breaker and ripe, respectively). The roots were harvested from plant grown indoor (22–28 °C, 16 h light and 8 h dark) and the rest tissues were harvested from plant grown in the outdoor field. Each bar represents mean value from three biological replicates from each type of tissues (n = 3). Error bars representing standard deviations (SD) are shown in each case. “*” and “**” means P < 0.05 and P < 0.001 respectively (Student’s t test). (B) Localization of GFP-UVR8 fusion protein transiently expressed in tobacco protoplasts. Upper panels, GFP-UVR8; middle panels, GFP; bottom panels, an untransformed protoplast as a negative control. Left to right: red, chlorophyll autofluorescence; green, GFP fluorescence; blue, nucleus stained with DAPI; merged, combined fluorescence from GFP, chlorophyll and DAPI. Scale bars = 25μm. (C) Western-blot analysis of transient expression samples from (B) by using anti-GFP antibody. The positions of protein ladders were marked on the left side of the gel figure.
acclimation to UV-B radiation. Over-expression of SlUVR8 could increase plant tolerance to UV-B stress by up-regulating SlHY5 expression and enhancing anthocyanin accumulation.

**SIUVR8 affects chloroplast development and nutrient quality of tomato fruits.** We grew the transgenic lines and WT plants in the outdoor fields, as described in “Materials and Methods”, with exposure to the natural sunlight. The SIUVR8Ri plants displayed normal growth without any phenotypes of hypersensitivity to UV radiation (data not shown). However, the SIUVR8Ri plants had pale green immature fruits (Fig. 4A). In contrast, SIUVR8OE plants had darker green fruits compared to WT plants (Fig. 4A). Accordingly, ripe fruits expressing SIUVR8 had a 20% increase in chlorophyll content (Fig. 4B) and a 25% increase in carotenoids (Lycopene and β-Carotene) contents (Fig. 4D), as compared with WT fruits. In addition, starch accumulation were boosted in fruits of SIUVR8OE plants but reduced in SIUVR8Ri plants (Fig. 4E). However, the anthocyanin contents in fruits were very low and indistinguishable between WT and transgenic fruits (Fig. 4C). The previous studies revealed that low accumulation of flavonoids (including anthocyanins) in tomato fruits was caused by low expression of chalcone isomerase (CHI) gene and other genes required for anthocyanin biosynthesis. Our results indicated that overexpression of SIUVR8 had no apparent effect on anthocyanin accumulation in tomato fruits, although SlHY5 expression was also up-regulated in fruits of SIUVR8OE plants (Supplemental Fig. 3).

Meanwhile, we investigated chloroplast compartment sizes in pericarp cells of mature green fruits from WT and transgenic plants. The results indicated that plastid number per cell showed no substantial difference between WT and transgenic plants (Table 1), but plastid size (plastid plan area) was altered significantly (Table 1 and Fig. 5A). Larger chloroplasts were developed in fruit pericarp cells of SIUVR8OE plants while smaller chloroplast were displayed in SIUVR8Ri plants, as observed by optical microscope (Fig. 5B). The quantitative measurements confirmed our observations (Table 1). These results revealed that SIUVR8 could influence chloroplast size but not chloroplast number in tomato fruits. In addition,
bigger starch grains were observed in chloroplasts from SlUVR8OE fruits while SlUVR8Ri fruits showed no obvious starch grains, as indicated by note “S” in Fig. 5B, in chloroplasts. This observation was consistent with our previous quantitative measurements of total starch content (Fig. 4D). It was noteworthy that we observed much less thylakoid stacks (grana) in the chloroplasts of SlUVR8Ri plants than in WT and SlUVR8OE plants (Fig. 5B), which implied that thylakoid membrane or photosynthesis apparatus in SlUVR8Ri plants might be damaged by solar UV radiation.

