Effects of UV-B radiation on the Patagonian 
*Jaborosa magellanica* Brisben

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**Abstract**

Treatment of *Jaborosa magellanica* with artificial UV-B radiation caused changes in plant growth, plant chemistry and increase DNA polymorphisms. Spectrophotometric analysis showed that UV-B radiation decreases the chlorophylls content, and increases the amount of UV-B absorbing compounds (e.g., phenylpropanoids). Other UV-induced alterations include reduction in leaf area, alterations in plant architecture, and DNA damage. Using random primers and PCR amplification procedure, a high degree of polymorphism was detected when treated plants were compared to non-irradiated plants. These biochemical changes may be interpreted as plant response to UV-B radiation stress and as an indicator of DNA damage.

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**Keywords:** Chlorophyll *a;* Plant response; Solanaceae; DNA damage

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1. **Introduction**

In the past few decades several climatic changes on the planet have been taken place. Most of these alterations are produced as a consequence of human activities. In this connection, the discovery of the “ozone hole” during the Antarctic springtime represents one of the most important atmospheric events. It is thought that the thinning of the ozone layer results in a substantial increase in the amount of UV-B radiation reaching the ground, especially in those wavelengths which are biologically more active. These assumptions have been corroborated by NASA and local measurements carried out by the Ozone Laboratory of the Physics Department of the Universidad de Magallanes [1]. They have determined that these reductions in the atmospheric ozone may result – at certain periods of the day – in a substantial increase of the amount of UV-B radiation striking the Magallanes Region. For instance, on October the 12th 2002, there was a low ozone occurrence of 41.8% which produced an 80% increase in the integral spectrum of the UV-B range. In general, for every 1% reduction in the ozone concentration, there is a 1.3–1.8% increase in the amount of radiation reaching the biosphere [2].

Several studies have demonstrated the harmful effects of this radiation on living organisms and therefore there is no doubt about the impacts on the local flora and fauna due to the increased UV-B radiation levels reaching the Patagonia Austral. Plants and other sessile organisms are specially affected. Besides other reactions, the effects on plant productivity (biomass reduction), photosynthesis and growth have been described in the literature [3–5]. These alterations are due to the high absorption by proteins and nucleic acids, which are
the major chromophores involved in these physiological and morphological plant responses to UV-B radiation.

Irradiation of DNA itself with UV radiation produces multiple DNA photoproducts [6] which may alter the nucleotide sequence and cause severe mutations during replication [7]. The most common DNA damage is the dimerization of adjacent pyrimidine bases which produces cyclobutane-type pyrimidine dimers (CPDs). This DNA photoproduce and the pyrimidine (6,4) pyrimidone are the most important UV-induced base pair changes [8]. DNA protein cross-links, and DNA strand breaks and deletion or insertion of base pairs can also be induced by UV exposure [9]. All these DNA structural changes may result in induced phenotype alterations, but they may also go undetected (silent mutations). In this latter case, changes may also have occurred in a portion of DNA apart from the gene which does not affect the gene product. On the other hand, changes on the gene may cause significant economic losses in agriculture, delay flowering and diminishing flower production and lifespan [10]. Nevertheless, this situation may be diminished if more stress-resistant plant varieties are identified.

When plants are exposed to UV-B radiation the enhancement of the secondary metabolite production has been detected. In fact, an increase in flavonoids and anthocyanins has been reported and it has been assumed that plants can use phytochemical defenses to avoid this harmful radiation [11–15].

Jaborosa magellanica is an herbaceous, perennial plant with a strong main root and stems which reach 5–30 cm in height. Leaves are arranged in bunches and axillary flowers in groups. Its fruit is a brown-grey berry with numerous seeds. Flowering starts in November and lasts until December. It is found in the open sandy coast of the Magellanes Region.

In this work, we set up experiments to study the effects of UV-B radiation on J. magellanica, a plant which grows in the Magallanes area directly below the “ozone hole” produced in the Austral springtime.

2. Materials and methods

2.1. Plant material

Seeds of J. magellanica were collected in 1999, near Seno Otway, in the suburbs of Punta Arenas city (52°59'S–71°14'W). They were maintained at 0 °C for two months as a chilling treatment. Seeds were sown on several plastic trays, containing a soil mixture of sand (40%) and compost (60%). After 60–65 days' growth, plants were transplanted into individual plastic pots, filled with the same compost mixture and were then moved into the UV-B chamber the following day. Plants were watered every 1–2 days and ambient daytime temperatures inside the UV-B chamber were 27–32 °C and relative humidity 38–42%. For all measurements leaf samples were taken from leaves developed in an enhanced UV-B ambient.

