1 **Functional differentiation in UV-B-induced DNA damage and growth inhibition between highland and**
2 **lowland ecotypes of two *Arabidopsis* species¹**

3
4 Qing-Wei Wang^{1*}, Soichiro Nagano², Hiroshi Ozaki¹, Shin-Ichi Morinaga^{3,4}, Jun Hidema¹, Kouki Hikosaka^{1,4}

5
6 ¹ Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan

7 ² Kazusa DNA Research Institute, Kisarazu, Chiba 292-0818, Japan

8 ³ College of Bioresource Sciences, Nihon University, Kanagawa 252-0880, Japan

9 ⁴ CREST, Japan Science and Technology Agency (JST), Japan

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11 * Corresponding author at: Graduate School of Life Sciences, Tohoku University, Aramaki Aoba 6-3, Aoba-ku,
12 Sendai 980-8578, Japan. Tel: +81 022 7957732

13 *E-mail address:* wangqw08@gmail.com (Q.-W. Wang)

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Abbreviations: UV-B, ultraviolet-B; UV-A, ultraviolet-A; UVB_{BE}, biologically effective UV-B radiation; CPD, cyclobutane pyrimidine dimer; UACs, UV-absorbing compounds; GR, growth rate; RGR, relative growth rate; TDM, total dry mass; AGDM, aboveground dry mass; BGDM, belowground dry mass; Fv/Fm, the maximal quantum yield of PSII; GA3, gibberellic acid-3.

Abstract:

Ultraviolet-B (UV-B; 280–315 nm) radiation has been shown to be more stressful for plants at higher elevations. Species inhabiting different origins may have evolutionarily altered UV tolerance to match their phenotypes to local conditions. However, little is known about UV adaptation patterns between high- and lowland. Here, we evaluated UV damage to DNA and growth in ecotypes of two species of *Arabidopsis* from different elevations, four ecotypes of *A. thaliana* and three ecotypes of *A. halleri* subsp. *gemmifera* under supplemental UV-B. Harvests were done before UV-B treatment and at early and late stages after the exposure to enhanced UV-B irradiation. The accumulation level of cyclobutane pyrimidine dimer (CPD) was determined as a measure of UV damage to DNA. At the early stage, lowland ecotypes of two species exhibited a higher CPD level and greater inhibition in biomass production, indicating that lowland ecotypes were more sensitive to increased UV-B than highland ecotypes. In contrast, at the later stage, CPD level and growth inhibition became similar or even lower in lowland ecotypes. These results suggest that the response to UV stress was constitutive in highland ecotypes but more inducible in lowland ecotypes. The relative growth rate was negatively related to CPD level. These ecotypic differentiations were common to two *Arabidopsis* species, suggesting that local adaptation occurred in parallel under a constraint of tradeoff between growth and UV tolerance.

Key words: cyclobutane pyrimidine dimer (CPD); relative growth rate (RGR); ecotypic differentiation; UV tolerance; constitutive and inducible defense; phenotypic plasticity

1. Introduction

Ultraviolet-B (UV-B; 280–315 nm) radiation is a minor fraction of the solar spectrum acting as a positive regulator of plant defense systems that stimulates specific signaling pathways (Heijde and Ulm, 2012; Hideg et al., 2013; Zavala et al., 2015). High doses of UV-B radiation can be harmful for organisms. At the molecular level, enhanced UV-B radiation damages plant DNA and proteins, impedes replication and transcription, and causes lethality or mutations (Britt, 1996; Jordan, 2002; Ries et al., 2000). At the whole plant level, it can inhibit photosynthesis, alter morphological development, and cause general growth reduction (Kataria and Guruprasad, 2012; Teramura and Sullivan, 1994). Such effects may potentially induce variations in species competition and ecosystem function (Caldwell et al., 2007; Hubner and Ziegler, 1998).

Plants have several tolerance mechanisms for preventing damage from UV-B radiation. UV-B protection, one of the best-documented mechanisms, is the accumulation of UV-absorbing compounds (UACs) in the vacuoles of epidermal cells as a screen against UV penetration (Neugart et al., 2012; Ruhland et al., 2013). Another major mechanism is photoreactivation (photorepair), which is mediated by a photolyase enzyme, using blue/ultraviolet-A (UV-A; 315–400 nm) light as the energy source to repair UV-induced DNA damage (Britt, 1996). Higher photorepair activity has been observed in Poaceae species (Hidema et al., 2007; Wang et al., 2016). It has been reported that UV-B protection is more efficient than photorepair across *Arabidopsis* ecotypes and soybean lines (Kalbina and Strid, 2006; Sullivan et al., 2010). In addition, morphological changes, such as in leaf hairs and leaf thickness, may also contribute to minimizing UV penetration (Albert et al., 2011; Zhang et al., 2014). Nevertheless, the intensity of UV-B radiation in the field varies dramatically with temporal and spatial dynamics. Plants may have specific protective strategies with respect to different background of ambient UV levels, because of their differences in management of cost and benefit in stress tolerance (Agrawal et al., 2010; Caldwell et al., 2007).

In stress biology, stress-protection responses are generally considered to be of two types: constitutive and induced. Each represents an optimal strategy in the allocation of resources between tolerance and plant growth in a given habitat (Fine et al., 2004). For example, in plant–herbivore relationships, selection will favor a constitutive defense when herbivory is constant (Ruiz et al., 2002) but induced defense when herbivory varies over time, because inducible responses are a cost-saving strategy (van Dam et al., 2000).

