Differential responses of the scavenging systems for reactive oxygen species (ROS) and reactive carbonyl species (RCS) to UV-B irradiation in *Arabidopsis thaliana* and its high altitude perennial relative *Arabis alpina*

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Abstract

The present work aimed to compare antioxidant response and lipid peroxide detoxification capacity of an arctic-alpine species *Arabis alpina* to its close relative model species *Arabidopsis thaliana* under acute short duration (3 h and 6 h) UV-B stress (4.6 and 8.2 W/m²). After 3 and 6 h exposure to UV-B, *A. alpina* showed lower lipid peroxidation and H₂O₂ accumulation when compared to *A. thaliana*. Moreover, F_v/F_m value of *A. thaliana* dropped to 0.70, while *A. alpina* dropped to 0.75 indicating better protection of PSII in this species. For elucidation of the antioxidant response, activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) were measured. SOD induction with 6 h of UV-B was more prominent in *A. alpina*. Also, *A. alpina* had higher chloroplastic FeSOD activity when compared to *A. thaliana*. APX activity was also significantly induced in *A. alpina*, while its activity decreased at 3 h or did not change at 6 h in *A. thaliana*. *A. alpina* was able to maintain constant CAT activity, but drastic decreases were observed in *A. thaliana* at both time points. Moreover, *A. alpina* was able to maintain or induce aldehyde dehydrogenase (ALDH), alkenal reductases (AERs) and glutathione-S-transferases (GST) activity, while an opposite trend was observed in *A. thaliana*. These findings indicate that *A. alpina* was able to maintain/induce its antioxidant defence and lipid peroxide detoxification conferring better protection against UV-B.

Keywords Antioxidant defence · *Arabidopsis thaliana* · *Arabis alpina* · Reactive carbonyl species · Reactive oxygen species · Ultraviolet-B

1 Introduction

UV-B radiation (280–315 nm) is an important growth regulator in natural environments and an abiotic stress factor depending on the severity of irradiation, developmental stage of the plant and availability of other environmental stress

factors [1]. Similar to abiotic stresses such as drought, salinity, heavy metals or high light, UV-B stress is also known to induce oxidative stress in plants as secondary stress [2]. It has been demonstrated that various reactive oxygen species (ROS) can be produced in plants treated with high UV-B doses by affecting various metabolic processes such as

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photosynthesis. Protein complexes involved in light reactions of photosynthesis and also Calvin–Benson–Bassham (CBB) cycle enzymes can be disrupted by UV-B. For example, UV-B is known to cause malfunctioning of electron transfer in PSII due to UV-B absorption of oxygen-evolving complex [3] or other quinone components [4], which eventually cause production of ROS such as O_2^- by PSI. ROS production during UV-B irradiation has been demonstrated with fluorescent ROS probes in spinach leaves [5]. Plants evolved various enzymatic or non-enzymatic antioxidant defences to counteract the detrimental effects of ROS. For example, superoxide dismutase (SOD) scavenges O_2^- to H_2O_2 , which is converted to H_2O by peroxidases (POX), ascorbate peroxidases (APX), thiol-peroxidases (peroxiredoxins) or catalases (CAT) in various compartments of the plant cell [6]. Moreover, UV-B radiation can convert H_2O_2 to highly reactive hydroxyl radical ($HO^\bullet$) by photo-conversion, thus changing the dynamics of ROS production and increasing the oxidative damage caused to biological molecules [7].

ROS such as $HO^\bullet$ and 1O_2 can react and oxidize polyunsaturated fatty acids (PUFAs) leading to production of lipid peroxyls and then lipid hydroperoxides (LOOHs). Since H_2O_2 can be converted to $HO^\bullet$ via Fenton reaction in the presence of metals such as Fe, increased H_2O_2 can also lead to oxidation of PUFA. LOOH break down to produce carbonyl radicals (aldehydes and ketones) of various size and structure [8]. These molecules are referred to as reactive carbonyl species (RCS) and are toxic at high concentrations due to their ability to oxidize sulfhydryl groups of proteins and modify proteins via Michael addition or Schiff base formation [9]. Among RCS, 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) are well known for their detrimental effects on metabolism at high concentrations. For example, it has been demonstrated that HNE can specifically inhibit decarboxylating dehydrogenases in the matrix of plant mitochondria including pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, NAD-dependent malic enzyme and the glycine decarboxylase [10]. On the other hand, RCS can activate caspase-3-like protease to induce programmed cell death [11]. Moreover, HNE and MDA are generally used as an indicator of lipid peroxidation and, in a broader sense, oxidative stress in both animal and plant systems [12]. In general, a high level of RCS scavenging capacity has been associated with stress tolerance in the literature for various stresses such as drought and salinity [13, 14], low temperature or cadmium [15]. When antioxidant defence becomes overwhelmed, lipid peroxidation increases and a higher capacity to detoxify RCS have been associated with increased stress tolerance [13–15]. RCS can be detoxified by enzymes such as aldehyde dehydrogenases (ALDHs), alkenal reductases (AERs) or glutathione-S-transferases (GSTs). Damaging and signalling roles of RCS have been an active area of research in recent years (reviewed in [9] and there

is limited knowledge on RCS detoxification mechanisms of plants under stressed conditions.