SlUVR8 enhances the accumulation of transcriptional factor SlGLK2 under UV-B radiation. Tomato GOLDEN2-LIKE (GLK) transcription factor SlGLK2 determines chlorophyll accumulation and chloroplast development in fruits through enhancing photosynthesis gene expression 28,33,34. Therefore, we checked the SlGLK2 protein abundance in the fruits of transgenic plants grown under sunlight in open fields by using a SlGLK2-specific antibody which doesn’t recognize SlGLK1 28. As shown in Fig. 6A, SlGLK2 proteins were over-accumulated in SlUVR8OE plants but less accumulated in SlUVR8Ri plants grown under sunlight. In addition, UV-B radiation could enhance the accumulation of SlGLK2 in fruits of WT plants grown in chamber with white light (Fig. 6B). These results indicated that SlUVR8 might affect chloroplast development through targeting transcription factor SlGLK2. Next, the qRT-PCR assays indicated that UV-B could increase the mRNA levels of SlGLK2 gene in WT fruits (Fig. 6C). It was noteworthy that UV-B radiation could increase SlGLK2 protein abundance by almost 7 times while mRNA level of SlGLK2 gene was increased by only 3 times (Fig. 6B and C). These results suggested that there possibly was a post-translational regulation in UV-B enhanced SlGLK2 accumulation. Also, SlGLK2 expression was increased in fruits of SlUVR8OE plants but decreased in fruits of SlUVR8Ri plants (Fig. 6D). We also randomly selected two SlGLK2-targeted genes (SlPsaL and SlPsbQ), which encode photosystem I subunit L and photosystem II subunit Q respectively, and checked their expression in fruits of transgenic plants (Fig. 6D). The results indicated that over-expression of SlUVR8 could increase the expression of transcriptional factor SlGLK2 and its target genes, while silencing of SlUVR8 led to suppression of SlGLK2 and its target genes in fruits, when plants were exposed to the sunlight.

Since SlGLK2 was also expressed in leaves 33,34, we also checked its expression in leaves of transgenic plants with or without UV-B treatment. The qRT-PCR assays indicated that, when plants were cultured in indoor

Figure 3. SlUVR8 mediates UV-induced gene expression and anthocyanin accumulation. (A) Leaves detached from 30-d-old 35S::SlUVR8Ri, Ailsa Craig (WT), and 35S::SlUVR8OE seedlings grown under white light (photoperiod: 16 h light and 8 h dark) with or without 3-days UV-B radiation (12 h/day). (B) Quantitative measurement of anthocyanin and chlorophyll content in the fully expanded leaves in (A). Values were shown as “means ± SD”; Error bars represent SD of ten biological replicates. “**” and “***” means P < 0.05 and P < 0.001 respectively (Student’s t test). (C) The quantitative RT-PCR analysis of UVB-induced genes SlHY5 and SlCHS of the seedlings in (A). Values were shown as “means ± SD”; Each bar represents mean value from three biological replicates from each line (n = 3). “***” means P < 0.001 (Student’s t test).
chamber with white light, SlUVR8 had no apparent effect on expression of SlGLK2 and its target genes (Fig. 7A). However, after UV-B treatment, SlGLK2 expression was significantly increased in WT and SlUVR8OE leaves but not in SlUVR8Ri leaves. In addition, the expression of the target gene SlPsaL and SlPsbQ showed no obvious difference under white light but was suppressed severely after UV-B treatment in the leaves of SlUVR8Ri plants compared with WT and SlUVR8OE plants (Fig. 7A). These results confirmed that SlUVR8 was required for UVB-enhanced expression of SlGLK2 and its target genes. We also checked the expression of SlGLK1 gene which was expressed only in leaves but not in fruits33,34. The qRT-PCR analysis indicated that UV-B radiation, as well as SlUVR8 expression, had no significant effect on SlGLK1 expression in leaves (Fig. 7B).

**Discussion**

As UV-B photoreceptor, UVR8 gene has been characterized in several plant species including Arabidopsis, *Populus euphratica*37, apple38, grape berry39, grapevine40 and radish sprouts41, and in *Chlamydomonas reinhardtii*42.

