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Antioxidative Enzymes in the Response of Buckwheat (*Fagopyrum esculentum* Moench) to Ultraviolet B Radiation

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The behavior of the enzymatic antioxidant defense system was studied in buckwheat leaves and seedlings subjected to short-term enhanced UV-B radiation. The effects of UV-B action were monitored immediately after irradiation as well as after recovery. The applied dose induced an increase in lipid peroxidation and total flavonoid content, a decrease in chlorophyll content, and a change in enzymatic digestibility of extracted DNA. The activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase, and soluble peroxidase, as well as the isoelectric focusing (IEF) pattern of peroxidase isoforms, was analyzed. In treated as well as recovered seedlings, soluble and ascorbate peroxidase activities were increased. The activity of SOD was not altered, whereas CAT activity was decreased. In contrast to seedlings, only CAT activity was increased in treated and recovered leaves.

KEYWORDS: Antioxidative enzymes; buckwheat; *Fagopyrum esculentum* Moench; flavonoids; UV-B radiation stress

1. INTRODUCTION

An important environmental factor affecting plant metabolism is increasing UV-B radiation, a consequence of the thinning ozone layer. UV-B (280−320 nm) can damage living organisms because their cellular components, such as proteins and nucleic acids, absorb this energy-rich radiation. UV-B radiation also produces oxidative stress by increasing reactive oxygen species (ROS), such as singlet oxygen, superoxide anions, hydrogen peroxide, and hydroxyl radicals. Many studies have shown deleterious effects of UV-B on plants, including reduced photosynthesis and decreased protein synthesis, together with damage to DNA, proteins, and lipids (*1*, *2*). Other kinds of abiotic and biotic stresses can also induce plants to produce ROS. Recent studies have confirmed that hydrogen peroxide is a signaling molecule, mediating responses to abiotic and biotic stresses in plants (*3*). Plants develop different systems to protect them from UV, including UV-absorbing compounds, and systems to protect from ROS, which are nonenzymatic and enzymatic. Rutin, quercetin, and other flavonoids are secondary plant metabolites that have many physiological functions in plants, including UV-B screening, antioxidant activity, and disease resistance. Nonenzymatic low molecular mass ROS scavengers, for example, glutathione, ascorbate, and tocopherols, have bbeen shown to act as antioxidants in detoxification of ROS. Antioxidants act as a cooperative network, employing a series of redox reactions. The enzymatic defense mechanism includes activities of enzymes regenerating the reduced forms of antioxidants such as ascorbate peroxidase (APX), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR) and ROS-interacting enzymes such as superoxide dismutase (SOD), catalase (CAT), nonspecific peroxidase (POD), ascorbate peroxidase (APX), etc. SOD accelerates the conversion of superoxide anionic radical (O^{2-}) to H_2O_2. CAT, APX, and a variety of general peroxidases catalyze the breakdown of H_2O_2. Antioxidative enzymes can exist in multiple molecular forms with different localizations in individual cell organelles (*4*). A specific role for each isoform in response to oxidative stress can be postulated. POD usually occurs as multiple molecular forms (isoenzymes). The function of these isoenzymes and their regulation remain largely unknown. POD requires H_2O_2 as an essential substrate and metabolizes it to H_2O. Plant peroxidases have been regarded as stress marker enzymes. Also it was shown that specific peroxidase from wheat flour seems to be an important allergen associated with baker's asthma (*5*). Anionic PODs are believed to utilize phenolic compounds to initiate the chain reaction that leads to lignification (*6*).

Common buckwheat is an important agricultural plant thriving at higher altitudes that is not suitable for cereals and rice (*7*). Buckwheat is the only field crop that contains considerable amounts of rutin (a flavonol glycoside) in the cotyledon, leaf, stem, flower, and seed (*8*). Its physiological role in buckwheat has been investigated (*9*, *10*). In a later study Suzuki et al. (*11*) treated the leaves of tartary buckwheat (*Fagopirum tataricum*) with UV-B radiation, cold, and desiccation and showed that rutin and rutin glucosidase activity may be related to enhancement of the defense system against stress. Gaberscik et al. (*7*) investigated the effects of enhanced UV-B radiation on photosynthetic activity, transpiration, and contents of pho-

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tosynthetic pigments and methanol-soluble UV-B-absorbing compounds and showed that UV-B-absorbing compounds and transpiration rate were increased whereas photosynthetic pigments were decreased. Antioxidant activity of buckwheat flavonoids and total phenolics has been well documented (*12*, *13*), and because of these properties buckwheat is an important source of antioxidants in functional foods as well as in modern phytopharmaceutics.