Previously, it has been demonstrated that UV-B can regulate antioxidant defence in various plant species such as *Arabidopsis thaliana* [16, 17], sunflower [18], rice [19], barley [20] or soybean [21]. In general, UV-B induces activities of antioxidant defence enzymes, but in some cases, activities are decreased depending on the severity of the UV-B irradiance and the duration of the stress. UV-B response also depends on plants species, developmental stage of the plant and even age of leaf rendering cross-study comparison difficult. However, UV-B stress adaptive antioxidant responses or contribution of antioxidant defence to UV-B stress tolerance, especially in plants naturally adapted to higher UV-B still needs to be revealed.

Arabis alpina is a diploid, self-compatible, perennial plant with a relatively small genome, which was recently sequenced [22]. It originates from central Anatolia (Turkey) and is widely used for ecological studies. The purpose of the present work was to compare antioxidant response and lipid peroxide detoxification capacity of an arctic-alpine species *Arabis alpina* to its close relative model species *Arabidopsis thaliana* under acute UV-B stress. Therefore, this work aimed to test (i) tolerance of arctic-alpine species *A. alpina* to high doses of UV-B in comparison with *A. thaliana*, (ii) involvement of antioxidant defence in UV-B tolerance, (iii) response of RCS detoxification metabolism and (iv) changes in ROS signalling. For this, F_v/F_m , lipid peroxidation levels and H_2O_2 levels were measured as parameters to determine oxidative effects of UV-B in these two species. Also, activities of SOD, CAT, POX, APX, glutathione reductase (GR), dehydroascorbate reductase (DHAR) and isoenzymes of SOD, CAT and POX were determined to elucidate the response of antioxidant defence of *A. alpina* and *A. thaliana* comparatively. Moreover, AER, ALDH and GST activities were measured to identify the involvement of RCS detoxification mechanisms in UV-B tolerance. In addition, plasma membrane-associated NADPH oxidase (NOX) activity was determined to reveal changes in NOX-mediated ROS signalling.

2 Materials and methods

2.1 Plant growth and stress treatments

Seeds of *Arabis alpina* were collected from Ağılbaşı (Engüzek) village, Malatya, Turkey. Collection site had calcareous marl rich soil and elevation was 1700 m. A new batch of seeds was produced from *A. alpina* seeds collected from nature and seeds obtained from a single individual were used in the experiments. *A. thaliana* Col-0 ecotype was used from laboratory stocks. Seeds of both

species were surface sterilized with 70% ethanol (30 s) and 4% bleach (10 min) and were washed five times with sterile water. Seeds were sown in pots containing 7:2:1 peat moss: vermiculite: perlite mixture and were stratified for two days at 4 °C in the dark in a plant growth chamber. Following stratification, plants were transferred to 22/20 °C (day/night) temperatures with a photoperiod of 12 h light/12 h dark and relative humidity of 60%. During the growth period, plants were sub-irrigated with 1/2 Hoagland solution every other day. For UV-B treatments, 3-week-old *A. alpina* and *A. thaliana* plants were used. Philips TL 20 W/01 RS SLV/25 bulbs (covered with cellulose acetate sheet) were used as UV-B source (for spectral output see Supplementary Fig. 1). UV-B source was positioned 30 cm above the plants. During UV-B treatments, plants were illuminated with light intensity used during the growth period (PAR 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$). UV-B exposures were 4.6 W/m² (3 h) and 8.2 W/m² (6 h). After 3 h and 6 h of UV-B treatments, leaves were harvested and stored at – 80 °C for further analysis.

2.2 Determination of TBARS and H₂O₂ levels

The level of lipid peroxidation in samples was determined by measuring thiobarbituric acid reactive substances (TBARS) according to [23].

H₂O₂ was determined according to [24] using eFOX reagent. This modified ferrous ammonium sulphate/xylene orange (FOX) assay was used due to its sensitivity and stability. In this assay, 1% ethanol is added to the reagent, which increases its sensitivity to H₂O₂ by 50% (i.e., eFOX). Samples were extracted using ice-cold acetone containing 25 mM H₂SO₄. Then, extracts were centrifuged for 5 min at 3000 g at 4 °C. For the reaction, 950 μl eFOX reagent (0.25 mM ferrous ammonium sulphate, 0.1 mM xylene orange, 0.1 mM μM sorbitol, 1% ethanol, v/v) was used for 50 μl of supernatant. Reaction mixtures were incubated at room temperature for 30 min and then absorbance at 550 nm and 800 nm was measured. H₂O₂ concentrations were calculated using a standard curve prepared with known concentrations of H₂O₂.