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**Table 1.** Cell and plastid characteristics of fruit pericarp cells from tomato cv. Ailsa Craig (WT), SlUVR8OE and SlUVR8Ri transgenic plants. Values are means ± SE. Cell index is calculated as total plastid area per cell plan area. Immature green fruit were studied at 25d post-anthesis.

|          | Cell plan area (μm², n = 30) | Plastid number per cell (n = 30) | Total plastid area per cell | Plastid density (per μm² cell plan area, n = 30) | Plastid plan area (μm², n > 1000) | Cell index |
|----------|-----------------------------|----------------------------------|-----------------------------|-----------------------------------------------|----------------------------------|-----------|
| WT       | 57579 ± 3707                | 817 ± 58                         | 10348 ± 811                 | 0.014                                         | 12.7 ± 0.8                       | 0.180     |
| UVR8-OE1 | 56494 ± 5344                | 859 ± 65                         | 13590 ± 799                 | 0.015                                         | 15.8 ± 1.1                       | 0.241     |
| UVR8-OE2 | 58665 ± 4740                | 928 ± 67                         | 13668 ± 785                 | 0.016                                         | 14.8 ± 1.3                       | 0.234     |
| UVR8-OE3 | 55966 ± 4265                | 846 ± 50                         | 12390 ± 829                 | 0.015                                         | 14.7 ± 0.8                       | 0.222     |
| UVR8-Ri1 | 54370 ± 4505                | 942 ± 75                         | 8368 ± 690                  | 0.017                                         | 8.9 ± 0.6                        | 0.154     |
| UVR8-Ri2 | 56956 ± 5406                | 885 ± 83                         | 7459 ± 789                  | 0.016                                         | 8.4 ± 0.9                        | 0.131     |
| UVR8-Ri3 | 59592 ± 4893                | 872 ± 68                         | 8782 ± 671                  | 0.015                                         | 10.1 ± 0.9                       | 0.148     |
Here, we isolated tomato SlUVR8 gene and identified its functions in plant’s adaptation to UV-B radiation. Transgenic evidences suggested overexpression of SlUVR8 increased plant tolerance whereas silencing SlUVR8 gene led to the hypersensitivity to UV-B stress (Fig. 2). In accordance with the previous studies in Arabidopsis, UV-B-induced HY5 and CHS expression, as well as anthocyanin accumulation, were impaired in SlUVR8Ri plants but were enhanced in SlUVR8OE plants compared to WT (Fig. 3). These results demonstrated that SlUVR8 played an essential role of orchestrating expression of key UV-responsive genes (such as SlHY5 and SlCHS) and accumulating UV-absorptive compounds with sunscreen functions of protecting plants from UV damage.

Sunlight provides energy for photosynthesis of chloroplast. However, solar UV radiation could damage photosynthesis and chloroplasts as well45. Numerous studies revealed that photosynthetic apparatus was susceptible to damage caused by UV-B46–50. The adverse effects from enhanced UV-B radiation on chloroplasts include but not limited to loss in integrity of the thylakoid membranes51, impaired activity of photosystem II (PSII)52,53, enhanced protein degradation50, inhibited carbon fixation54 and reduced content of starch and chlorophyll53,55. Therefore, plants must employ some strategies or mechanisms, some of which remain to be elucidated yet, to respond to these problems and to maintain photosynthesis and chloroplast functions. A few studies suggested that UVR8 is required for maintaining the photosynthesis efficiency under enhanced UV-B radiation in Arabidopsis49,50. The underlying mechanism, however, has not been revealed yet. In our work, we proved that SlUVR8 promote chloroplast development in tomato fruits when plants were grown in outdoor fields and exposed to natural sunlight (Fig. 4). Silencing SlUVR8 caused some developmental issues in fruits including reduced size of chloroplasts and decreased contents of chlorophyll and starch, while overexpression of SlUVR8 led to larger chloroplasts and increased contents of chlorophyll and starch (Fig. 5). The previous studies revealed that low accumulation of flavonoids (including anthocyanins) in tomato fruits resulted from low expression of chalcone isomerase (CHI) gene43 and other genes44 required for anthocyanin biosynthesis. Our results also showed that the anthocyanin contents in fruits were very low and indistinguishable between WT and transgenic fruits (Fig. 4C). It is conceivable that deficiency of anthocyanins was not responsible for chloroplast abnormality in fruit cells from SlUVR8Ri plants. In our case, SlUVR8 might play another role in affecting chloroplast development of tomato fruits rather than its role in accumulating UV-absorbing compounds. Therefore, we speculated that SlUVR8 could enhance chloroplast development, facilitate the recycling of damaged chloroplasts, and thereby contribute to maintain photosynthesis under UV-B stress.