Intense UV-B radiation is a typical characteristic of subalpine and alpine environments, because of the shorter path length of solar radiation through the atmosphere at high elevation (Bais et al., 2015; Caldwell et al., 1980). However, UV-B is thought to exert minimal influence on plant survival and growth at higher elevations (Caldwell, 1968; Shi et al., 2004; Sullivan et al., 1992). Numerous studies have found that traits related to growth such as photosynthesis, growth rate, and biomass of highland plants are less inhibited by UV supplementation than those of lowland plants under the same (e.g., common garden) conditions (Pukacki and Modrzyński, 1998; Rau and Hofmann, 1996; Ren et al., 2006). Moreover, many plants growing high-elevation sites often exhibit high constitutive UV protection (Barnes et al., 2015; Barnes et al., 2016a; Barnes et al., 2016b). For example, epidermis UV transmittance of plants originating from high elevation was generally lower and less variable than those of plants from low UV-B environments (Barnes et al., 2000; Nybakken et al., 2004; Robberecht et al., 1980; Ziska et al., 1992). In contrast, Jansen et al. (2010) studied variation in constitutive and inducible UV-B tolerance in 224 *Arabidopsis thaliana* accessions and found no significant correlation between the degree of protection of photosystem II (PSII) and habitat elevation of the accessions. It is still unclear whether UV tolerance and its inducibility differ between lowland and highland plants.

Such differences in the UV tolerance among studies may be also derived from the assessment methods. Although the maximal quantum yield of PSII (F_v/F_m) is a convenient indicator of plant stress tolerance, it is also sensitive to other environmental factors such as high light and low temperature (Havaux, 1992; Kato et al., 2003; Tsonev and Hikosaka, 2003). Plant growth and morphology under stressful conditions may reflect mixtures of constitutive and inducible protection responses (Boelen et al., 2006; Ziska et al., 1992).

DNA damage is a useful parameter to detect UV tolerance, because DNA is the primary target of UV-B (Britt, 1996; Giordano et al., 2003; Hidema et al., 2007; Wang et al. 2016). UV-B can directly alter DNA structure and specifically induce photoproducts between adjacent pyrimidines on the same strand. Cyclobutane pyrimidine dimer (CPD), as the largest component of photoproducts, has frequently been used as a direct indicator of UV tolerance in plants and has become recognized as the principal cause of UV-B induced growth inhibition (Hidema et al., 2007; Rousseaux et al., 1999). In our previous study, we showed that *in situ* CPD content in leaves of perennial plants was greater at higher than at lower elevation, indicating that UV stress is more severe at higher elevation (Wang et al., 2014). However, few studies have compared DNA damage between high- and lowland plants grown under the same conditions. Exceptionally, Casati and coworkers have

1 studied UV response in maize lines adapted to high- and lowland cultivation and found that highland maize
2 showed a more adaptive response to enhanced UV-B; for example, highland maize showed greater
3 accumulation of UACs and higher expression of genes associated with DNA repair (Casati et al., 2006; Casati
4 and Walbot, 2005; Casati et al., 2005; Lario et al., 2011; Rius et al., 2012). However, they reported that CPD
5 levels did not differ between the lines (Casati et al., 2006), and it is not clear how the plant growth rate
6 responded to UV enhancement.

7 In the present study, we examined the adaptation pattern to UV in ecotypes of two *Arabidopsis* species
8 that originate at high and low elevations. Use of ecotypes may be effective for elucidating adaptive changes,
9 compared with use of different species, because ecotypes have adapted to the respective environment more
10 recently, relative to the occurring time of interspecific differentiations. *Arabidopsis* as a model plant species is
11 well suited to this type of investigation, owing to the availability of natural accessions from different
12 elevational origins, as well as known results of ecological, physiological and genetic studies. As plant
13 materials, we selected *Arabidopsis* ecotypes from 4-degree latitude zones, including four ecotypes of *A.*
14 *thaliana* (two highland ecotypes: DZI and Sha, and two lowland ecotypes: Tsu-1 and Col-0), and three
15 ecotypes of *A. halleri* subsp. *gemmifera* (300, 760, and 1300 m above sea level (a.s.l.) in Mt. Ibuki, Japan). *A.*
16 *halleri* subsp. *gemmifera* is one of the species most closely related to *A. thaliana*. We harvested samples at
17 early and late stages after the exposure to enhanced UV-B irradiation, determined CPD level as a measure of
18 DNA damage and the concentration of UACs as a measure of UV protection, and then compared UV damage
19 to DNA and growth between elevations. We hypothesized that (1) growth suppression and DNA damage are
20 smaller in high- than in lowland ecotypes, (2) UV tolerance was constitutive in highland ecotypes but more
21 inducible in lowland ecotypes.

2. Materials and Methods

2.1. Plant materials

Arabidopsis has been used as a model system for analysis of molecular mechanisms in higher plants. Because of their adaptation to specific elevations, *Arabidopsis* ecotypes are useful for studying the interaction of plants with their natural UV-B radiation environment. *A.thaliana* predominantly displays a “winter annual” life history, germinating in autumn, overwintering as rosettes, and then growing, flowering and setting seeds in the spring (Griffith et al., 2004). In this study, four ecotypes of *A. thaliana* were obtained from a seed bank: the Arabidopsis Information Resource (TAIR, <https://www.arabidopsis.org>). Two lowland ecotypes, Col-0 and Tsu-1, grow naturally at below 100 m a.s.l., and two highland ecotypes, DZI and Sha at 3400 m. The present study considered that Poland was the origin of Col-0 (Koornneef and Meinke, 2010).