In contrast to evidence about the action of rutin and other flavonoids in the antioxidative response of buckwheat to stress, there are no data about the role of antioxidative enzymes in this process. The aim of this work was to investigate the roles of antioxidative enzymes in the response of common buckwheat leaves and seedlings to enhanced short-term UV-B radiation. The activities of SOD, CAT, APX, and PODs and POD isoform patterns were determined. Besides free radical-dependent damage to membranes (lipid peroxidation), changes in chlorophyll and flavonoid contents, as well as digestibility of DNA, were also investigated in order to understand the overall buckwheat response to UV as a potential source of oxidative stress.

2. MATERIALS AND METHODS

2.1. Plant Material and Treatments. Buckwheat (*Fagopyrum esculentum* Moench), cv. Darja seeds were surface-sterilized with NaClO (0.4% active Cl_2) for 10 min and then thoroughly rinsed with distilled water. They were germinated in Petri dishes, in the dark, at 25 °C. Six days after germination, seedlings were divided into three groups. One group was used as the control and was frozen in liquid nitrogen after 90 min of adaptation in ambient light and then stored at −70 °C until use. The other two groups of seedlings were treated with supplemented UV-B light (radiation 290−320 nm) for 90 min by use of a UV-B lamp (HPQ 100 W Phillips) installed 15 cm above the seedlings. UV light was filtered trough 0.13 mm thick cellulose acetate filters, which cut out the UV-C range, and plants received 49 kJ m^{-2} biologically effective UV-B radiation. After UV-B exposure, one group was immediately frozen in liquid nitrogen, while the other group of seedlings was frozen after 240 min of recovery in the ambient light. Frozen plant material was stored at −70 °C until use.

Buckwheat plants were grown in the experimental field of the Institute of Molecular Genetics and Genetic Engineering in Belgrade. Plants at the stage just before flowering were pulled out from the soil and subjected to UV-B radiation under the same conditions.

2.2. Chlorophyll Content. Extracts for determination of chlorophyll content were prepared from 0.5 g of plant leaves, homogenized in methanol in the dark. Extracts were centrifuged for 10 min at 3500*g*. Supernatants were analyzed spectrophotometrically and the total chlorophyll content was calculated from equations according to ref *14* and expressed in relative units with respect to control (100%).

2.3. Total Flavonoid Content. For determination of total flavonoid content in leaves, freeze-dried samples (100 mg) were extracted with methanol/acetic acid/water (100:2:100 by volume) at room temperature for 1 h. The samples for spectrophotometric analyses were diluted 50-fold. By the method described in ref *9*, 0.2 mL of 5% $AlCl_3$ in methanol or 0.2 mL of solvent was added to 2 mL of diluted sample. After 30 min, the absorbance at 420 nm was measured in both solutions. The concentration was calculated from the difference between each measurement and expressed in relative units with respect to control (100%).

2.4. Crude Extract Preparation for Assay of Enzyme Activity. Frozen leaves and seedlings were ground in liquid nitrogen and the powder was suspended in extraction buffer, containing 50 mM potassium phosphate (pH 7.0) and 0.1 mM EDTA. The homogenates were centrifuged at 15000*g* for 20 min, and the supernatant fraction was used for the assays of enzyme activity. All steps were carried out at 4 °C. Protein concentration in the extracts was determined according to ref *15* by use of a Bio-Rad assay kit and bovine serum albumin as the standard.

2.5. Lipid Peroxidation. The level of lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction as described in ref *16*. The crude extract was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 3500*g* for 10 min, the absorbance of the supernatant was monitored at 532 nm. The MDA content was calculated per milligram of protein in the reaction mixtures, and the level of lipid peroxidation was expressed with respect to control (100%).

2.6. Enzyme Assays. Catalase (EC 1.11.1.6) activity was assayed in a reaction mixture (1.5 mL) composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 2 mM H_2O_2, and 20 μL of crude extract. The reaction was started by adding H_2O_2, and the activity was followed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption (*17*). Catalase activity was expressed as ΔA_{240} min^{-1}(mg of protein)$^{-1}$.

Ascorbate peroxidase (EC 1.11.1.11) activity was assayed as described in ref *18* with a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, and 20 μL of crude extract. H_2O_2-dependent oxidation of ascorbate was followed by monitoring the absorbance decrease at 290 nm. Ascorbate peroxidase activity is expressed as ΔA_{290} min^{-1} (mg of protein)$^{-1}$.

