

Epidermal Lignin Deposition in Quinoa Cotyledons in Response to UV-B Radiation[¶]

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ABSTRACT

UV-B radiation (280–320 nm) is harmful to living organisms and has detrimental effects on plant growth, development and physiology. In this work we examined some mechanisms involved in plant responses to UV-B radiation. Seedlings of quinoa (*Chenopodium quinoa* Willd.) were exposed to variable numbers of UV-B radiation doses, and the effect on cotyledons was studied. We analyzed (1) cotyledons anatomy and chloroplast ultrastructure; (2) peroxidase activity involved in the lignification processes; and (3) content of photosynthetic pigments, phenolic compounds and carbohydrates. Exposure to two UV-B doses induced an increase in the wall thickness of epidermal cells, which was associated with lignin deposition and higher activity of the peroxidase. The chloroplast ultrastructure showed an appearance typical of plants under shade conditions, likely in response to reduced light penetration into the mesophyll cells due to the screening effect of epidermal lignin deposition. Exposure to UV-B radiation also led to (1) enhancement in the level of phenolics, which may serve a protective function; (2) strong increase in the fructose content, a fact that might be related to higher requirement of erythrose-4P as a substrate for the synthesis of lignin and phenolics; and (3) reduction in the chlorophyll concentration, evidencing alteration in the photosynthetic system. We propose that the observed lignin deposition in epidermal tissues of quinoa is a resistance mechanism against UV-B radiation, which allows growing of this species in Andean highlands.

INTRODUCTION

UV-B radiation (280–320 nm) on the earth's surface fluctuates with the changes in sun position, altitude, cloud cover, atmospheric

turbidity and stratospheric ozone concentration (1). Anthropogenic depletion of the stratospheric ozone layer at the polar zone and also at earth midlatitudes is leading to considerable increment in UV-B radiation on the earth's surface (2–4).

UV-B radiation has been reported to affect the morphology, anatomy, biochemistry and physiology of plants at different levels (5–7). These effects include: reduction in plant height and leaf area (6); increase in palisade parenchyma thickness and spongy intercellular spaces (8,9); increase in the cell length and number in both palisade and spongy mesophylls (10); changes in chloroplast structure, such as increase in granal stacks, dilation of thylakoid membranes and disintegration of the envelope (8,11–13); alteration in cellular metabolism, such as impairment in the photosynthetic function; increase in the antioxidant capacity and accumulation of phenolic compounds (14,15).

Because different plant species exhibit different degrees of UV-B attenuation, depending on their leaf structure and pigment composition, variation in their susceptibility to enhanced UV-B radiation is expected. The response of each species depends on the UV-B radiation intensity and the exposure period (16). Plant responses also seem to depend on whether high or low fluxes of photosynthetically active radiation (PAR) are given concurrently with the UV-B radiation (17).

Most research has been conducted on agricultural herbaceous plants, and little is known about the effects of UV-B radiation on native species that may have developed protective mechanisms (18,19). Despite the large amount of research, several questions remain open to debate because of our poor understanding of the targets and mechanisms involved in plant responses to UV-B radiation (6).

In the present work the effect of UV-B radiation on quinoa, an Andean highland crop, was studied at the anatomical and biochemical levels. Our major finding was the UV-B-induced lignin deposition in epidermal cell walls, which may act as an effective screener against the radiation.

MATERIALS AND METHODS

Plant growth and light treatments. Quinoa (*Chenopodium quinoa* Willd. cv. sajamá) seeds were germinated and grown in plastic boxes containing vermiculite moistened with mineral nutrient solution (Hoagland 1:8) under the following conditions: temperature, 25–28°C; ambient relative humidity,

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Abbreviations: PAR, photosynthetically active radiation.