2.3 Maximum efficiency of PSII (F_v/F_m)

Chlorophyll fluorescence measurements (maximum efficiency of PSII, F_v/F_m) of plants for each group were measured with Plant Efficiency Analyzer of Hansatech, UK. Leaves were dark-adapted for 30 min with leaf clips before measurements. Preliminary studies with different time points

demonstrated that 30 min was sufficient for *A. alpina* leaves for dark adaptation.

2.4 Extraction and assays of antioxidant enzymes

All extractions were performed at 4 °C or on ice. 100 mg leaf samples were ground to a fine powder in liquid nitrogen and then homogenized in 500 μl of 50 mM Tris–HCl, pH 7.8 buffer, containing 0.1 mM EDTA, 0.1% (w/v) Triton X-100, polyvinylpyrrolidone (PVPP, 1% w/v) and 1 mM phenylmethylsulfonyl fluoride (PMSF). For the determination of APX activity, 5 mM ascorbate was added into the homogenization buffer to prevent inhibition of chloroplastic APX isoforms. Samples were centrifuged at 10,000 $\times g$ for 10 min, and supernatants were used for the determination of antioxidant enzyme activities and protein contents. Total soluble protein contents of the enzyme extracts were determined with Bradford reagent according to the directions of the manufacturer (Sigma, B6916).

SOD (EC 1.15.1.1) activity was assayed by its ability to inhibit the photochemical reduction of NBT at 560 nm [25]. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photoreduction. CAT (EC 1.11.1.6) activity was estimated according to the method of [26], which measures the rate of decomposition of H₂O₂ at 240 nm. The decrease in the absorbance was followed for 1 min and 1 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1}$ was defined as 1 unit of CAT. POX (EC 1.11.1.7) activity was based on the method described by [27]. The increase in the absorbance at 465 nm due to oxidation of diaminobenzidine (DAB) was followed for 1 min in Na-phosphate-citrate buffer (pH 4.4). One unit of POX activity was defined as 1 $\mu\text{mol H}_2\text{O}_2$ decomposed in 1 min. APX (EC 1.11.1.11) activity was measured according to [28]. The assay depends on the decrease in absorbance at 290 nm as ascorbate was oxidized. The concentration of oxidized ascorbate was calculated using an extinction coefficient of 2.8 mM^{–1} cm^{–1}. One unit of APX was defined as 1 μmol ascorbate oxidized min^{–1}. GR (EC 1.6.4.2) activity was measured according to [29]. NADPH oxidation was followed at 340 nm. The activity was calculated using the extinction coefficient of NADPH (6.2 mM^{–1} cm^{–1}). One unit of GR was defined as 1 $\mu\text{mol GSSG}$ reduced min^{–1}. DHAR (EC 1.8.5.1) activity was determined according to [28]. NOX (EC 1.6.3. 1) activity was measured according to [30]. The assay medium contained 50 mM Tris–HCl buffer, pH 7.5, 0.5 mM XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide), 100 μM NADPH and 20 μg of protein. After the addition of NADPH, XTT reduction was followed at 470 nm. The corrections of background production were determined in the presence of 50 U SOD. The activity was calculated using the extinction coefficient, $2.16 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

2.5 Extraction and assays of RCS scavenging enzymes

To determine RCS scavenging enzyme activities; ALDH, AER and GST enzyme activities were assayed. Samples were ground to a fine powder with liquid nitrogen and homogenized with 50 mM pH 7.0 K-Pi buffer containing 1% PVPP at 4 °C. Homogenized samples were centrifuged at 8000×g for 10 min and supernatants were collected in new tubes to perform further analysis. ALDH activity was determined according to the modification of [31]. NADPH formation was observed at 340 nm in the presence of 100 mM sodium pyrophosphate (pH 9.5), 1.3 mM NAD⁺, 10 mM acetaldehyde. Alkenal reductase (AER) activity was measured according to [32]. NADPH oxidation was observed at 340 nm, in the presence of 50 mM MES-NaOH (pH 6.0), 0.1 mM NADPH and 0.1 mM diamide. Glutathione-S-transferase activity was determined according to [33], with 0.1 Mm Na-P (pH 6.5), 1 mM GSH, 1 mM CDNB (1-Chloro-2,4-dinitrobenzene) reaction mixture.