In tomato, silencing SIHY5 also caused abnormalities in both organization and abundance of thylakoids26. However, no reports yet showed that overexpression of SIHY5 could lead to larger chloroplasts and high pigment of fruits in tomato. Therefore, we surmise that other genes are also involved in the UVR8-mediated chloroplast development. Our results disclosed that UV-B could enhance accumulation of SIGLK2, which was a key regulator of fruit chloroplast development33,34, and the UVB-enhanced SIGLK2 accumulation was dependent on SlUVR8 gene. Several studies demonstrated that SIGLK2 influenced chloroplast development and chlorophyll level by increasing fruit photosynthesis and chloroplast gene expression33,34. The accumulation of carbohydrates

Figure 5. SlUVR8 affects chloroplast development in fruit cells. (A) Isolated pericarp cells from immature green tomato fruits (25 DPA) of SlUVR8OE, wild-type (WT) and SlUVR8Ri plants grown in the outdoor field. Bars = 50 μm. (B) Transmission electron microscopy images of immature green fruit (25 DPA) chloroplasts from SlUVR8OE, wild-type (WT) and SlUVR8Ri plants grown in the outdoor field. Bars = 1 μm. Red “C” indicated Chloroplasts, Red “S” indicated Starch in chloroplast.
and carotenoids were elevated in fruits of SlGLK2 overexpression plants. Our results showed that more chlorophyll, starch, and carotenoids were accumulated in fruits of SlUVR8OE plants (Fig. 4), similar to the phenotypes of fruits expressing SlGLK2. In addition, our results indicated that SlGLK2 proteins were over-accumulated in SlUVR8OE fruits but less-accumulated in SlUVR8Ri fruits under sunlight (Fig. 6). Further studies revealed that expression of SlGLK2 and its randomly-selected target genes were elevated in SlUVR8OE plants under UV-B radiation (Figs 6 and 7). Silencing SlUVR8 abolish UVB-induced expression of SlGLK2 and its target gene (Figs 6 and 7). These results suggested that SlUVR8 gene is required for UVB-enhanced SlGLK2 expression. Therefore, we proposed an unknown pathway that photoreceptor UVR8 promote chloroplast development in tomato under UV-B stress by enhancing accumulation of SlGLK2. Of course, we can’t exclude the role of SlHY5 in UV-mediated chloroplast development26. A study about the root greening in Arabidopsis56 showed that GLK2 could induce the accumulation of HY5 and combination of GLK2 and HY5 mediated the coordinated expression of many key genes of chloroplast biogenesis. This study might shed some light on the mechanism that both SlHY5 and SlGLK2 are possibly required for UVB-mediated chloroplast development. The previous study showed that expression of GLK2 was also responsive to photooxidative chloroplast damage and retrograde signals from plastid in Arabidopsis57, indicating that GLK2 might play an additional role in adjusting photosynthetic capacity in changing environmental conditions. It will be interesting to check whether silencing SlGLK2 alone could affect UVB-mediated chloroplast development and plant response to UV-B stress in further investigations.