2.2. Light sources

In addition to greenhouse sunlight (PAR: 1200 μmolm⁻²s⁻¹), UV radiation was provided by four fluorescent tubes (Philips TL 20W/12; 1220 mm×1000 mm). In order to keep the spectral quality of irradiance output relatively constant, these tubes were 70 h pre-burnt [16]. The UV-B radiation supplementation was obtained by shielding these tubes with a presolarized (7 h) cellulose acetate film (0.075 mm thickness) which cuts off all wavelengths below 280 nm. To avoid UV photo degradation of acetate film, the filters were changed after 90 h exposure [16,17].

2.3. UV treatments

Plants were continuously exposed to 9 h of daily UV-B irradiation and harvested after 51 days of exposure. Lamps were placed on a mobile rack and hung at 0.30 m above the plants. We chose this distance because it produces a daily UV-B radiation fluence rate of 4.5 mWm⁻², which is within the average values measured in Punta Arenas area during 2000. For instance, under “normal ozone” conditions, the irradiance at 300 nm on 15 October was 2.23 mWm⁻². On the other hand, under “perturbed ozone” conditions 14.21 mWm⁻² was detected [1]. After irradiation, the UV absorbing films were removed in order to give the same light conditions to all plants.

2.4. Radiation measurements

The spectral irradiance levels of plant height below the lamps were measured with a photometer/radiometer (Solar Light Co. PMA 2200 1.10). The spectral distribution of UV radiation provided by these UV-B tubes has been previously reported [12,13]. The radiometer was calibrated using a NIST traceable 1000 W tungsten filament quartz halogen lamp.

2.5. Experimental design

The experimental pattern used in this study corresponded to a randomized complete block design with several variables. Twenty plants (60–65 days old) were distributed in two groups inside the experimental chamber: control group (−UVB) and treated group (+UVB). A UV absorbing plastic film was used to isolate both groups. Pot positions were randomized within each group every two days to minimize position effects. The
lamp rack height was adjusted to maintain the UV irradiance levels once a week.

2.6. Growth variables

Leaf area of adaxial epidermis and leaf length were determined by an imaging processor (IMAGE-PRO PLUS 4.1) of digital photographs (MAVICA MVC-FD 71). Leaf fresh weight was measured in a digital balance (PRECISA XT 120A). Stem elongation (plant height) was measured from soil (pot) level to the top of the plant. Leaves used in fresh weight, area and length measurements were collected from the top half of plants (first stage). Each harvest was carried out on five different plants.

2.7. Photosynthetic pigments

Several leaf samples from the top half of plants were analyzed. Total chlorophylls and carotenoids were extracted from individual leaf disks (1 cm²) with 5 ml of DMSO for 12 h at 65 °C in the dark. Absorbance was determined at 664, 648 and 470 nm on 1 ml of samples and absorbance spectrum recorded between 200 and 700 nm (Shimadzu UV-160A spectrophotometer). Photosynthetic pigment concentrations were calculated according to equations given in Chapelle et al. [18].

2.8. UV-B absorbing compounds

Internal UV-B absorbing phenolics were extracted from leaves by grinding them using a pestle and mortar with 2 ml of MeOH:H₂O:HCl = 79:20:1 (v/v). Homogenates, combined with further washing of the pestle and mortar with 1 ml of the same solvent mixture were centrifuged at 3000 rpm for 10 min. Supernatants were then filtered (Whatman N° 1) and evaporated to dryness at 40 °C. Residues were redissolved in MeOH. Quantitation of UV-B absorbing compounds was carried out following the procedure of Caldwell [19] and Mirecki and Teramura [20].

2.9. Isolation of chromosomal DNA from leaves

Total plant DNA for PCR was isolated from first stage leaves using a CTAB protocol described by Doyle and Doyle [21] and modified as follows. Frozen tissue (0.5–0.75 g) was ground in a pestle and mortar in liquid nitrogen and homogenized in 5 ml of preheated (60 °C) extraction buffer containing: 1 M Tris–HCl (pH 8.0); 1.4 M NaCl; 20 mM EDTA; CTAB 3% (w/v). The mixture was incubated for 20 min at 65 °C with occasional mixing by gentle swirling. DNA was extracted twice with an equal volume of chloroform:isoamyl alcohol = 24:1 (v/v) and precipitated with one volume of cold isopropanol. The precipitated DNA was extracted with a glass hook, washed in 70% (v/v) ethanol for 5 min, dried at room temperature and re-suspended in autoclaved water. Contaminating RNA was removed by digestion with RNase A. The DNA was further purified using chloroform/isoamyl alcohol extraction and re-precipitated with ethanol and re-suspended in autoclaved water.