Although perennial

Arabidopsis halleri subsp. *gemmaefera* is a self-incompatible, clonal, and perennial herb widely distributed in Asia (Al-Shehbaz and O’Kane Jr, 2002) and is one of the species most closely related to *A. thaliana*. *A.halleri* typically flowers in the spring and sets seeds in June, simultaneously produces new rosettes after flowering, grows vegetatively for the rest of a year, and then overwinters as rosettes (Kawagoe and Kudoh, 2010). The morphological traits of this model plant are well known to vary with elevation. On Mt. Ibuki (35° 25’ 04” N, 136° 24’ 22” E, 1377 m a.s.l. at the summit) in central Japan, the lowland ecotype grows at 300–1050 m with no trichomes, the intermediate ecotype at 740–1050 m with some trichomes, and the highland ecotype at 1050–1300 with dense trichomes. However, recent studies have found that lowland and highland ecotypes share a similar genomic structure, but their adaptive divergence occurred at the microgeographic scale (Ikeda et al., 2010; Kubota et al., 2015). Seeds were collected along elevational gradients on Mt. Ibuki at 300, 760, and 1300 m for the lowland ecotype, the intermediate ecotype, and the highland ecotype, respectively, on July 3, 2012 (Table 1).

2.2. Historical climate data collection

We collected local climatic variables including temperature, precipitation, cloud cover, frost frequency, and wind speed. Based on the ecotype location supplied by TAIR, data for *A. thaliana* were obtained from the Climate Research Unit (CRU), University of East Anglia (<https://crudata.uea.ac.uk/cru/data/hrg/>), CRU CL 1.0

climate dataset, averaged over 1961–1990. Solar radiation on a horizontal surface at geographical coordinates of *Arabidopsis* ecotypes was obtained from Surface meteorology and Solar Energy (SSE) of National Aeronautics and Space Administration (NASA), which provides time-series of daily irradiation from January 1st 1983 to December 31th 2005 for cells of 1 degree by 1 degree (<https://eosweb.larc.nasa.gov/cgi-bin/sse/homer.cgi?email=skip@larc.nasa.gov>). For *A. halleri subsp. gemmifera*, mean air temperature was estimated at points near meteorological stations of the Japan Meteorological Agency: Nagahama station (low elevation) and Ibuki station (high elevation), averaged over 1980–2000 assuming a lapse rate of 0.6 °C/100 m. Solar radiation on a horizontal surface at 760 and 1300 m a.s.l. were estimated based on the data of that at 300 m a.s.l., assuming a lapse rate of 9 % /1000 m (Wang et al., 2014).

2.3. Growth conditions

Seeds of the two species were germinated separately in growth cabinets (NK System Biotron), because of their different germination processes and growth rates. Therefore, the treatment of *A. halleri subsp. gemmifera* was performed 27 days earlier than that of *A. thaliana*. For *A. halleri subsp. gemmifera*, seeds were vernalized with gibberellic acid-3 (GA3) solution and then sown on a double paper filter moistened with 100-ppm GA3 solution in a Petri dish at 22/22°C with a 16/8 h (fluorescent) light/dark regime. Germinated seeds were washed with purified water and transferred into the same set of petri dishes, soaked in water, and incubated under low (fluorescent) light at 22 °C. After second-leaf expansion, seedlings were transplanted into 87.5 cm³ pots with a fertilized soil:perlite:vermiculite mixture at one plant per pot and moved to a growth house under UV-B lamps for treatment. For *A. thaliana*, seeds were vernalized for 4 days at 4°C (in the dark), and then sown in 87.5 cm³ pots with a fertilized soil:perlite:vermiculite mixture at one plant per pot. The germination condition was 22/18°C with 12/12 h light (fluorescent light)/dark day length. After second-leaf expansion (at around 5 days), the seedlings were moved into the same growth house. Eighty plants per ecotype were prepared for growth and biochemical analyses.

Plants were grown under visible radiation with or without supplemental UV-B radiation in the growth house (12h photoperiod, day/night temperatures 22 /18°C, 65% air relative humidity), according to previous experiments (Hidema et al., 2007; Hidema et al., 2005). Visible light was supplied by metal halide lamps (MT 400DL/BUD, Iwasaki Electric Ltd Co.; <http://www.iwasaki.co.jp>) positioned at the top of the house with a

1 heat-absorbing filter (Tabai Espec Ltd. Co.) that eliminated radiation below 350 nm (Fig. 1). Photosynthetic
2 active radiation (PAR) was adjusted to $190 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was determined with a PAR sensor (LI-190SA;
3 Li-Cor, Lincoln, NE) and a data logger (LI-1000; Li-Cor) and not different between UV-B supplementation
4 treatment (UV+) and control treatment (UV-) (Fig. 1). For UV-B treatment, UV-B lamps (FL20SE; Toshiba
5 Electric Ltd Co., Tokyo, Japan) were placed 30 cm above the plants. Under UV-B bulbs, a UV31 glass filter
6 (Toshiba Glass Co., Shizuoka, Japan; <http://www.jgc.co.jp>) reduced 310 nm radiation by 50% and excluded
7 UV-C region (<280 nm) (Hidema et al., 1996). Spectral distribution was determined with a spectroradiometer
8 [USR-45DA, USHIO Inc., Tokyo, Japan, within 0.2% vs. full scale (at 25°C)], shown in Fig. 1. Biologically
9 effective UV-B radiation (UV-B_{BE}) was calculated by the plant action spectrum of (Caldwell, 1971)
10 normalized to unity at 300 nm. The dose was set at $3.28 \text{ kJ m}^{-2} \text{ day}^{-1}$ for UV-B enhancement treatment
11 according to our preliminary experiment (Fig. S1).

12 UV-B supplementation was simultaneous with visible light when the fourth leaves had expanded fully at
13 around 10 and 22 days after transplantation for *A. thaliana* and *A. halleri subsp. gemmifera*, respectively. The
14 pots were moved every day to receive a similar dose of radiation. Plants were watered every 3 days and
15 fertilized (Hyponex 6-10-5, 1.2 mL/L, 7.5 mL/pot) once a week.