Soluble peroxidase activity was determined in the crude extract by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol, in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM guaiacol, and 20 μL of crude extract. The reaction was started by adding H_2O_2 (final concentration 10 mM). POD activity was expressed as ΔA_{470} min^{-1} (mg of protein)$^{-1}$.

Superoxide dismutase (EC 1.15.1.1) activity was measured according to ref *19*. Crude extract (50 μL) was added to the reaction mixture (1.5 mL) containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 13 mM methionine, 2 μM riboflavin, and 75μM nitro blue tetrazolium (NBT). Riboflavin was added last and the tubes were shaken. The reaction was started by exposing the mixture to cool white fluorescent light. After 15 min the light was switched off, the tubes were mixed, and the absorbance was measured at 560 nm. SOD activity was expressed as ΔA_{560} min^{-1} (mg of protein)$^{-1}$.

2.7. Enzyme Extraction and PAL Activity Assay. Extraction and assays of PAL activity were performed as described in ref *20*. Samples of fresh tissue were homogenized with chilled Tris-HCl (0.05 M, pH 8.8, 4 mL g^{-1} FW), supplemented with β-mercaptoethanol (0.8 mM final concentration), with the addition of 100 mg of hydrated PVP [insoluble poly(vinyl pyrrolidone)]. Extracts after centrifugation were used as the source of crude enzyme. Protein content in the extracts was determined according to ref *15* with bovine serum albumin as the standard.

2.8. DNA Preparation and Enzymatic Digestion. Genomic DNA from leaves was isolated with the Wizard genomic DNA purification kit (Promega). DNA digestions were carried out with *Bgl*II and *Sae*3 restriction endonucleases at 37 °C overnight. Products of DNA digestions were further analyzed by electrophoresis on 1.5% agarose gel.

2.9. POD Isoenzyme Determination. Extracts from each sample containing approximately 10 μg of soluble protein were loaded onto Immobiline gel (Pharmacia, pH range 3−10), and the gel was run by use of a Multiphor II electrophoresis unit (Amersham Pharmacia Biotech). The cathode and anode strips were soaked in 1 M NaOH and 1 M H_3PO_4 respectively, and were placed directly onto the ends of the gel. Prefocusing was carried out for 30 min at 1500 V, and the gel was focused for 90 min at 1500 V at 16 °C. After electrophoresis, the gel was equilibrated with potassium phosphate buffer (50 mM, pH 7.0) for 30 min. The POD isoenzymes were visualized by incubating the gel in 50 mM potassium phosphate buffer containing 5 μM α-naphthol and 0.33 mM H_2O_2 for 15 min (*21*).

To determine the isoelectric points (p*I*s), markers ranging from 4.45 to 9.6 (Bio-Rad, CA) were run on the same gel.

2.10. Statistics. Chlorophyll and flavonoid content, level of lipid peroxidation, and enzyme activities were determined on three independent experiments with three replications each. All data obtained

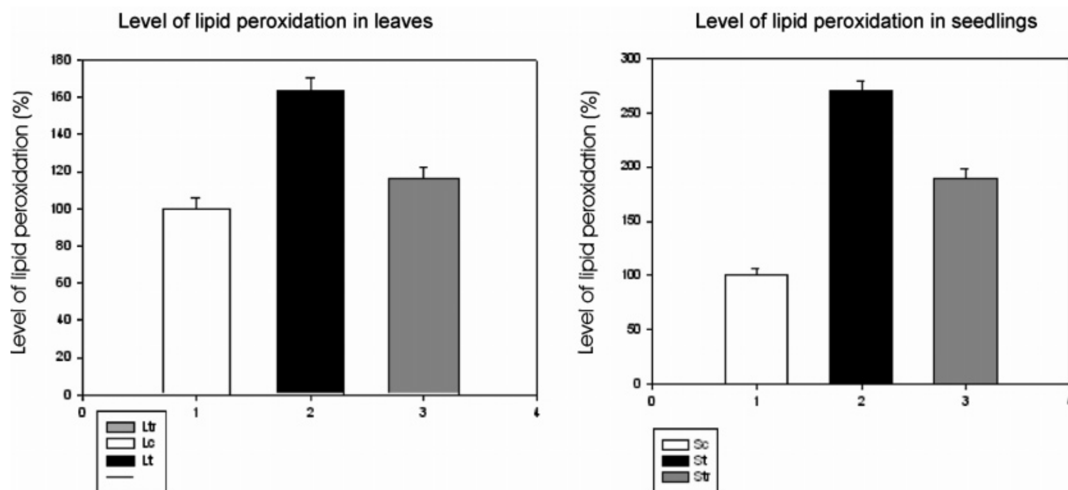

Figure 1. Effect of UV-B treatment on level of lipid peroxidation in buckwheat seedlings and leaves. Values are the means of three different experiments with three replicated measurements ± SE and are shown as a percentage relative to the control (100%). Significant differences $p < 0.05$. Sc, untreated seedlings; St, UV-treated seedlings; Str, UV-treated and recovered seedlings; Lc, untreated leaves; Lt, UV-treated leaves; Ltr, UV-treated and recovered leaves.

were subjected to statistical analysis by the Sigma stat program. Comparisons with $p < 0.05$ were considered significantly different. In all the figures, the spread of values is shown as error bars representing standard errors of the means.