2.6 Identification of isoenzymes

Samples containing equal amounts of protein were subjected to native polyacrylamide gel electrophoresis (native-PAGE) as described by [34]. For the separation of SOD isoenzymes 12.5% separating gels were used. SOD activity was detected as described by [35] with NBT. The different types of SOD were determined by incubating gels in different SOD inhibitors before staining, for example with 2 mM KCN to inhibit Cu/ZnSOD activity and 3 mM H₂O₂ to inhibit Cu/ZnSOD and FeSOD activities as described by [36] (MnSOD is resistant to both inhibitors). CAT isoforms were detected according to [37]. The electrophoretic separation was performed using 7.5% separating gel. The gels were incubated in 0.01% H₂O₂ for 5 min. After incubation, the gels were washed with distilled water twice and incubated for 5 min in a staining solution containing 1% FeCl₃ and 1% K₃Fe(CN)₆. POX isoforms were detected according to [38]. For the separation of POX isoenzymes, 10% separating gels were used. The gels were incubated for 30 min at 25 °C in 200 mM Na-acetate buffer (pH 5.0) containing 1.3 mM diaminobenzidine (DAB) and 3% hydrogen peroxide. Gels were photographed with a Vilber Lourmat gel imaging system and then analysed with ImageJ software.

2.7 Statistical analysis

Each data point was the mean of three technical replicates from three separate biological replicates ($n=9$). Gels were repeated three times. The results were expressed as mean and error bars were used for showing standard error of the mean ($\pm$ SEM). Groups of each species were compared

using one-way ANOVA and Tukey's HSD. ANOVA was performed separately for each species. Significant differences ($p<0.05$) were marked with different letters (a–d) in the charts.

3 Results

Lipid peroxidation and H₂O₂ levels were measured to evaluate the degree of oxidative damage. *A. thaliana* showed increased levels of TBARS, an indicator of lipid peroxidation when compared to *A. alpina* under UV-B stress (Fig. 1A). Lipid peroxidation increased by 13% ($p<0.005$) after 3 h of UV-B exposure while it induced by 30% ($p<0.0001$) after 6 h UV exposure in *A. thaliana*. Both 3 h and 6 h UV-B exposure did not cause any statistically significant change the lipid peroxidation levels of *A. alpina* as compared to its controls.

H₂O₂ accumulation was observed in both species by UV-B stress but later and lesser in *A. alpina* when compared to *A. thaliana* (Fig. 1B). H₂O₂ levels of 3 h and 6 h UV-B exposed *A. thaliana* plants were enhanced by 57% ($p<0.0001$) and by 41% ($p<0.0005$), respectively. On the other hand, the levels of H₂O₂ in *A. alpina* did not changed with 3 h UV-B exposure, while it was induced with 6 h UV-B exposure by 17% ($p<0.0001$).

The maximum quantum yield of PSII (F_v/F_m) was measured to evaluate the damage on PS II caused by UV-B stress. The decrease in F_v/F_m was more prominent in *A. thaliana* compared to *A. alpina* (Fig. 1C). F_v/F_m of *A. thaliana* decreased to 0.708 and 0.703 ($p<0.0005$ and 0.0001) at 3 and 6 h UV-B exposure, respectively, while control F_v/F_m values were 0.85. On the other hand, UV-B exposure decreased F_v/F_m of *A. alpina* to 0.753 and 0.750 for 3 and 6 h treatments ($p<0.005$ and 0.005), respectively, while F_v/F_m values of controls were similar to that of *A. thaliana* (0.85). Inter-species comparison of F_v/F_m values (with one-way ANOVA) also indicates that *A. alpina* was able to maintain higher F_v/F_m capacity ($p<0.05$).

Among antioxidant enzymes, SODs are named as the first line of defence against oxidative stress due to their ability to scavenge O₂^{•−} enzymatically [39]. There are three types of SOD isoforms divided according to the type of the metal found in their active site, namely MnSOD, FeSOD and Cu/ZnSOD. Among these generally, MnSOD is located in mitochondria, FeSOD in chloroplasts and Cu/ZnSOD mainly in cytosol and chloroplasts. Constitutive levels of SOD activity were higher in *A. thaliana*, when compared to *A. alpina* (Fig. 2A, B). During the experiment, total SOD activity changed both with UV-B treatments and circadian rhythm in both plant species. SOD activity decreased at 6 h in controls of both species when compared to 3 h. SOD activity of *A. thaliana* decreased

Fig. 1 The effect of UV-B exposure on **A** TBARS, **B** H₂O₂, **C** the maximum quantum yield of PSII. Letters (a–d) above the bars indicate statistically significant difference at $P < 0.05$ (one-way ANOVA

and Tukey's HSD). ANOVA was performed separately for each species. Y-axis of **C** starts from 0.5 to emphasize the difference in the response to treatments