16 To assess response to UV-B radiation, new fully expanded leaves were collected twice during
17 development. Owing to differences in growth rate, we harvested *A. thaliana* leaves after 6 and 20 days of
18 UV-B treatment and *A. halleri subsp. gemmifera* leaves after 10 and 40 days of UV-B treatment. For each
19 treatment, we used at least three replicate per ecotype. The leaf sample was immediately placed in liquid
20 nitrogen and then stored at -80°C for biochemical assays.

21 2.4. Growth

22 At least four individuals were harvested before the onset of UV-B treatment and at early and late stages
23 after the exposure to enhanced UV-B irradiation (hereafter 0, 6, and 20 days after the treatment for *A. thaliana*,
24 and 0, 10, and 40 days for *A. halleri subsp. gemmifera*). *A. thaliana* was harvested earlier than *A. halleri subsp.*
25 *gemmifera* because its growth rates were faster. Individual plants were divided into aboveground and
26 belowground (root) parts. The roots were washed with tap water to remove soil. These parts were dried at
27 60°C for at least 72 h and the dry mass (DM) was measured.

2.5. Quantification of UV absorbance

The concentration of UACs was estimated by UV absorbance as described by Teranishi et al. (2004). Briefly, frozen leaf discs were homogenized in buffer (50 mM sodium phosphate, pH 7.2 and 10% (v/v) glycerol) at a leaf: buffer ratio of 1:15 (g:ml) using a chilled mortar and pestle. UACs and carotenoids were extracted with 1% (v/v) HCl in 70% (v/v) methanol (final concentration) at 4°C for 24 h. The extract was centrifuged at 1,800× g for 15 min at room temperature. Absorbance was measured at 330 nm with a Jasco V-550 UV/VIS spectrophotometer (Jasco Ltd. Co., Tokyo, Japan). The final concentrations were expressed as units per leaf area (cm⁻²).

2.6. DNA extraction, preparation of agarose plugs, treatment of DNA with UV endonuclease, and alkaline gel electrophoresis

All procedures for DNA extraction, preparation of agarose plugs, and the treatment of DNA with UV endonuclease have been described in detail (Hidema et al., 2000; Teranishi et al., 2004). UV endonuclease was purified from *Micrococcus luteus* by streptomycin and ammonium sulfate precipitation. The activity was 7 × 10⁻¹⁴ CPD cleaved μl⁻¹ h⁻¹. According to their single-strand molecular lengths, DNA molecules were separated by alkaline agarose gel (0.7%) electrophoresis using static-field and biased sinusoidal-field gel electrophoresis (Genofield; Atto Co., <http://www.atto.co.jp>). The molecular length markers were DNA from *Hansenula wingei* chromosomes (smallest, 1.05 Mb; Bio-Rad; <http://www.bio-rad.com>), T4 (170 kb), λ DNA (48.5 kb), and a *Hind* III digest of λ DNA (23.1, 9.4, 6.6, 4.3, and 2.3 kb).

2.7. CPD analysis

CPD frequencies were determined with a DNA damage analysis system constructed by Tohoku Electric Co., as previously described (Hidema et al., 2000; Teranishi et al., 2004). CPD frequencies were calculated from the quantity of DNA at each migration position shown in quantitative image data based on a molecular length standard curve (Freeman et al., 1986). CPD frequencies were expressed in units of CPD Mb⁻¹.

2.8. Statistics

Growth rate (GR) was calculated as the difference of DM between two stages divided by the time interval: GR = (M₂ - M₁) / (t₂ - t₁), where M₁ and M₂ are the values of DM at harvest times t₁ and t₂,

1 respectively. Mean DM (M_{mean}) was estimated by assuming exponential growth at each harvest time: $M_{\text{mean}} =$
2 $(M_2 - M_1) / [\ln(M_2) - \ln(M_1)]$. Relative growth rate (RGR) was expressed as GR divided by M_{mean} according
3 to Hirose (1988): $\text{RGR} = (1 / M_{\text{mean}}) \times \text{GR}$. The ratio of total dry mass (TDM) under UV+ to that under UV-
4 treatment was estimated at each stage.

5 A generalized linear model was used to assess effects of ecotype, treatment, and harvest time (early or late)
6 on CPD level, concentration UACs, and plant growth. Growth parameters included GR, RGR, TDM,
7 aboveground DM (AGDM), belowground DM (BGDM), and AGDM: BGDM ratio. Significant difference
8 among ecotypes within species across UV treatments, were assessed according to Tukey's multiple range test
9 ($P < 0.05$). Simple linear regression was used to estimate effects of CPD on RGR and UACs. R 3.0.2 was used
10 for statistical analysis.

3. Results

3.1. Climate data

The habitat of highland ecotypes was apparently characterized by low air temperature (Table 1). Local conditions varied inconsistently between the two species. For the habitats of *A. thaliana*, precipitation, wind speed, and cloud cover generally decreased with elevation, whereas the opposite trends were observed for the habitats of *A. halleri subsp. gemmifera*. Ecotypes of *A. halleri subsp. gemmifera* grow in warmer and more humid habitats than *A. thaliana*. Differences were noted even between ecotypes in similar categories, such as between Tsu-1 and Col-0 and between DZI and Sha. Although monthly averaged solar radiation was calculated on a horizontal surface, it was distinctly higher at geographical coordinates of DZI and Sha than that of others, and increased with elevation in Mt. Ibuki (Fig.S2). Seasonal variations of solar radiation differed markedly among origins of Tsu-1, Col-0, and IB-L ~ IB-H.