3. RESULTS

3.1. Lipid Peroxidation. The level of peroxidation in buckwheat leaves and seedlings was measured as MDA content, and the effect of UV-B radiation was expressed as the percentage of increase relative to the control (**Figure 1**). MDA content increased 63.5% in treated buckwheat leaves after 90 min of UV-B irradiation with respect to the untreated control. When plants were allowed to recover for 4 h, the MDA content remained 16.18% higher when compared to the control. On the other hand, buckwheat seedlings showed an increase of 169.6% immediately after UV-B treatment and an 89.8% increase after recovery for 4 h in comparison with untreated seedlings. These results indicate the presence of more efficient antioxidative systems in leaves of adult buckwheat plants than in seedlings.

3.2. Chlorophyll Content. After 90 min of UV-B illumination, the chlorophyll content in buckwheat leaves had decreased by 14.3%. After 4 h of recovery, the chlorophyll content was still 3.4% lower than in the control plants (**Figure 2**).

3.3. Flavonoid Content. The results obtained showed that flavonoids were increased by 150% in UV-B-treated leaves and by 30% in treated seedlings. After 4 h of recovery, flavonoid content was still 41% higher in leaves and 9% higher in seedlings, with respect to the control (data not shown).

3.4. Effect of UV-B on Digestibility of Extracted DNA. All investigated extracts from control as well as irradiated leaves contained high-molecular-weight DNA, but DNA digestibility was much greater in extracts prepared from UV-B-treated plants compared with that from nontreated plants. Thus, DNA extracted from treated leaves was digestible by the restriction endonucleases *Sae*3 and *Bgl*II in all 20 individual extracts, whereas DNA from nontreated plants was digestible in 7 out of the 20 investigated extracts (not shown).

3.5. Enzyme Activities. Different behavior of the enzymes with peroxidase activity was observed after UV-B treatment. In leaves, APX and POD activities (**Figures 4** and **5**) remained unaltered after the applied UV-B radiation dose and recovery, but CAT (**Figure 3**) showed increased activity in treated plants

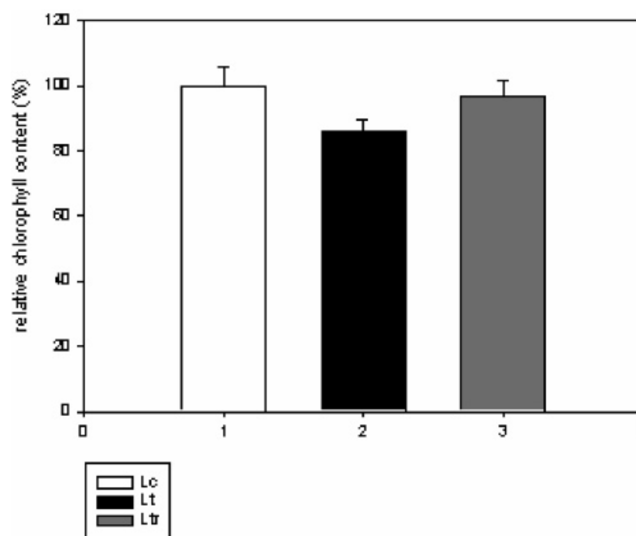

Figure 2. Effect of UV-B treatment on chlorophyll content. Values are the means of three different experiments with three replicated measurements ± SE. Significant differences $p < 0.05$. Abbreviations are defined in the caption for Figure 1.

(about 120% in respect to the control) and even more in recovered plants (about 200%). On the other hand, in seedlings, CAT activity (**Figure 3**) showed no significant difference ($p >$ 0.05) between control and treated plants, while APX and POD (**Figures 4** and **5**) gave similar responses. Thus, the activity of APX and POD increased in the treated plants by about 80% and 40%, respectively, while in the recovered plants the increase was about 150% and 100%, respectively.

The activity of superoxide dismutase was not altered in treated leaves and seedlings compared to the control group (data not shown).