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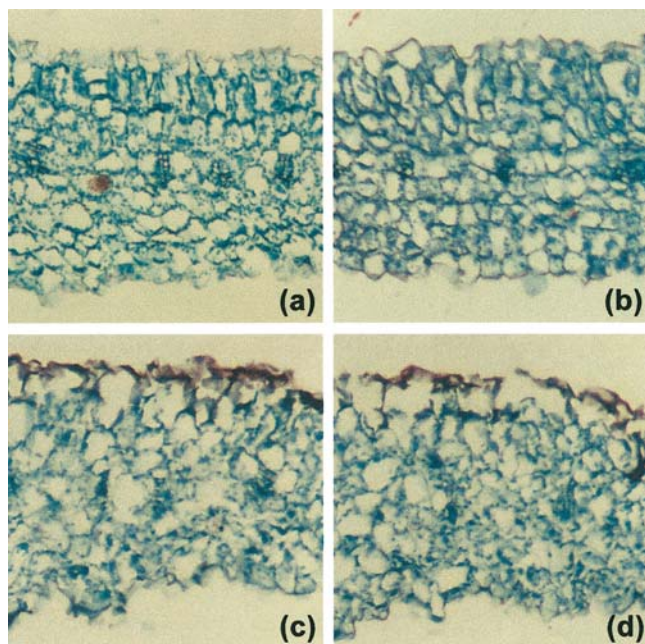


Figure 1. Light photomicrographs of cross sections from quinoa cotyledons with or without UV-B treatment. a: Zero dose (control). b: One dose. c: Two doses. d: Three doses. (200 \times).

60%; photoperiod, 12 h; PAR (400–700 nm), 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the plants.

The UV-B radiation was supplied by fluorescent lamps (UVB-313, Q-Panel, Cleveland, OH) mounted horizontally 60 cm above the tops of the plants. The radiation for control or treated plants was filtered through Mylar or cellulose acetate, respectively, to exclude or transmit UV-B radiation. The irradiance at plant level was 7.5 W m^{-2} as determined with a radiometer (Series 9811, Cole-Parmer Instrument Co., Chicago, IL). Seedlings were UV-B irradiated for 4 h at the middle of the photoperiod (in the absence of PAR radiation) at Day 4 (one dose), or at Days 4 and 5 (two doses) or at Days 4, 5 and 6 (three doses). The cotyledons were harvested at Day 7, at the middle of the photoperiod, to be analyzed as indicated below.

Light and electron microscopy. For light microscopy, cotyledons were fixed in FAA (formalin 33%, acetic acid 100% and ethanol 70% in a ratio of 5:5:90 [vol/vol/vol]) and then embedded in paraffin according to Johansen (20). Cross sections (10 μm) were prepared with a microtome and stained with safranin-fast green (20) before observation with a Carl Zeiss light microscope. The wall thickness was determined from images of the cotyledon cross sections digitized with a charged-coupled device 200E video camera (Videoscope International, Washington, DC) coupled with a Macintosh Quadra 700 computer. Image analysis and quantitation were performed with NIH Image 1.45 software (Rasband W, National Institutes of Health, Bethesda, MD).

For electron microscopy, cotyledons were fixed with 3% glutaraldehyde for 6 h at 4°C and then postfixed overnight with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2). The samples were dehydrated with a graded series of ethanol, ending with 100% acetone and then embedded in Spurr's medium (21) and polymerized overnight in a 60°C oven. Cross sections were cut using an ultramicrotome, collected onto formvar-coated copper grids and stained with uranyl acetate and lead citrate. Sections were observed and photographed in a JEOL JSM35 CF transmission electron microscope.

Peroxidase activity. Peroxidase activity was determined as described by Peyrano *et al.* (22) using syringaldazine as substrate of syringaldazine oxidase, a peroxidase associated with lignification (23). The activity was expressed as the increase in A_{530} per minute and milligram of fresh weight.