3.2. UV-induced DNA damage

CPD levels varied significantly depending on ecotype, treatment, harvest time, and their interactions (Table 2). Increased UV-B radiation induced higher CPD levels than those of the control group (Fig. 2). Under increased UV-B, CPD levels varied between ecotypes originating at high and low elevations with significance depending on the harvest time. At the first harvest, the CPD level of lowland ecotypes was higher than that of highland ecotypes in both species (Fig. 2a, b). Towards the second harvest, it decreased dramatically to a value similar to or lower than that of highland ecotypes, particularly in *A. thaliana* (Fig. 2c, d).

3.3. UV protection

Concentrations of UACs of *A. thaliana* varied significantly depending on ecotype, treatment, and harvest time, whereas that of *A. halleri subsp. gemmifera* depended mainly on the harvest time (Table 2). They were higher in plants under increased UV-B than in the control group at the first harvest, but similar or lower in the plants under increased UV at the second harvest (Table 3). Variation in UAC tended to be greater across ecotypes, but without elevation trends, except for a decrease with increasing elevation in *A. halleri subsp. gemmifera* at 40 days.

3.4. Growth response

Growth rate and biomass production varied significantly depending on ecotype, treatment, harvest time, and their interactions, with a few exceptions (Table 4, S1). Increased UV-B generally reduced growth compared with the control group (Table S2, 3). In plants exposed to UV-B, TDM, growth rate (GR) and RGR were lower for lowland than for highland ecotypes at the early stage, but were similar or higher at late stage (Tables S2, 3; Fig. 3), although there were exceptions. However, interspecific difference occurred in the ratio of AG: BG. It was higher in lowland ecotypes than highland ecotypes in *A. thaliana*, while the observation was contrary in *A. halleri* (Table S2, 3, Fig.S3).

The ratio of TDM in UV+ to that in UV- treatments was clearly lower in lowland than in highland ecotypes at the first harvest (Fig. 4a, b), and higher, in contrast, at the second harvest (Fig. 4c, d).

3.5. The relationship of DNA damage with growth and UACs

CPD levels were negatively associated with RGR rather than with the ratio of TDM (Fig. 5). There was no significant relationship between CPD levels and UACs ($P > 0.05$; data not shown).

4. Discussion

UV induced DNA damage and growth inhibition differed significantly between highland and lowland ecotypes, and the difference depended strongly on the harvest time. Lowland ecotypes appeared more sensitive to enhanced UV-B radiation than highland ecotypes at the early stage, with higher CPD levels and suppressed RGRs. Toward the late stage, interestingly, CPD levels declined and RGR increased dramatically in lowland ecotypes, to levels comparable to those in highland ecotypes. These results suggest that lowland ecotypes are relatively susceptible to UV just after the exposure but acquire UV tolerance after acclimation to a UV-enhanced environment; thus, that UV tolerance is inducible as was hypothesized. In contrast, highland ecotypes maintained similar levels of CPD and RGR throughout the exposure, suggesting that they have constitutive UV tolerance irrespective of growth environment.

Phenotypic plasticity is commonly considered to be the primary mechanism enabling plants to adapt phenotypes to local conditions and cope with environmental stresses (Nicotra et al., 2010; Valladares et al., 2007; Valladares et al., 2006). Our results reflect different patterns of UV stress at different elevations. In lowland areas, plants are generally exposed to low UV-B radiation and/or easily shaded by neighbors. In this case, selection will favor inducible protection. In this way, plants can save resistance costs and use resources for growth or competition when UV-B is lower. Additional investment may be made only when UV stress is present. Such a plastic response to UV stress confers a fitness advantage, resulting in reducing susceptibility to damage (Figs. 2 and 4). In contrast, constitutive UV tolerance is observed in highland ecotypes (such as Sha, Dzi, and IB-H). Higher plasticity is not necessarily beneficial in highland areas, where plants are constantly subjected to high UV stress and other harsh influences during their lifespans. Constant resistance has no lag time in the highland UV environment, although it may be costly owing to sustained investment in protection mechanisms, which potentially slows RGR. Thus, on the whole, constitutive UV tolerance may be more advantageous for plants inhabiting high-UV environments. There is a potential tradeoff between high growth rate and UV tolerance.

Common ecotypic differentiations in the two *Arabidopsis* species suggest that local adaptation occurred in parallel, possibly owing to historical distribution and evolutionary adaptation of species. *A. thaliana* and *halleri* subsp. *gemmifera* expanded from refuges in central Europe to eastern Asia during the glacial period

(Beck et al., 2008; Koch et al., 2007). Despite this historical distance, the genome-wide investigation indicates that ecotypes successfully colonizing different elevations in Asian mountains, such as *A. halleri subsp. gemmifera* in Mt. Ibuki, still share a similar genomic structure (Ikeda et al., 2010). Interestingly, recent morphological divergence between the highland and the lowland ecotypes has been shown to have occurred, suggesting that adaptive divergence has taken place relatively recently. Although gene flow generally erodes and prevents divergence among populations on a small scale, recent studies have found that it can occur even among populations with high gene flow if selective pressure is sufficient (Wright, 1969). For example, Kubota et al. (2015) identified one gene, *GSL8* (*AT2G36850*) associated with UV adaptation in *A. halleri subsp. gemmifera* ecotypes involved in “meristem initiation,” “trichome morphogenesis,” and “telomere maintenance in response to DNA damage” on two distinct mountains, Ibuki and Fujiwara. This genome scan work supplies strong evidence that adaptive divergence occurs at such microgeographic scales and also suggests that UV environment is an important selective pressure for plants. Further work would reveal the regulation and expression of the candidate genes of ecotypes responding to UV-B variation and shed light on their evolutionary dynamics during local adaptation.