Although PAL is an enzyme included in flavonoid biosynthesis, no significant changes in PAL activity were found in buckwheat seedlings and leaves upon UV-B treatment and recovery (not shown).

3.6. Electrophoretic Pattern of POD Isoforms. The isoenzyme profiles of nonspecific soluble peroxidases obtained from buckwheat seedlings and leaves are shown in **Figure 6**. It is evident that UV-B induced the appearance of basic isoforms in

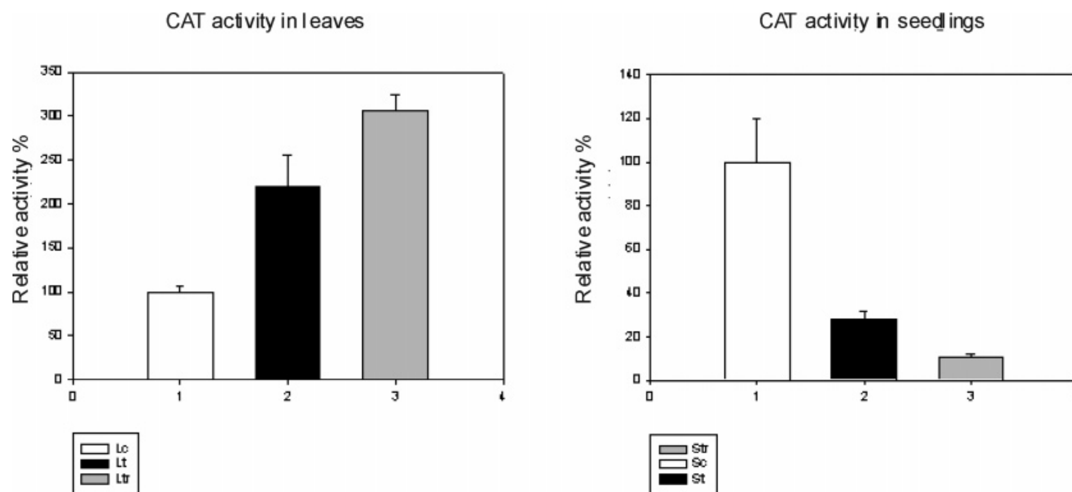

Figure 3. Effect of UV-B treatment on CAT activity in buckwheat seedlings and leaves. Values are mean ± SE based on three replicates and shown as a percentage relative to the control (100%). Abbreviations are defined in the caption for Figure 1.

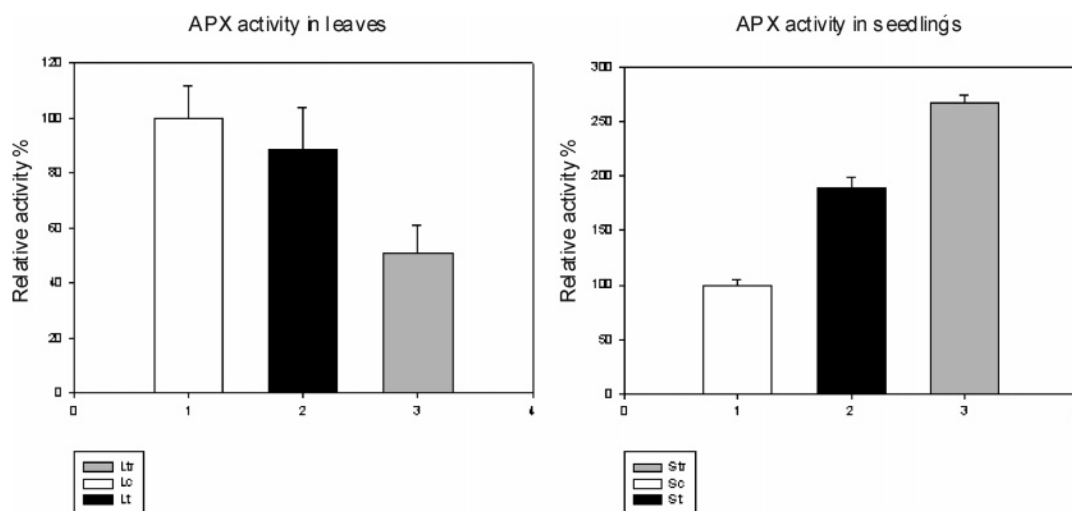

Figure 4. Effect of UV-B treatment on APX activity in buckwheat seedlings and leaves. Values are mean ± SE based on three replicates and shown as a percentage relative to the control (100%). Abbreviations are defined in the caption for Figure 1.