**Figure 6.** SIUVR8 enhances SIGLK2 accumulation under UV radiation. (A) Immunoblot analysis of SIGLK2 levels among Ailsa Craig (WT), 35S::SIUVR8Ri and 35S::SIUVR8OE transgenic lines. The proteins extracted from immature fruits (25 DPA) from field-grown plants was resolved by SDS-PAGE, then probed with anti-SIGLK2 antibody and anti-β-actin antibodies. (B) Immunoblot analysis of SIGLK2 levels between Micro Tom (WT) plant grown in white light and plant grown in white light with added 3 days of UV-B irradiation. The proteins extracted from immature fruits (15 DPA) was resolved by SDS-PAGE, then probed with anti-SIGLK2 antibody and anti-β-actin antibodies. Blots were quantitatively analyzed by software ImageJ 1.46s. Values were shown as "means ± SD" from results of three independent experiments. (C) Quantitative RT-PCR analysis of SIGLK2 mRNA levels in immature fruits (15 DPA) from Micro Tom (WT) with different UV-B irradiation time (0 d, 1 d and 3 d respectively). Values were shown as "means ± SD"; Error bars represent SD of 3 biological replicates. “*” and “**” means P < 0.05 and P < 0.001 respectively (Student’s t test). (D) Quantitative RT-PCR analysis of chloroplast development related genes SIGLK2, SLPsaL and SLPsbQ in Ailsa Craig (WT), 35S::SIUVR8Ri and 35S::SIUVR8OE transgenic lines. Total RNAs were extracted from fruit pericarps of immature fruits (25 DPA) from field-grown plants. Values were shown as "means ± SD"; Error bars represent SD of 3 biological replicates. “*” and “**” means P < 0.05 and P < 0.001 respectively (Student’s t test).
In Arabidopsis, transcription factor HY5 is regulated by UVR8 at both transcriptional and post-translational levels\(^8,17,21\). Here, our immunoblot analysis showed that UV-B radiation could increase SlGLK2 protein abundance by about 7 times while mRNA level of SlGLK2 gene was increased by only 3 times, as compared with untreated condition (Fig. 6B and C). These results suggested that a post-translational modification, which requires further studies, might exist in this case. In Arabidopsis, UVR8 were associated with chromatin in the region of the HY5 promoter and regulate HY5 transcription\(^8,58,59\). However, a recent study\(^60\) indicated that UVR8 didn't bind to chromatin in vivo or in vitro, possibly because of lacking critical histone- and DNA-interaction residues. Therefore, the exact mechanism of UVR8 regulating transcription remains controversial and elusive. In our study, there is

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**Figure 7. SlUVR8 is required for UV-induced SlGLK2 expression.** (A) Quantitative RT-PCR analysis of chloroplast development related genes SlGLK2 and its target genes (SlPsaL and SlPsbQ) in Ailsa Craig (WT), 35S:SlUVR8OE and 35S:SlUVR8Ri transgenic lines with or without 3-d UV-B irradiation. Total RNAs were extracted from totally expanded leaves of 30-d old seedlings grown in white light. Values were shown as “means ± SD”; Error bars represent SD of 3 biological replicates. “*” and “**” means P < 0.05 and P < 0.001 respectively (Student's t test). (B) Quantitative RT-PCR analysis of SlGLK1 gene in the same samples as described in (A). “***” means P < 0.001 (Student's t test).
Materials and Methods

Plant Materials and Growth Conditions. Tomato (Solanum lycopersicum) cv Ailsa Craig (LA2838A) were acquired from the Tomato Genetics Resource Center (UC Davis, USA). Tomato plants were grown under artificial conditions (22–28°C, 16 h light and 8 h dark) for several weeks and then transplanted into the outdoor field (located at the suburbs of Chengdu, China) for 4 months (from May to August). During this time, the maximum value of UV-B irradiance at noon (1 p.m.) is 9.5 μmol/m²/s and the minimum value is 4.3 μmol/m²/s, as measured by TBQ-ZW-2 UV-B (280–320 nm) light meter (Shanghai Minyin Electrics co., Ltd, China). For photosynthetic UV-B treatment, seedlings were grown under white light (photoperiod: 16 h light and 8 h dark) supplemented with Philips TL20W/01RS narrowband UV-B tubes (23 μmol/m²/s) for 12 hours per day.

Generation of transgenic tomato. The Agrobacterium EHA105 strains containing vectors pBI121-SlUVR8 or pBI121-SlUVR8-RNAi were used to transform tomato cv Ailsa Craig by using Agrobacterium tumefaciens-mediated transformation67. Primary transformants (T0) and their offspring were cultivated under artificial conditions for 4 weeks and then transplanted into the outdoor field (located at the suburbs of Chengdu, China). The T-DNA insertions of transgenic plants were identified by PCR using NPTII-specific primers and the expression of SlUVR8 gene was validated by real-time RT-PCR using SlUVR8-specific primers. Three independent transgenic lines were used for phenotypic and molecular analysis.