Our results clearly support the hypothesis that highland ecotype has a constitutive UV response. However, it is not necessarily consistent with previous studies. For instance, Jansen et al. (2010) found no significant association between UV tolerance in PSII and elevational origin across *A. thaliana* accessions. However, the present study focused on the effect of UV on DNA, the primary target of UV stress, whereas Jansen et al. (2010) evaluated the protection of PSII. Because PSII is sensitive not only to UV-B but also to other environmental stresses such as high visible light and low temperature (Havaux, 1992; Kato et al., 2003; Kato et al., 2002), PSII tolerance may include adaptation to other environmental factors. Further study of UV tolerance in PSII and DNA is needed.

Regression analysis indicated that RGR was negatively associated with CPD levels at each stage (Fig. 5). This result is consistent with previous results in rice (Hidema et al., 2007) and in barley (Mazza et al., 1999). These results confirm that CPDs induced by UV-B are important causes of growth inhibition. UV-induced CPDs may influence the biological processes of plants by interfering with transcription and replication processes (Sancar et al., 2004; Takeuchi et al., 2002). Alternatively, CPDs may act indirectly as the initiator of a series of UV-B signal transduction pathways involving up- or downregulation of gene expression (Hidema et

1 al., 2007; Li et al., 2015). Thus, the accumulation of CPDs is a valuable indicator of the effects of UV-B on
2 plant growth and ecophysiological characters, and can be applied in agricultural research that select and breed
3 UV-resistant cultivars.

4 The adaptation of a species to UV-B is generally ascribed to its tolerance mechanisms. However, our
5 study did not detect increasing concentrations of UACs with elevation, suggesting that UACs do not explain
6 UV damage observed among ecotypes. Similar results were observed in previous common-garden studies (e.g.
7 Nybakken et al. 2004). An explanation may involve the effects of other habitat stresses, given that elevational
8 variation of phenolics is induced not only by higher UV-B, but also by other factors such as low temperature
9 (Albert et al., 2009; Bilger et al. 2007; Spitaler et al., 2008). Because habitats at higher elevations were
10 generally exposed to more forms of stress (Table 1). It is questionable whether the response of UACs to
11 enhanced UV-B depends on conditions in growth cabinets. Another possible explanation may involve the
12 maximal expression of UACs of *Arabidopsis* ecotypes. For instance, UACs are costly or constrained, or it may
13 be that there is no benefit to additional tolerance beyond some upper threshold (Agrawal et al., 2010). Further
14 investigation of molecular processes and energy consumption of UACs would help understand the mechanism
15 of adaptation of species to highland UV stress.

16 Enhanced UV-B can generally alter growth allocation and RGR (Barnes et al., 1990; Bussell et al., 2012).
17 In the present study, highland ecotypes of *A. thaliana* allocated more biomass belowground than lowland
18 ecotypes, while it was contrary in *A. halleri* (Table S2, 3; Figure S3). It may be explained by their life-history
19 strategies and seasonal variation of solar radiation at origins (Fig.S2). *A.thaliana* is typically a winter annual,
20 which germinates in fall, overwinters as a vegetative rosette, and flowers in the spring (Griffith et al., 2004).
21 Increase in root production may offset spring water deficit and escape the harm from increasing UV-B
22 exposure with snow melt. In contrary, *A.halleri*, as a perennial, not only flowers in the spring, but also
23 produces new rosettes after flowering and then overwinters (Kawagoe and Kudoh, 2010). Increase in shoot
24 production may be of great benefit to improve tolerance in heavy UV-B stress in summer and freezing in
25 winter, and also contribute to interspecific competition during the vegetative stage. Although changes in
26 root:shoot ratio have important implications for plant responses to future UV-B environment and plant–plant
27 competitive balance (Robson et al., 2015), the underlying molecular mechanisms are not clear.

5. Conclusions

The present study showed that UV-induced DNA damage and growth varied significantly with ecotype and the duration after the onset of UV-B enhancement. Constitutive and inducible UV tolerances reflect specific phenotypic plasticity in response to different patterns of UV stress; lowland ecotypes showed more inducible UV tolerance, whereas highland ecotypes tended to show constitutive UV tolerance. This differentiation may be a result of parallel adaptation to local UV environments under a constraint of tradeoff between growth and UV tolerance. It also suggests that constitutive defense is a more advantageous strategy than inducible defense for highland plants in highlands, where UV stress is constantly high.

1 **Author contributions**

2

3 K.H., J. H. and Q.-W.W. designed the experiment, S.N., S.-I. M., J.H.and H.O supplied plant materials

4 and determination technique, Q.-W.W. performed the experiments. Q.-W.W. and K.H. analyzed the data and

5 wrote the article.

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1 **Table 1. Summary of climatic information for ecotypes of *Arabidopsis thaliana* and *A. halleri* subsp. *gemmifera* used in this study.** Seeds of *A. thaliana* were obtained
 2 from the Arabidopsis Information Resource: two ecotypes from highland, Dzi and Sha, and two ecotypes from lowland, Tsu-1 and Col-0; those of *A. halleri* were collected
 3 from Mt. Ibuki (35° 25' 04" N, 136° 24' 22" E) in central Japan: three ecotypes from 300, 760, and 1300 m above the sea level, IB-L, IB-M, IB-H, respectively. Climate data
 4 at the geographic origins of *A. thaliana* ecotype was obtained from the Climate Research Unit (CRU CL 1.0 climate dataset, averaged over 1961–1990). Mean value of
 5 monthly mean temperature (MEMT) of *A. halleri* subsp. *gemmifera* was estimated near meteorological stations of the Japan Meteorological Agency: Nagahama (low
 6 elevation) and Ibuki (high elevation) (averaged over 1980–2000) assuming a lapse rate of 0.6°C per 100 m.
 7