Phenolic compounds. Total phenolic compounds were extracted with 90% methanol and quantified using Folin-Ciocalteu reagent as described by Smith (24). Flavonoids were extracted using acidified methanol (79:20:1 vol/vol/vol) according to the procedure of Mirecki and Teramura (25) and assessed and quantified spectrophotometrically at 305 nm. Total betalains

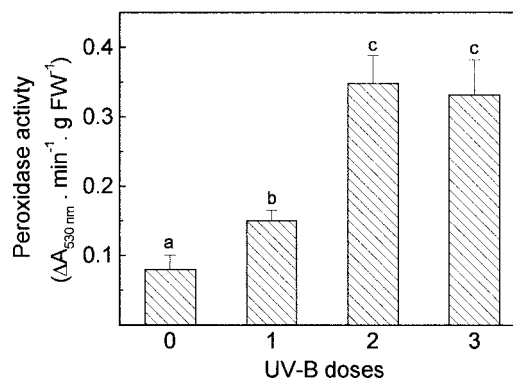


Figure 2. Effect of UV-B radiation on the peroxidase activity in quinoa cotyledons. Each value is the mean \pm standard deviation of three separate measurements. Different letters indicate significant differences between means.

(present in quinoa instead of anthocyanins) were extracted using 50% methanol and quantified spectrophotometrically at 540 nm.

Carbohydrates. Soluble sugars were extracted according to Prado *et al.* (26). Glucose was determined by the glucose-oxidase-peroxidase coupled assay according to Jorgensen and Andersen (27). Total fructose was measured by the method of Roe and Papadopoulos (28) and sucrose by a former modification (29).

Photosynthetic pigments. Chlorophyll was extracted using dimethyl sulfoxide, during 12 h in the dark at 45°C, as described by Chapelle and Kim (30). Chlorophyll *a* and *b* contents were calculated from absorbances at 665 and 649 nm, according to Wellburn's procedure (31).

Data analysis. Data are expressed as means \pm standard deviation. Results were subject to analysis of variance, and differences among means were estimated by Tukey's test ($P < 0.05$).

RESULTS

Effect of UV-B radiation on cotyledons anatomy

We studied the effects of UV-B radiation on cotyledon anatomy by observing safranin-fast green-stained sections under a light microscope. The appearance of cotyledons with one UV-B dose was similar to the control (Fig. 1a,b). They exhibit a dorsoventral structure with adaxial and abaxial epidermis without any alteration. The palisade-spongy cells ratio was 0.3. On the other hand, the cotyledons exposed to two and three UV-B doses showed an increase in the wall thickness of epidermal cells due to lignin deposition as indicated by specific safranin staining (Fig. 1c,d). The epidermal wall thickness in control and one-dose-treated cotyledons was $1.8 \pm 0.2 \mu\text{m}$, whereas that in two- and three-doses-treated cotyledons was $9.3 \pm 1.1 \mu\text{m}$, as quantified with an image analyzer (see Materials and Methods). Thus, there was a five-fold enhancement in the epidermal wall thickness because of UV-B-induced lignin deposition. In cotyledons exposed to two and three UV-B doses, a decrease in the empalisade-spongy cells ratio was observed (Fig. 1c,d).

Peroxidase activity

To evaluate whether the UV-B radiation induces lignification processes, we analyzed the peroxidase activity using syringaldazine as substrate. The level of enzyme activity was slightly increased with one UV-B dose and was enhanced about three-fold with either two or three UV-B doses (Fig. 2).