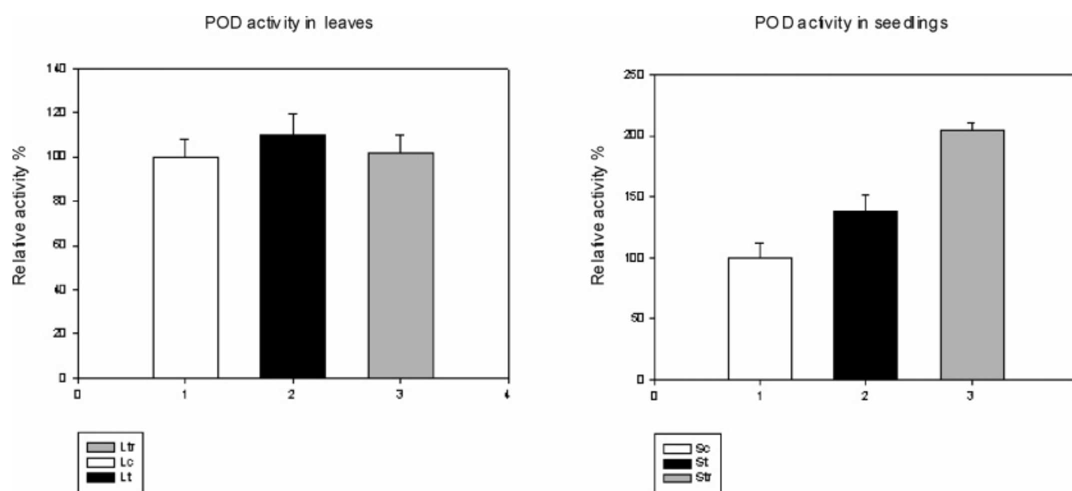

Figure 5. Effect of UV-B treatment on POD activity in buckwheat seedlings and leaves. Values are mean ± SE based on three replicates and shown as a percentage relative to the control (100%). Abbreviations are defined in the caption for Figure 1.

the treated seedlings. Thus, after 90 min of treatment the neutral isoform of p*I* 6.5 disappeared and new isoforms of p*I* 6.2 as well as basic p*I* range of p*I* 8.5−8.8 emerged. Upon recovery, the former forms remained. In leaves, the disappearance of some acidic forms of POD was the main characteristic of the UV-B response. After irradiation for 90 min, forms of p*I* 4.0, 4.2, and 6.3 disappeared. Upon recovery, forms of p*I* 6.7 and 8.8 appeared and form p*I* 4.4 was lost. The appearance of the p*I* 6.7 form correlated with buckwheat leaves recovery, whereas the disappearance of the p*I* 6.3 form was a mark of UV-B action.