with 3 h UV-B exposure by 42% ($p < 0.0001$), while in *A. alpina*, it did not change significantly. SOD activity was induced by 6 h UV-B exposure in both species, induction was 30% ($p < 0.05$) for *A. thaliana* and 67% ($p < 0.0001$) for *A. alpina*. Overall, the decrease of SOD activity at 3 h was lower in *A. alpina*, while induction at 6 h was higher when compared to *A. thaliana*. Total of five and four different SOD isoenzymes was detected for *A. thaliana* and *A. alpina* representing all three types of SOD, respectively. These isoenzymes were named according to their migration in the native-PAGE gels (Fig. 2A). In *A. thaliana*, one MnSOD (MnSOD2), one FeSOD (FeSOD2) and three Cu/ZnSOD (Cu/ZnSOD2, 4 and 5) isoenzymes were detected. On the other hand, in *A. alpina*, one MnSOD (MnSOD1), one FeSOD (FeSOD1) and two Cu/ZnSOD (Cu/ZnSOD1 and 3) isoenzymes were observed. Among the type of isoenzymes investigated, Cu/ZnSOD had the highest activity in both species.

H₂O₂ produced via dismutation of O₂⁻ or via other metabolic processes such as photorespiration is detoxified by a set H₂O₂ scavenging enzymes including CAT, POXs and APXs [39]. CAT activity of 3 h and 6 h UV-B exposed *A. thaliana* plants were decreased by 40% and 25% ($p < 0.0001$ and 0.005), respectively, as compared to controls (Fig. 3A, B). No differences with UV-B exposure were detected in

the activity of CAT in *A. alpina*. One CAT isoenzyme was detected in both species.

POX activity of *A. thaliana* was sixfold higher than that of *A. alpina* at constitutive levels (Fig. 3C, D). UV-B treatment for 3 h decreased POX activity in *A. thaliana* by 32% ($p < 0.0001$) as compared to its control, while at 6 h control and UV-B treated plants had similar activities. On the other hand, there were no significant changes with UV-B treatment at both time points in *A. alpina*. Only one isoenzyme was observed in native-PAGE gels for both *A. thaliana* and *A. alpina* and isoenzyme pattern did not change with UV-B treatments.

APX is an important component of the antioxidant system, which uses ascorbate as its electron donor to scavenge H₂O₂ [40]. APX is thought to play an essential role in scavenging ROS and protecting cells in higher plants and has a higher affinity for H₂O₂ than CAT and POX [41]. Constitutive level of APX activity in *A. alpina* was 2.2 fold higher than that of *A. thaliana* (Fig. 4A). APX activity was decreased in *A. thaliana* at 3 h by 41% ($p < 0.0001$), while at 6 h, no significant changes were detected when control and UV-B-treated plants were compared. On the other hand, APX activity was increased at 3 and 6 h in *A. alpina* by 37% ($p < 0.0001$) and 35% ($p < 0.0001$), respectively. In *A. thaliana*, GR activity was induced by 17% ($p < 0.0001$) at 6 h,

Fig. 2 The activity staining (**A**) and total activity of SOD (**B**) and contribution of isoenzymes to total SOD activity (**C**) as %. Letters (a–c) above the bars in panel (**B**) indicate statistically significant dif-

ference at $P < 0.05$ (one-way ANOVA and Tukey's HSD). ANOVA was performed separately for each species

while there was no change in *A. alpina* at 6 h (Fig. 4B). In *Arabidopsis*, DHAR activity was not changed at 3 h, while it was induced by 62% ($p < 0.005$) at 6 h (Fig. 4C). However, in *A. alpina*, DHAR activity was induced significantly at both 3 and 6 h treatments by 53% ($p < 0.0001$) and 100% ($p < 0.0001$), respectively.

Similar to ROS, levels of RCS are also controlled by the balance between RCS production and scavenging. While

RCS production seems to be an uncontrolled process catalyzed by oxidation of PUFA with ROS, its scavenging is controlled by enzymes such as AER, ALDH or GST in different cellular compartments [9]. AER activity of *A. thaliana* significantly decreased with UV-B treatment, decreases were 40% ($p < 0.0001$) and 30% ($p < 0.0001$) for 3 and 6 h exposures (Fig. 5A). On the other hand, a slight increase in AER activity by 12% ($p < 0.05$) was observed in *A. alpina*

Fig. 3 The activity staining of CAT isoenzymes and the activity of CAT (A, B), the activity staining of POX isoenzymes and the activity of POX (C, D). Letters (a–c) above the bars in panel (B and D) indi-

cate statistically significant difference at $P < 0.05$ (one-way ANOVA and Tukey's HSD). ANOVA was performed separately for each species

at 3 h. A similar response to AER was observed for ALDH activity in both species (Fig. 5B). No statistically significant change was detected in ALDH activity at either 3 h or 6 h in *A. thaliana*, while it was increased in *A. alpina* at 3 h by 32% ($p < 0.005$). In contrast to AER and ALDH, GST activity measured with CDNB as substrate was maintained in both *A. thaliana* and *A. alpina* with both 3 and 6 h of UV-B treatment (Fig. 5C). However, it should be noted that not all GST isoenzymes have the capacity to scavenge RCS, while most can use CDNB as a substrate [42]. Therefore, GST data should be interpreted with caution.