Quantitative RT-PCR analysis. Total RNAs were isolated using Trizol reagent (Invitrogen). The genomic DNAs in total RNAs were erased and first-strand cDNAs were synthesized with oligo-dT(18) primers by using cDNA Synthesis kit, according to the manufacturer's protocol (TransGen, Beijing, China). Quantitative PCR was conducted on the ABI StepOnePlus PCR System by using the TransStart Green qPCR SuperMix (TransGen). Gene expression was normalized by the expression of UBI3 gene. All the primers used are listed in Table S1.

Subcellular localization analysis. The coding sequence of the SlUVR8 gene was cloned into the expression vector pART27-mcs:GFP and generated the pART27-GFP-SlUVR8 construct. Plasmid vectors pART27-GFP-SlUVR8 and pART27-GFP were introduced into protoplasts of tobacco (Nicotiana benthamiana) according to the methods described previously62,63. After incubation at 25°C for 12–16 h, protoplasts were observed under a Leica TCS SPII confocal microscope using 488 and 633 nm excitation wavelengths and three-channel measurement of emission: 435 nm (blue/DAPI), 522 nm (green/GFP) and 680 nm (red/chlorophyll).

Protein extraction and immunoblot analysis. Tissue was ground in liquid N₂ and was treated in boil water for 5 min with SDS-PAGE loading buffer to extract total proteins. Immunoblot analysis was performed as described previously64. The SIGL2-specific antibodies recognized the SIGL2 N-terminal unique sequence (synthetic peptide SSSLSYKNERENYD, 5–18), but not SIGL1 protein, as described in the previous study28. The secondary antibody Goat-anti-rabbit IgG conjugated to horseradish peroxidase (HRP) were purchased from Hangzhou HuaAn Biotechnology Co., Ltd.

Plastid Analysis and Transmission Electron Microscopy. The fruit tissues used for plastid analysis were harvested from plants grown in outside open fields and processed as previously reported65. In brief, the fruit pericarp tissues were immersed in 3.5% glutaraldehyde for 1 hour at least, then incubated in 0.1 M Na₂-EDTA for 30 min at 60°C. The sliced and smashed samples were imaged with a Leica DM2500 microscope, and cell plan area was measured by Image-Pro Plus. For transmission electron microscopy, outer pericarp tissues were prefixed in 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C. Tissues were post fixed in 1% osmium tetroxide, dehydrated in series acetone, infiltrated in Epox 812 for a longer, and embedded. The semithin sections were stained with methylene blue and ultrathin sections were cut with diamond knife, stained with uranyl acetate and lead citrate. Sections were examined with a Transmission Electron Microscope (TEM; HITACHI, H-600IV, Japan).

Anthocyanin, chlorophyll and carotenoid assays. Anthocyanin was assayed according to the procedures described previously66. Chlorophyll and green fruits’ total carotenoid was extracted into 80% acetone, and their content was calculated by using Lichtenthaler’s formulas, in which chlorophyll a equals 12.21A₆₆₃-2.81A₆₄₅, chlorophyll b equals 20.13A₆₄₅-5.03A₆₆₃, total carotenoids equals (1000 A₅₄₀-3.27Cₐ-104Cb)/229. The red fruits’ carotenoid contents were measured by HPLC as previously described29, with analytical reagent lycopene and β-carotene as standards.

Starch Analysis. Starch quantification was determined using a starch assay kit (STA20; Sigma-Aldrich) following the manufacturer’s protocol34.
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Author Contributions

H.L., S.W. and Y.L. designed the experiments. H.L., Y.L., H.D., X.S., A.W., Y.G., N.Z., L.W. and S.Y. conducted the experiments. H.L. and S.W. analyzed the data. S.W., Y.L. and H.L. wrote the manuscript.

Additional Information

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