2.10. RAPD and inter simple sequence repeat PCR

Random decamer oligonucleotide primers purchased from Life Technology were used as single primers for PCR. The primers were designed according to previous published reports [22]. The reaction mix for a 25 µl reaction comprised 20 ng of template DNA, 0.5 mM primer, 0.2 mM each of the dNTP’s and 0.5 units Taq polymerase (Perkin–Elmer) in 1x reaction buffer supplied by the company. Amplification by PCR was performed using two thermocyclers a MJResearch (PTC-100) and a Perkin–Elmer Gene Amplifier (PCR system 2400) according to the following programme: denaturation 4 min (94 °C), then 40 cycles of 1 min at 94 °C, 1 min at 34 °C, and 2 min at 72 °C.

ISSR primers were obtained from The University of British Columbia (Vancouver, Canada) set #9 and listed in Table 4. They were used for PCR amplification with the following protocol. PCR reaction mixture (20 µl) containing: one unit of Taq DNA polymerase, 1.5 mM MgCl₂ buffer, 0.2 mM of each dNTP, with 0.3 µM of a single primer and 10 ng of genomic DNA. PCR amplifications were performed with a MJR PTC-100 Thermocycler using the following program: 30 cycles of 1 min at 94 °C, 2 min at 55 °C and 30 s at 72 °C, with a final 5 min period at 72 °C.

2.11. Reproducibility of amplification products

The amplification procedure was carried out in duplicate each time. Bands were only considered reproducible when the same DNA pattern was obtained in at least two amplification runs using DNA isolated on different occasions. Two thermocyclers were used to determine variation between machines.

2.12. Separation of PCR products

RAPD products were separated on agarose gel 2% and ISSR–PCR products were separated by electrophoresis on 5% urea polyacrilamide gels and stained with silver to visualize the bands [22].

2.13. Data analysis

ISSR markers were treated throughout as 'genetic' phenotypes. The DNA profiles were recorded with presence (1) or absence (0) of bands and the results assembled in a data matrix table. A distance measurement
was calculated from the binary ISSR data matrix table using correlation coefficient (CORR). These distances were used to carry out principal components analysis (PCA) using NTSYSpc [23].

2.14. Statistical analysis

On the basis of the experimental design utilized in this study, the data were analyzed using procedures for a randomized complete block design. Statistical assessments (ANOVA, LSD test) were performed for all measurements using the SAS 8.2 System Statistical Package.

3. Results

3.1. Internal UV-B absorbing compounds

The UV spectrum of MeOH extracts of the internal leaf phenolics showed two major absorption peaks at 293 and 332 nm and two minor peaks at 403 and 410 nm. TLC analysis of MeOH extracts revealed the presence of several unknown compounds. Using the typical fluorescence under UV/NH₃ as a basis, 2–3 compounds (RF=0.8 and 0.6) were partially identified as hydroxycinnamoyl derivatives. Because of their absorption spectra ($\lambda_{max}$ 270–310 nm), these compounds are likely to participate in the protection of the plant against UV-B radiation. In order to compare the effects of UV-B irradiation on these internal UV-B absorbing compounds, quantitation was carried out by measuring the MeOH extracts absorbance. Table 1 shows the absorbance at 300 nm of control and treated plants after 51 days of UV-B treatment. A clear increase in the absorbance of irradiated plants is observed. This is a well known plant response to UV-B radiation which is based on the absorption bands of hydroxy-cinnamic esters being within the UV-B range.

3.2. Photosynthetic pigments

The photosynthetic pigments, chlorophyll $a$, chlorophyll $b$ and carotenoids have decreased values in treated plants compared with controls. Table 2 shows the effect of the irradiance treatment after 51 days of exposure. The highest decrease is observed in both chlorophyll $a$ (64.2%) and chlorophyll $b$ (63.3%) contents. The carotenoids were not damaged (4.5% decrease but differences are not statistically significant) as chlorophylls, although carotenoids help to protect the chlorophylls from photodestruction [24]. Therefore, the biosynthetic pathway of chlorophylls might be more influenced by UV-B radiation than that of carotenoids. The reduction in the chlorophylls content may also be related to the photomorphogenic effects found on plant biomass as shown by the major alterations in plant height (Fig. 2), leaf shape and leaf area of the irradiated plants (Fig. 1).

3.3. Growth

In addition to the effects on the photosynthetic pigments, other important physiological processes determining morphology and plant growth were severely affected by UV-B radiation (Fig. 1). Clearly, irradiated

![Fig. 1. Comparison between leaves from non-irradiated plants (left) and leaves from irradiated plants (right).](image-url)
Plants have smaller leaves than control plants. Table 3 shows that leaf area was decreased by about 92%, leaf length decreased by up to 72%, and leaf fresh weight was decreased by about 88% in irradiated plants. These results indicate that the physiological development of leaves is affected by UV-B radiation. This negative effect on plant production is characteristic of “sun adapted” plants which are smaller, with less leaf, stem, and root, therefore, total biomass [25].