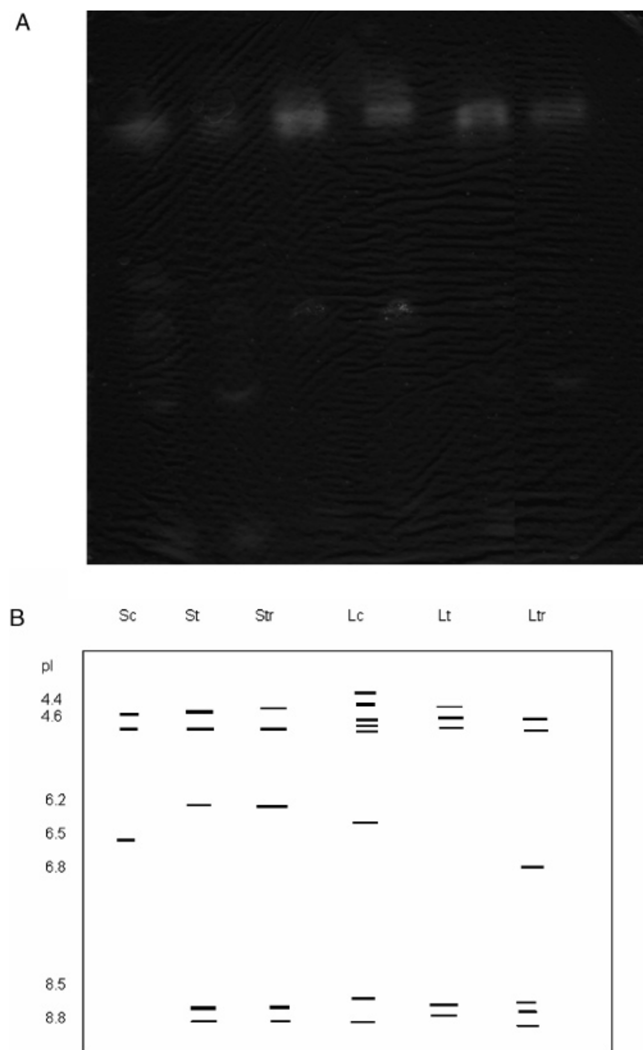

Figure 6. (A) Electrophoretic pattern of soluble peroxidases extracted from the buckwheat seedlings and leaves obtained by native isoelectric focusing. About 15 μg of total protein extract of each sample was loaded on the gel, and staining for POD activity was performed as indicated under Materials and Methods. The data were saved on CCD. (B) Schematic peroxidase isoenzyme patterns obtained by isoelectrofocusing from UV-B-treated buckwheat leaves. Abbreviations are defined in the caption for Figure 1.

4. DISCUSSION

UV-B radiation represents one type of abiotic stress. The effect of exposure to that type of radiation on free radical production and scavenging, as well as on cell membranes in plants, has been well documented (*22*−*24*). It is becoming evident that reactive oxygen species, which are generated during abiotic stress, are recognized by plants as a signal for triggering defense responses. The highly cytotoxic nature of reactive oxygen species requires their accumulation to be under tight control. One type of ROS, hydrogen peroxide, is a moderately long-lived molecule that can diffuse some distance from its production site. H_2O_2 may inactivate enzymes such as those of the Calvin cycle and SOD by oxidizing their thiol groups (*25*, *26*). UV-B radiation may affect many biomolecules in plant cells. In this study it was found that the applied dose of UV-B radiation, given for 90 min, provoked changes in chloroplast, which was exhibited as a 14.3% decrease in chlorophyll content in comparison with the control (**Figure 2**). Decrease in chlorophyll content and disruption of photosynthesis were also observed in common buckwheat treated with enhanced UV-B radiation (*7*). Since after recovery for 4 h the chlorophyll content was only 3.4% less than in control plants, specific mechanisms must have been activated that were able to repair the changes in chlorophyll content. It is already known that free radicals cause peroxidation of membrane lipids as a reflection of stress-induced damage at the cellular level (*27*). The level of MDA produced during peroxidation of membrane lipids is often used as an indicator of oxidative damage. The lower level of lipid peroxidation observed in buckwheat leaves compared with seedlings (**Figure 1**) suggests that leaves may have more efficient protection mechanisms against oxidative damage under UV-B stress than seedlings. Thus, oxidative stress induced by irradiation provoked more serious damage to seedling membranes and, in addition, it seems that seedlings also had less efficient mechanisms to repair membrane damage than leaves. Disruption of membrane integrity by lipid peroxidation and damage to DNA are two different types of molecular damage proposed to cause UV-B-induced growth inhibition in plants (*28*). The most frequent types of DNA lesions induced by UV-B radiation are the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6−4) pyrimidone adducts, commonly referred to as 6−4 photoproducts (6−4PPs). In the present work we analyzed some other potential effects of UV-B radiation on DNA. The results obtained for digestibility of DNA with restriction endonucleases indicate UV-B radiation can modify DNA accessibility to enzymes such as nucleases. The exact mechanism causing this appearance should be examined.

The methanol extract of soluble buckwheat flavonoids contained principally four flavonol glycosides: rutin, quercetin, kaempferol-3-rutinoside, and flavonol triglycoside (*29*). These compounds are produced along the phenylpropanoid pathway, which is unique to plants, and they play diverse roles in essential plant processes, including growth, defense, protection from UV light, and reproduction (reviewed in refs *30*−*34*). UV-B-absorbing compounds belong mainly to flavonoids and contribute to the filtering of harmful rays and mitigate against the effects of free radicals (*35*). UV-B applied treatment induced a considerable increase in methanol-soluble flavonoids, in agreement with previously reported data (*9*). The higher level of flavonoids observed in buckwheat leaves compared with seedlings could be responsible for lower susceptibility of leaves to enhanced UV radiation. In spite of the increased flavonoid content, the activity of PAL was unchanged, suggesting absence of enhanced flavonoid biosynthesis during short-term UV-B action (90 min) and 4 h afterward. Disagreement between the soluble phenolic content and PAL activity was also observed in soybean chilling seedlings (*36*). No change observed in PAL activity in our experiment could be explained by (1) short time of plant exposure and recovery that is not sufficient for de novo synthesis and accumulation of PAL and (2) existence of flavonoids in insoluble forms and structures that could be released upon UV radiation, serving as antioxidants. The exact mechanism responsible for the observed increase in flavonoid content should be further investigated.