Stock No.	Abb.	Location	Coordinate	ELE	MEMT	MAMT	MIMT	MP	MC	MF	MW
<i>Arabidopsis thaliana</i>											
CS22681	Col-0	Poland, Gorzow	N53, E15	1/100	8.68	12.71	4.68	1.50	67.37	8.54	3.87
CS22693	Tsu-1	Japan, Tsushima	N34, E129	1/100	14.00	18.13	9.91	5.18	66.43	5.88	3.37
CS22690	Sha	Tajikistan, Pamiro-Alay	N37/39, E68/74	3400	3.87	9.85	−2.11	1.32	51.00	11.08	1.95
CS28980	Dzi	Tajikistan, Dzhiland	N38, E73	3400	−3.89	1.60	−9.40	1.34	53.51	14.62	1.90
<i>Arabidopsis halleri</i> subsp. <i>Gemmifera</i>											
—	—	Japan, Nagahama	N35, E136	95	13.59	23.34	4.07	4.27	59.2	—	1.85
—	—	Japan, Ibuki	N35, E136	1376	6.36	16.00	-1.53	6.64	63.52	24.83	8.76
—	IB-L	—	N35, E136	300	11.90	—	—	—	—	—	—
—	IB-M	—	N35, E136	760	9.30	—	—	—	—	—	—
—	IB-H	—	N35, E136	1300	6.25	—	—	—	—	—	—

8
 9 *Note:* ELE, elevation (m a.s.l., above sea level); MEMT, mean value of monthly mean temperature (°C); MAMT, Mean value of monthly maximum temperature (°C); MIMT,
 10 Mean value of monthly minimum temperature (°C); MP, mean daily precipitation (mm d^{−1}); MC, mean diurnal cloud cover (% d^{−1}); MF, mean monthly frost frequency (day);
 11 MW, mean wind speed (m/s). “—” represents no data.

1 Table 2 . Effects of ecotype, treatment, harvest time, and interactions on cyclobutane pyrimidine dimer (CPD,
2 CPD Mb⁻¹) and UV-absorbing compounds (UACs, OD cm⁻²) in *Arabidopsis thaliana* and *A. halleri* subsp.
3 *gemmifera* ecotypes. df and *F* values in each factor are shown.

Source	df	CPD (<i>F</i>)		df	UACs (<i>F</i>)	
<i>Arabidopsis thaliana</i>						
Ecotype	3	7.64	***	3	42.88	***
Treatment	1	428.99	***	1	9.50	**
Harvest	1	33.66	***	1	16.96	***
Ecotype × harvest	3	16.48	***	3	208.52	***
Ecotype × treatment	3	18.38	***	3	3.61	*
Treatment × harvest	1	0.83	n.s.	1	61.42	***
Ecotype × treatment × harvest	3	29.33	***	3	8.93	**
Error	32			32		
<i>Arabidopsis halleri</i>						
Ecotype	2	2.42	n.s.	2	2.24	n.s.
Treatment	1	228.11	***	1	2.56	n.s.
Harvest	1	11.33	**	1	105.58	***
Ecotype × harvest	2	6.16	**	2	4.45	*
Ecotype × treatment	2	1.65	n.s.	2	2.81	n.s.
Treatment × harvest	1	3.46	n.s.	1	3.49	*
Ecotype × treatment × harvest	2	7.07	**	2	0.01	n.s.
Error	30			48		

5
6 Note: Significance levels: *** *P* < 0.001; ** *P* < 0.01; * *P* < 0.05; “n.s.” *P* > 0.05.

7

1 **Table 3. Variation in concentration of UV-absorbing compounds (UACs, OD cm⁻²) among *Arabidopsis* ecotypes**
2 **and between UV treatments.** Seedlings were grown with (UV+) and without (UV-) enhanced UV-B (3.28 KJ m⁻²
3 day⁻¹) when the fourth leaf was fully expanded after emergence. New fully expanded leaves were harvested to
4 determine UAC at early and late stages after the exposure to enhanced UV-B irradiation as 6 days and 20 days in *A.*
5 *thaliana* and as 10 days and 40 days in *A. halleri* subsp. *gemmifera*. See Table 1 for ecotypes of origin.

Species	Harvest	Ecotype	UV-		UV+	
			mean	SE	mean	SE
<i>Arabidopsis thaliana</i>	6 d	Col-0	1.12	0.01 b	1.79	0.02 a
		Tsu-1	0.38	0.02 e	0.96	0.03 b
		Dzi	0.51	0.02 de	0.77	0.03 c
		Sha	0.62	0.07 cd	1.07	0.05 b
	20 d	Col-0	1.32	0.01 a	0.69	0.06 c
		Tsu-1	1.25	0.16 ab	1.09	0.04 ab
		Dzi	1.22	0.06 ab	0.92	0.04 bc
		Sha	0.91	0.06 bc	0.74	0.02 c
<i>Arabidopsis halleri</i>	10 d	IB-L	0.68	0.06 a	1.12	0.29 a
		IB-M	0.65	0.03 a	0.63	0.05 a
		IB-H	0.75	0.09 a	1.11	0.32 a
	40 d	IB-L	1.80	0.02 abc	1.97	0.21 ab
		IB-M	2.02	0.02 a	1.48	0.06 c
		IB-H	1.57	0.05 bc	1.37	0.09 c

7
8 *Note:* Means and standard errors are shown (mean ± SE, $n \geq 3$). For each stage, values with different letters denote
9 significant difference among ecotypes (including UV+ and UV-) at $P < 0.05$, Tukey's multiple range test.

1 **Table 4 . Effects of ecotype, treatment, harvest time, and interactions on growth in *Arabidopsis thaliana* and *A.***
2 ***halleri* subsp. *gemmifera* ecotypes.** df and *F* values for each factor are shown.