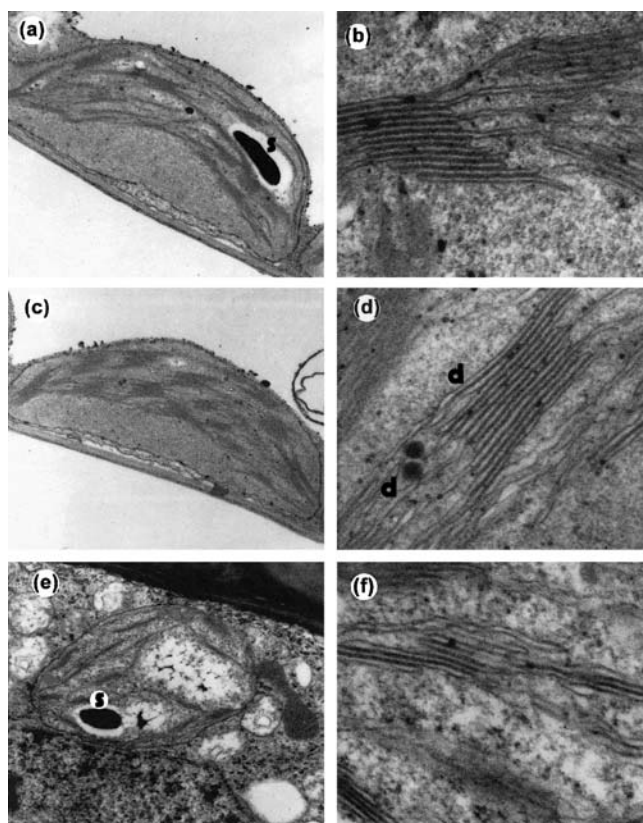


Figure 3. Transmission electron micrographs of palisade mesophyll chloroplasts of quinoa cotyledons with or without UV-B treatment. (a) and (b): zero dose; (c) and (d): two doses; (e) and (f): three doses. s, Starch; d, dilation in the luminal space. Left panels (12000 \times), right panels (56000 \times).

Chloroplast ultrastructure

To evaluate the UV-B effects on the photosynthetic apparatus, we analyzed the chloroplasts anatomy in cotyledons from quinoa seedlings. Figure 3 shows the changes induced by UV-B radiation on chloroplasts of palisade mesophyll cells. Figure 3a,b shows the structure of a control chloroplast. In chloroplasts exposed to one UV-B dose, the grana and stroma thylakoids organization was similar to that in the control (not shown). Exposure to two UV-B doses induced a notable increase in the grana–stromatic lamella ratio (Fig. 3c) and luminal space dilation of the stromatic lamellas (Fig. 3d). Three UV-B doses caused a strong disturbance in the whole lamellar system, including a decrease in the grana thylakoids amount as can be seen in Fig. 3e,f.

Phenolic compounds

We evaluated the effect of UV-B radiation on the content of phenolic compounds from quinoa cotyledons (Fig. 4). As can be seen, total phenols (Fig. 4A) and the flavonoid subclass (Fig. 4B) increase significantly after one UV-B dose, whereas the betalains remain without change (Fig. 4C).

Soluble sugars

Because carbohydrates are central compounds in cellular metabolism and important regulators of different chloroplast functions, we analyzed the effects of UV-B radiation on the soluble

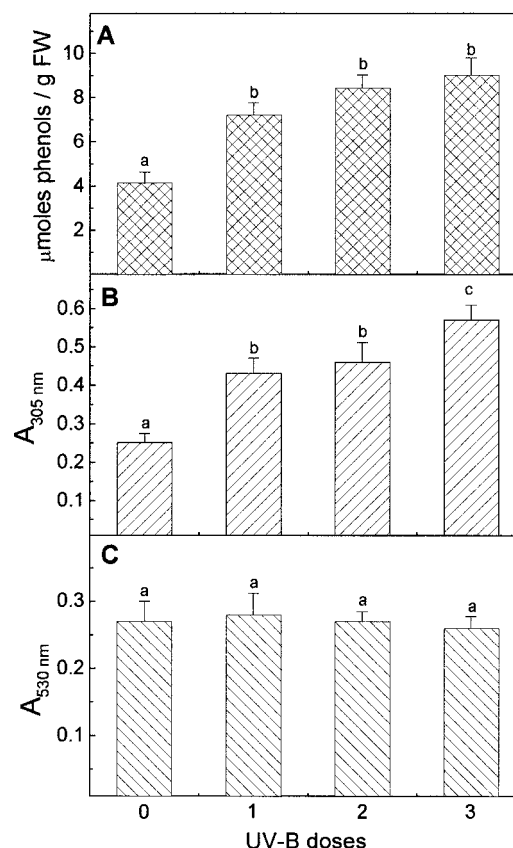


Figure 4. Effect of UV-B radiation on the concentration of phenolic compounds in quinoa cotyledons. A: Total phenols. B: Flavonoids. C: Betalains. Each value is the mean \pm standard deviation of three separate measurements. Different letters indicate significant differences between means.

carbohydrate content of quinoa cotyledons. As shown in Fig. 5A, the glucose content significantly increased with one UV-B dose, whereas with two and three doses the glucose content was similar to that of the control. The fructose content strongly increased (around 10-fold) and the sucrose content decreased with all the UV-B doses tested (Fig. 5B,C).