NOXs plasma membrane-localized proteins involved in production of O_2^- at the apoplast, which is converted to H_2O_2 for signalling purposes [6]. NOX activity of *A. thaliana* decreased in both 3 h and 6 h UV-B exposure by 22% ($p < 0.01$) and 31% ($p < 0.0001$), respectively (Fig. 6). The decrease in NOX activity under UV-B stress might be attributed to excess ROS production and oxidative stress in *A. thaliana*. However, the activity of NOX was induced by 15%

($p < 0.0001$) in *A. alpina* after 3 h of UV-B exposure, while it was decreased by 16% ($p < 0.0001$) at 6 h.

4 Discussion

In this study, we aimed to reveal mechanisms involved in acute UV-B stress tolerance of *A. alpina*, a species that is closely related to *A. thaliana* and adapted to arctic–alpine environments. We hypothesized that *A. alpina*, which can cope with water limitation, fluctuating temperature, and extremely low nutrient availability of arctic–alpine environments [43], should also confer a higher UV-B tolerance than *A. thaliana* and investigated if it is related to maintaining its ROS and RCS detoxification capacity. Indeed, our comparative study demonstrated that *A. alpina* was able to cope with the detrimental effects of UV-B better than *A. thaliana* by maintaining and/or inducing its antioxidant and

Fig. 4 The activity of **A** APX, **B** GR, and **C** DHAR. Letters (a–d) above the bars indicate statistically significant difference at $P < 0.05$ (one-way ANOVA and Tukey's HSD). ANOVA was performed separately for each species

RCS detoxifying enzymes leading to more efficient redox regulation and oxidative stress protection.

Exposure of plants to abiotic stress factors such as drought, salinity, sub-optimal temperatures, or heavy metals can result in an uncontrolled accumulation of ROS, which leads to peroxidation of lipid membranes in the cell [41]. Increased ROS production and lipid peroxidation following damaging UV-B doses is well-documented [2, 44–47]. In line with these previous reports, 3 and 6 h of UV-B exposure drastically promoted H_2O_2 and TBARS in *A. thaliana* (Fig. 1A and B). Although 6 h of UV-B exposure elevated H_2O_2 also in *A. alpina*, the increment was more prominent in *A. thaliana*. Moreover, the change in lipid peroxidation of *A. thaliana* was similar to that of H_2O_2 , while such correlation was not observed in *A. alpina*. These findings imply that *A. alpina* can control the detrimental effects of oxidative stress caused by high-dose UV-B exposure. This was also supported by decrease in the maximum quantum yield of PSII (F_v/F_m), which is an indicator of damage to the photosynthetic machinery under UV-B stress [48]. Decrease in F_v/F_m was more pronounced in *A. thaliana* than *A. alpina* (Fig. 1C). Taken together, our results suggest that *A. alpina*

can control the accumulation of ROS better than *A. thaliana*, which prevents the detrimental effect of oxidative stress caused by UV-B stress, evidenced by TBARS and F_v/F_m results.

Quantification of the activity of the enzymatic components of the antioxidant system has been widely used to unveil the ability of the plants to cope with the oxidative pressure caused by diverse environmental stresses. Previously, it has been reported that UV-B stress can either promote [49] or diminish [18] the activity of SOD. The SOD activity decreased in both species at 3 h, however, the decrease in *A. thaliana* was more prominent (Fig. 2B). This drastic decrease in SOD activity in *A. thaliana* after 3 h of UV-B exposure might lead to the accumulation of $O_2^{\cdot-}$ which can cause peroxidation of lipid membranes (Fig. 1A). However, the SOD activities of 6 h exposed plants were induced again in both species indicating activation of adaptive responses, this time induction was higher in *A. alpina*. Although the total activity of *A. thaliana* at 6 h was higher than *A. alpina*, the contribution of different types of SOD to the total activity was different. In *A. thaliana*, 81% of total activity was Cu/ZnSOD, while 14% was FeSOD and the

Fig. 5 The activity of **A** AER, **B** ALDH, and **C** GST. Letters (a–c) above the bars indicate statistically significant difference at $P < 0.05$ (one-way ANOVA and Tukey's HSD). ANOVA was performed separately for each species

Fig. 6 The activity of NOX. Letters (a–c) above the bars indicate statistically significant difference at $P < 0.05$ (one-way ANOVA and Tukey's HSD). ANOVA was performed separately for each species

remaining 5% was MnSOD. In *A. alpina*, contributions of different isoenzymes were 58%, 35% and 7% for Cu/ZnSOD, FeSOD and MnSOD, respectively, indicating a clear difference between two species in SOD profiles. Moreover, in terms of enzyme activity, FeSOD activity of *A. alpina* was

twice ($0.22 \text{ U mg}^{-1} \text{ protein}$) that of *A. thaliana* ($0.11 \text{ U mg}^{-1} \text{ protein}$). When higher F_v/F_m values of *A. alpina* are considered, these findings indicate that a higher capacity of FeSOD in chloroplasts might contribute to the UV-B tolerance of *A. alpina*.