3

Source	df	TDM (<i>F</i>)		GR (<i>F</i>)		RGR (<i>F</i>)	
<i>Arabidopsis thaliana</i>							
Ecotype	3	16.78	***	17.73	***	5.11	**
Treatment	1	1912.16	***	1842.91	***	534.69	***
Harvest	1	3831.73	***	2407.73	***	1498.56	***
Ecotype × harvest	3	6.69	**	1.42	n.s.	12.27	***
Ecotype × treatment	3	5.90	**	6.02	**	0.15	n.s.
Treatment × harvest	1	1527.46	***	1055.29	***	165.64	***
Ecotype × treatment × harvest	3	3.99	*	4.31	**	5.07	**
Error	64						
<i>Arabidopsis halleri</i>							
Ecotype	2	47.71	***	47.11	***	27.07	***
Treatment	1	297.69	***	298.26	***	93.63	***
Harvest	1	1151.70	***	900.32	***	77.09	***
Ecotype × harvest	2	43.79	***	36.44	***	39.14	***
Ecotype × treatment	2	6.02	**	6.93	**	6.81	**
Treatment × harvest	1	264.43	***	206.61	***	57.07	***
Ecotype × treatment × harvest	2	3.84	*	1.52	n.s.	25.20	***
Error	36						

4 *Note:* TDW, total dry mass; GR, growth rate; RGR, relative growth rate of the whole individual. Significance levels:

5 *** *P* < 0.001; ** *P* < 0.01; * *P* < 0.05; “n.s.” *P* > 0.05.

1 **Figure**

2

3

4 **Fig. 1. Spectra of groups with or without UV-B radiation in the growth house.** Plants were grown with (UV+,
5 solid line) and without (UV-, dotted line) enhanced UV-B.

6

Fig. 2. Cyclobutane pyrimidine dimer (CPD) induction in *Arabidopsis* ecotypes exposed to supplementary UV-B radiation. *Arabidopsis* seedlings were grown with (UV+, closed bar) and without (UV-, open bar) enhanced UV-B (3.28 kJ m⁻² day⁻¹) when the fourth leaf was fully expanded after emergence. New fully expanded leaves were harvested to determine the CPD accumulation at early and late stages after the exposure to enhanced UV-B irradiation as 6 days (a) and 20 days (c) in *A. thaliana* ecotypes (Col-0 and Tsu-1 from low elevation below 100 m a.s.l., and Dzi and Sha from high elevation around 3400 m a.s.l.), and as 10 days (b) and 40 days (d) in *A. halleri* subsp. *gemmifera* ecotypes (IB-L, IB-M, IB-H from 300, 760, and 1300 m a.s.l. respectively). Different letters indicate significant difference among ecotypes (including UV+ and UV-) at $P < 0.05$, Tukey's multiple range test.. Bars represent means ($n \geq 3$) and vertical bars represent standard errors.

Fig. 3. Relative growth rate (RGR) of whole plants of *Arabidopsis* ecotypes exposed to supplementary UV-B radiation. *Arabidopsis* seedlings were grown with (UV+, closed bar) and without (UV-, open bar) enhanced UV-B ($3.28 \text{ kJ m}^{-2} \text{ day}^{-1}$) when the fourth leaf was fully expanded after emergence. Growth was determined at early and late stages after the exposure to enhanced UV-B irradiation as 6 days (a) and 20 days (c) in *A. thaliana* ecotypes (Col-0 and Tsu-1 from low elevation below 100 m a.s.l., and Dzi and Sha from high elevation around 3400 m a.s.l.), and as 10 days (b) and 40 days (d) in *A. halleri* subsp. *gemmaifera* ecotypes (IB-L, IB-M, IB-H from 300, 760, and 1300 m a.s.l. respectively). Different letters indicate significant difference among ecotypes (including UV+ and UV-) at $P < 0.05$, Tukey's multiple range test. Bars represent means ($n \geq 3$) and vertical bars represent standard errors.

Fig. 4. Ratio of total dry mass (TDM) in UV+ to UV- treatments in *Arabidopsis* ecotypes. Seedlings grown with (UV+) and without (UV-) enhanced UV-B ($3.28 \text{ kJ m}^{-2} \text{ day}^{-1}$) when the fourth leaf was fully expanded after emergence. Net total biomass and the ratio of net biomass between (UV+) and (UV-) was determined at early and late stages after the exposure to enhanced UV-B irradiation as 6 days (a) and 20 days (c) in *A. thaliana* ecotypes (Col-0 and Tsu-1 from low elevation below 100 m a.s.l., and DZI and Sha from high elevation around 3400 m a.s.l.), and as 10 days (b) and 40 days (d) in *A. halleri subsp. gemmifera* ecotypes (IB-L, IB-M, IB-H from 300, 760, and 1300 m a.s.l. respectively). Different letters indicate significant difference among ecotypes at $P < 0.05$, Tukey's multiple range test. Bars represent means ($n \geq 4$) and vertical bars represent standard errors.

Fig. 5. Relationship between cyclobutane pyrimidine dimers (CPD) level and relative growth rates (RGR) in *Arabidopsis* ecotypes exposed to supplementary UV-B radiation. a) *A. thaliana* ecotypes included Col-0 (square), Tsu-1 (circle) from lowland below 100 m a.s.l., DZI (triangle), and Sha (diamonds) from highland around 3400 m a.s.l.; b) *A. halleri subsp. gemmifera* ecotypes included IB-L (square), IB-M (circle), IB-H (triangle) from 300, 760, and 1300 m a.s.l., respectively. Open symbols, early stage after the exposure to enhanced UV-B irradiation (6 and 10 d for *A. thaliana* and *A. halleri subsp. gemmifera*); closed symbols, late stage after the exposure to enhanced UV-B irradiation (20 and 40 d for *A. thaliana* and *A. halleri subsp. gemmifera*). The results of linear regression analysis are shown, including r and p .