Photosynthetic pigments

To determine whether the UV-B-induced alterations in the thylakoid membranes were associated with changes in the level of photosynthetic pigments, we analyzed the chlorophyll *a*–*b* content of cotyledons. As can be seen in Fig. 6A, exposure to one UV-B dose caused little effect on chlorophyll *a*, but with two or three UV-B doses there was a significant decrease of 46%. Figure 6B shows that chlorophyll *b* decreased significantly about 30% with one UV-B dose, and 59% with either two or three doses.

DISCUSSION

Plants exhibit avoidance responses to adverse environmental conditions, such as high levels of UV-B radiation, through mechanisms that trigger morphological and biochemical changes to resist (32,33). The most basic mechanism of UV-B resistance is radiation attenuation within cells and tissues by absorption or

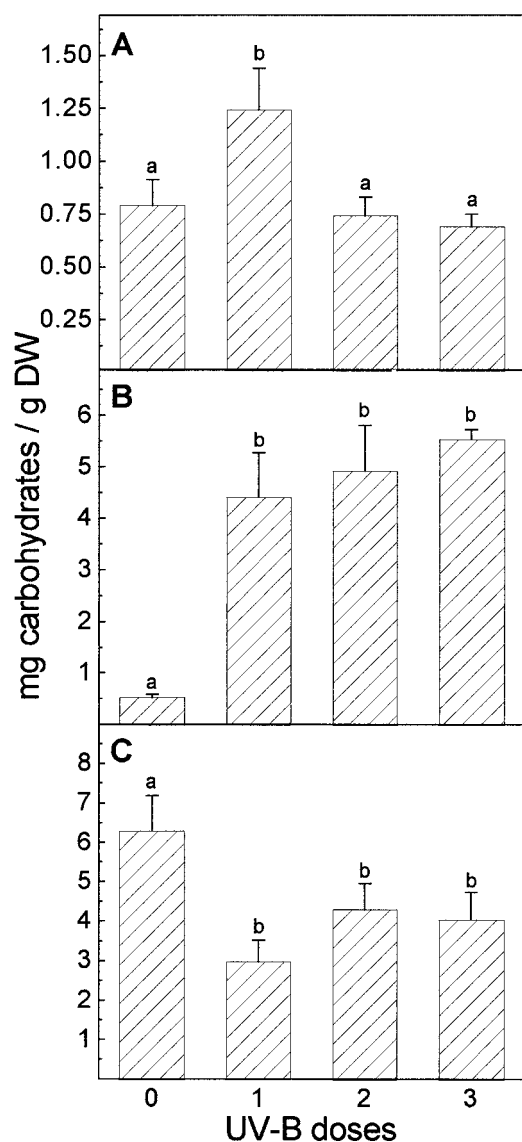


Figure 5. Effect of UV-B radiation on the carbohydrate content in quinoa cotyledons. A: Glucose. B: Fructose. C: Sucrose. Each value is the mean \pm standard deviation of three separate measurements. Different letters indicate significant differences between means.

reflection (34,35). An increase in the leaf content of lignin under enhanced UV-B has been reported (36), and it has been suggested that lignin may be an effective UV screener (6). Data in the present paper support this assumption evidencing UV-B-induced lignin deposition in leaf epidermic cells, which is coincident with increased activity of a specific peroxidase involved in the lignification processes (Figs. 1 and 2). There are not available data about any effect of other source of light (different than UV-B) on the lignin deposition. It has recently been shown that lignification does not only occur in the secondary walls of particular tissues but can also be observed in the primary walls of juvenile actively growing tissues (37). We propose that the observed lignin deposition in epidermal tissues of quinoa cotyledons is a resistance mechanism that attenuates the radiation and allows the plant to grow in the Andean region.