In this work we also investigated the activity of some antioxidative enzymes in buckwheat leaves and seedlings. The combined action of antioxidative enzymes is supposed to eliminate reactive oxygen species efficiently and, consequently, protects cellular components against serious damage (*37*). There are many studies on the effects of UV-B radiation on the activity of antioxidative enzymes in different plants (*38*, *25*, *6*) but not in buckwheat. In addition, there is no clear correlation between the activity of these enzymes and the fluctuations in chlorophyll

content, flavonoid content, and the level of lipid peroxidation. According to data obtained, the activities of antioxidative enzymes seem to be differently regulated in different plant organs as well as at different stages of development.

A higher level of flavonoids may also be a signal for activation of rutin glucosidase (flavonol 3-glucosidase; rutin-degrading enzymes). This enzyme converts rutin to its aglycon quercetin, which is a suitable substrate for guaiacol peroxidase (*39*, *40*). In buckwheat, peroxidases from tartary buckwheat bran (*41*) and from buckwheat seed (*42*) have been characterized. In buckwheat seed, two peroxidase isoforms with different substrate specificity have been found. Regarding the fact that plant PODs exist in many different isoforms, we investigated the relationship between UV-B stress and POD isoform pattern in common buckwheat seedlings and leaves. The activity of PODs was increased in treated seedlings (**Figure 5**) and this increase correlated with the appearance of new basic POD isoforms (**Figure 6**). These isoforms were induced by UV-B radiation, and their role could be repair of damage provoked by enhanced radiation. In leaves there are no quantitative changes in POD isoform activity, but changes in POD isoenzyme pattern are evident (**Figure 6**). Observed qualitative changes in POD isoform activity could be attributed to their specific functions in the response to UV stress. Determination of POD isozymes was carried out with α-naphthol (*21*), which is a more sensitive substrate for isoperoxidases than guaiacol.

The different activities of antioxidant enzymes (CAT, APX, POD), which were observed in the treated plants, suggests that leaves and seedlings have different mechanisms of response to oxidative stress caused by enhanced UV-B radiation. The increased CAT activity found in treated and recovered leaves indicates that CAT (which disrupts H_2O_2 to H_2O and O_2) may be the main antioxidative enzyme in leaves. Moreover, the reaction chain, responsible for producing reactive oxygen species during UV-B irradiation of leaves, probably terminates by forming hydrogen peroxide, the natural substrate for CAT. In contrast to leaves, seedlings showed different steps during the production of ROS. The estimated increased level of APX activity indicated that the reaction chain is terminated by forming ascorbate peroxide, an essential substrate for ascorbate peroxidase, which transforms that kind of peroxide to monodehydroxyascorbate. This suggests that ascorbate could play an important role in the response of buckwheat seedlings to UV-B stress. Since the activity of SODs (dismute O^{2-} to O_2) was not altered either in the leaves or in the seedlings, it seems that the part of reaction chain in which SOD is included was omitted or maybe blocked. Studies carried out in rice (*Oryza sativa*) leaves demonstrated that, after UV-B irradiation, CAT and SOD activities were enhanced but no difference was observed in APX activity (*38*). In sunflower cotyledons, the induction of antioxidant enzymes with peroxidase activity indicated that hydrogen peroxide participates actively in the plant response to UV-B (*23*).

In conclusion, this work has demonstrated that short-term UV-B irradiation induced different defense responses in leaves and seedlings of affected buckwheat plants. Among antioxidative enzymes, the main role in the leaves' response could be attributed to CAT, especially during recovery. On the other hand, the main role in seedlings' response to UV could be attributed to APX and soluble PODs. Such differences in antioxidative enzyme activities could be correlated with different developmental and ecophysiological states of seedlings and leaves and especially with different importance of total flavonoids and low molecular mass antioxidants. Qualitative changes in POD activity observed in this work could be explained by considering the specific function these isoforms might play in the buckwheat response to UV stress. The role of particular components of overall antioxidative mechanisms, especially of antioxidative enzyme isoforms and low molecular mass antioxidants, should be further investigated.

ABBREVIATIONS USED

APX, ascorbate peroxidase; CAT, catalase; DNA, deoxyribonucleic acid; EDTA, ethylenediaminotetraacetate; GPX, guaiacol peroxidase; GR, glutathione reductase; IEF, isoelectric focusing; MDA, malondialdehyde; NBT, nitro blue tetrazolium; PAL, phenylalanine ammonia lyase; p*I*, isoelectric point; POD, peroxidase; PVP, poly(vinyl pyrrolidone); ROS, reactive oxygen species; SE, standard error; SOD, superoxide dismutase.

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