CAT plays a crucial role in the detoxification of H_2O_2 [39]. Previously, it has been demonstrated that CAT activity can increase [18, 50], decrease [51], or remain unchanged [16] following UV-B stress. In the present work, one isoenzyme for each species was detected in native-PAGE activity gels (Fig. 3A). While the activity of CAT was not affected by UV-B in *A. alpina*, we observed a reduction in the CAT activity of UV-B exposed *A. thaliana* seedlings (Fig. 3B), coherent with the elevated H_2O_2 in these plants (Fig. 1B). Therefore, in this *A. thaliana* vs *A. alpina* comparison, the ability to maintain CAT activity seems to be associated with high UV-B tolerance. POX is also a major scavenger of H_2O_2 in plant cells [41]. Promotion of POX activity due to UV-B irradiation has been shown in a variety of plant species [49]. In the current work, one isoform of POX was detected in both species (Fig. 3C), indicating that POX family related to H_2O_2 scavenging is not diverse in these

species. We also observed that the response of POX activity to UV-B radiation was similar to that of CAT in both of the plants (Fig. 3D). Taken together, it seems CAT and POX are not involved in the regulation of ROS in *A. alpina* under UV-B stress as no statistically significant change was observed in UV-B exposed plants. Moreover, the reduction of the activity of CAT and POX could explain the susceptibility of *A. thaliana* against the oxidative stress caused by UV-B exposure.

In our study, UV-B exposure significantly induced APX activity in *A. alpina* (Fig. 4A). Contrarily, a decline was observed in the activity of APX in *A. thaliana* following 3 h of UV-B exposure. It is also worth noting that the constitutive activity of APX was higher in *A. alpina* than that in *A. thaliana*. Previously, it was shown that APX activity increased in the leaves of potato [50] but remained unchanged in sunflower cotyledons [18] with UV-B treatment. Therefore, the contribution of APX to the regulation of oxidative damage during high-dose UV-B seems to be specific to plant species and developmental stage. Moreover, it has been demonstrated that chloroplastic APXs are inhibited when reduced ascorbate levels are low in the presence of H_2O_2 [52]. Therefore, ROS burst caused by high-dose UV-B could have inhibited chloroplastic APX activity in *A. thaliana* via depletion of reduced ascorbate in the chloroplasts. GR is an important enzymatic component of the antioxidant system as it sustains the glutathione pool in a reduced state [41]. It has been demonstrated that GR activity was induced by UV-B irradiation [53]. On the other hand, in the current work, the activity of GR was not increased with UV-B exposure neither in *A. thaliana* nor *A. alpina* (Fig. 4B). This inconsistency can be due to differences in antioxidant responses between species, and between leaves of different age, and/or developmental stage. DHAR is responsible for the regeneration of dehydroascorbate to its reduced form ascorbate and regulation of the cellular ascorbate redox state, which is crucial for tolerance to various abiotic stresses [41]. It has been reported that DHAR overexpression improves plant tolerance against salinity, drought, ozone, and low temperature [54–57]. Here, we showed that the activity of DHAR was remarkably increased following UV-B treatment in *A. alpina* (Fig. 4C). Furthermore, there was a high correlation between activities of APX and DHAR (Fig. 6), indicating efficient induction of the Asada–Halliwell–Foyer pathway in this species. In *A. thaliana*, however, DHAR did not respond to UV-B and there was no correlation between APX and DHAR activity. Again, these findings underpin the difference of these two plant species in their antioxidant capacity to deal with UV-B-induced oxidative stress.

When activities of enzymes involved in scavenging of H_2O_2 is evaluated in conjugation with SOD activity, which is a major source of H_2O_2 , it can be seen that in *A. thaliana*

there are overlapping responses to some extent. For example, at 3 h of UV-B exposure drastic decrease in SOD activity was accompanied by decreases in CAT, APX and POX indicating a link between their regulation. For efficient protection from oxidative stress, H_2O_2 scavenging system should adapt to changes in SOD activity and it has been demonstrated that overexpression of chloroplastic Cu/ZnSOD can induce both transcription and activity of APX in tobacco [58]. On the other hand, when FeSOD activity was decreased with RNAi, interestingly, APX activity did not follow a similar pattern and its activity was again induced [59]. These reports indicate that increase or decrease in activity and even type of SOD can differentially affect the H_2O_2 scavenging system and the relationship between two systems is also compartment specific. In our case, decrease of SOD accompanied by CAT, APX and POX resulted in increased H_2O_2 and lipid peroxidation in *A. thaliana*. This might indicate various possible scenarios (i) processes other than SOD that produces H_2O_2 , such as photorespiration, was induced, (ii) coordination between SOD and H_2O_2 scavenging was decoupled and a higher decrease in H_2O_2 scavenging capacity occurred, (iii) site of ROS production was shifted to a different compartment where H_2O_2 scavenging capacity was lower.