The ultrastructural organization of the chloroplast grana after exposure to two UV-B doses (Fig. 3c) resembles that typical of

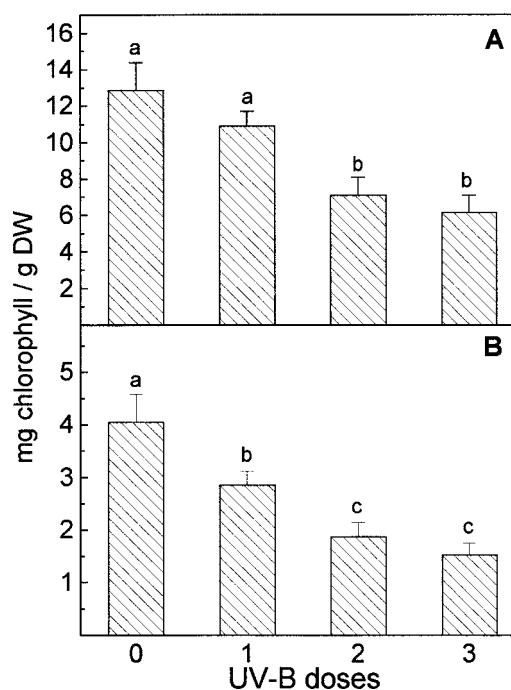


Figure 6. Effect of UV-B radiation on the chlorophylls content in quinoa cotyledons. A: Chlorophyll a. B: Chlorophyll b. Each value is the mean \pm standard deviation of four separate measurements. Different letters indicate significant differences between means.

plants under shade conditions (38). This phenomenon has also been observed in *Brassica napus* exposed to UV-B radiation (12). We assume that this chloroplast organization is a response to the reduction in light penetration into mesophyll cells due to the screening effect of the epidermal lignin deposition.

UV-B radiation reduced the chlorophyll levels in quinoa cotyledons, especially after two or more UV-B doses (Fig. 6). This effect has also been observed in other species (6,13,39).

UV-B radiation increased phenolic concentrations, including the flavonoid subclass (Fig. 4A,B). These are UV-absorbing compounds that have received much attention because of their presumed protective screening function. These compounds may also serve a protective function as antioxidants (6,34). The betalain content remained unchanged under the UV-B treatment (Fig. 4C). Betalains are present in members of the plant order Caryophyllales, such as quinoa, instead of the antocyanins found in members of most plant families (40). Our results are consistent with the idea that antocyanins–betalains do not have an important role in the plant responses to UV-B radiation (6).

Many studies on the effects of UV-B on carbohydrates have been carried out but they have been contradictory, some indicating increases in response to UV-B (13,41,42) and others indicating decreases (43,44). This may reflect the different levels of UV-B irradiation and the diversity of tissues and experimental conditions used in those studies. Under the experimental conditions of the present work, the glucose content increased significantly after one UV-B dose but showed no difference with the control after two or three doses (Fig. 5A). The sucrose content decreased with all the UV-B doses tested (Fig. 5C). To our knowledge, there is no previous report on the effect of UV-B radiation on the fructose content in plants. An interesting finding of the present study is the large enhancement in the fructose concentration with all the UV-B

doses tested (Fig. 5B). This fact might be a consequence of enhanced activity of the pentose phosphate pathway to supply high levels of erythrose 4-phosphate, which is used as a substrate for the synthesis of lignin and phenolics compounds in the shikimate pathway (45). Increased production of erythrose 4-phosphate implies high production of fructose-P because both of them are synthesized in the same reaction catalyzed by transaldolase in the pentose phosphate pathway.

The effect of UV-B radiation on the pentose phosphate pathway activity is an interesting aspect that should be further analyzed in future studies, as well as the UV-B-induced process of epidermal lignin deposition.

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