NOX, the main producer of ROS during oxidative signalling, have essential roles in signal transduction and abiotic stress tolerance [60]. It has been demonstrated that NOXs are also involved in regulation of antioxidant defence and *rbohdf* double mutants of Arabidopsis had decreased antioxidant capacity when compared to wild type [61]. Induction of NOX activity was only observed in *A. alpina* at 3 h, while in *A. thaliana*, it was decreased at both time points. This indicates that *A. thaliana* down-regulates NOX-mediated signalling probably due to excess ROS accumulation, wherein *A. alpina* apoplastic H_2O_2 provided by NOX might still act as a signal molecule at 3 h.

Due to their highly reactive nature, RCS can modify proteins via sulfhydryl oxidation, Michael addition or Schiff base formation causing loss of activity. Therefore, accumulation of high levels of RCS is toxic for the cells and can cause triggering of programmed cell death [62]. For regulation of RCS levels plants utilize different types of enzymes such as ALDH, AER and GST. In *A. alpina* RCS scavenging enzymes were induced or were at the same level with controls under UV-B stress. However, an opposite trend was observed in *A. thaliana*. Since accumulation of lipid peroxides upon UV-B exposure were lower in *A. alpina*, it can be suggested that the ability to maintain high levels of RCS scavenging capacity might be considered as a vital component of UV-B stress tolerance. Similarly, it was previously shown that transgenic plants with higher RCS scavenging capacity was more tolerant to UV-B stress [45]. Moreover, it has been demonstrated that addition of H_2O_2 can inactivate

carbonyl reductase activity in cell-free extracts (Biswas et al. 2020) [11] indicating that increased H_2O_2 can induce RCS levels not only by increasing lipid peroxidation but also by inactivating the RCS scavenging system. When results obtained in our study are considered, these findings are compatible with increased H_2O_2 and decreased RCS scavenging enzyme activity that has been observed in *A. thaliana* UV-B exposure.

We also performed a principal components analysis (PCA) to compare the effect of UV-B exposure on *A. thaliana* and *A. alpina* plants (Fig. 7). In the PCA, the first two axes (PC1 and PC2) together explained a large fraction of the variation (73.7%). As expected, species was the largest source of variation, *A. thaliana* and *A. alpina* were separated along PC1. PC2 also explained a major fraction of the variation (33.5%) and control and UV-B-treated plants were separated at this axis. Especially, the separation between control and UV-B-treated samples was very clear in *A. thaliana*, while *A. alpina* samples that were exposed to UV-B for 3 h were clustered together with control plants. *A. alpina* plants exposed to UV-B for 6 h were diverted from control and 3 h UV-B treated plants, but this separation was small in comparison to that observed in *A. thaliana* plants. This result shows that the effect of UV-B on *A. thaliana* was much more drastic in comparison to that on *A. alpina*. It seems *A. alpina* plants treated with UV-B for 3 h were stable and the effect of 6 h of UV-B exposure was relatively small.

Fig. 7 First two axes (PC1 and PC2) of the principal component analysis performed on the mean values of all the parameters measured in the study. Mean values of each parameter of each group were converted to z scores before applying *prcomp* function in R

Overall, findings related to indicators of oxidative stress (TBARS, H_2O_2) and photosynthetic efficiency (F_v/F_m) indicate that *A. alpina* is more resistant to UV-B induced oxidative stress than *A. thaliana*. Although CAT, GR and POX remained unchanged in *A. alpina*, a significant increase in the activity of SOD (at 6 h), APX and DHAR following UV-B exposure provide evidence for strategies used by this species to counteract oxidative stress. On the other hand, activities of some antioxidant enzymes (CAT, POX, APX) even decreased in *A. thaliana*. In this sense, the ability of *A. alpina* to maintain the activity of these enzymes under stress seems to be vital for its resistance to UV-B. Besides, *A. alpina* was able to mitigate the consequences of oxidative stress by detoxifying lipid peroxidation-derived RCS via ALDH, AER and GST enzymes better than *A. thaliana*. However, it should be noted that the effect of UV-B on plants is a function of both avoidance mechanisms (ex: absorption by pigments) as well as detoxification and repair mechanisms including ROS scavenging. In this case, our work has a limitation in that it does not include information related to avoidance mechanisms, which requires further scrutiny. Another limitation of the current study is the possible interference due to epigenetic adaptation of *A. alpina* to higher UV-B levels when compared to *A. thaliana*, which has been produced under lab conditions for many generations.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The study is in compliance with ethical standards.

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