The daily rate of growth is given in Fig. 2 from which it is clear that although irradiated plants grow until day 14, they do so more slowly than controls and at 14 days start to actually decline. On the contrary, non-irradiated plants continue to grow even after 28 days and at that point the difference between both groups is quite clear. This effect of reducing plant height is a well known response of sensitive plants and has also been found in Cucumis sativa [26], Glycine max [27], Hordeum vulgare [28] and Phaseolus vulgaris [29]. These plants have shown that these changes are fluence and wavelength dependent, and the most effective period occurs during the transition from vegetative to reproductive stage.

### 3.4. Analysis of the polymorphisms

DNA was extracted from the different leaves and bulked for PCR analysis. The amplifications were performed in triplicates. Eleven out of twenty-two different primers tested were able to generate amplification products. Polymorphism was detected when irradiated and non-irradiated DNAs were compared using both RAPD and ISSR primers. New fragments were amplified on irradiated samples, but in some cases the loss of fragments was also detected on the gel (Fig. 3). When a dissimilarity index was calculated, taking into account all of the bands amplified, a 60% differences was found between the bulked samples. This provides clear evidence of the direct effect of UV-B radiation on DNA structure.

<table>
<thead>
<tr>
<th></th>
<th>Area</th>
<th>Length</th>
<th>Fresh weight</th>
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<tr>
<td>Control</td>
<td>9.44±0.3</td>
<td>11.00±0.23</td>
<td>0.904±0.025</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.72±0.3</td>
<td>3.10±0.23</td>
<td>0.110±0.025</td>
</tr>
</tbody>
</table>

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**Table 3**

Effects of UV-B radiation on leaf area, leaf length and leaf fresh weight.

- Mean area (cm²; N=15), mean length (cm; N=15) and mean fresh weight (g; N=15) and standard error, after 51 days of treatment.
- Means are statistically significant as determined by LSD in ANOVA: DF=1; F=432.50; P<0.0001.
- Means are statistically significant as determined by LSD in ANOVA: DF=1; F=607.30; P<0.0001.
- Means are statistically significant as determined by LSD in ANOVA: DF=1; F=509.78; P<0.0001.

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**Fig. 2.** Daily increase in stem elongation (growth rate) for control and treated plants. Each point represents the average of five plants. Means are significantly different at P<0.05 level by LSD in ANOVA. DF=1; F=53.42; P<0.0001.

**Fig. 3.** Amplifications profile from irradiated and non-irradiated bulked samples. Lane (a): non-irradiated samples. Lane (b): irradiated samples. RAPD primers used P3, P5; ISSR primers used 855–840–825–811. (M) molecular marker 100 bp ladder.
4. Discussion

After 51 days of UV-B treatment, J. magellanica plants, showed visible manifestations such as chlorosis or bronze leaf discoloration. In addition to these well-known damaging effects, there were other physiological and morphological changes which will be discussed in more detail.

The effect of UV-B radiation on the light harvesting complexes has often been reported [25,30,31]. However these studies showed inconsistent results in the relative change in the constituent photosynthetic pigments. In this study, the photosynthetic pigments seem to be altered after UV-B irradiation. It can be observed that the treated group has decreased values in the chlorophylls content. On the contrary, carotenoids were not affected (differences between groups are not statistically significant). Since the ratio chlorophyll a:chlorophyll b is similar in both groups of plants these responses may be interpreted as a regulation process rather than a damage result. However, the experimental evidence resulting from the negative effect on plant morphology, decrease in leaf area, size, stem elongation and plant biomass production indicates significant plant damage. This interpretation is well supported by literature where a decrease in plant biomass production has been well correlated with a decrease in the photosynthetic pigment content [32]. On the other hand, the positive responses or regulation processes have often been related to an increase in the chlorophylls and carotenoids content. These responses are regarded as a plant strategy to dissipate this harmful radiation from inner tissues avoiding irreparable damage to photosynthetically important membrane systems [33,34]. In this connection, Middleton and Teramura [25] have pointed out that an increase in the light harvesting complexes in UV-B irradiated plants might also provide enhanced photo-protection via higher concentration of xanthophylls.

Morphogenic modifications observed in this study (reduction in leaf area and leaf length) indicate how plants try to avoid penetration of this radiation to the inner tissues by decreasing the exposure area of their main absorptive structures. Milthorpe and Newton [35] have pointed out that leaf size is determined by three factors: (1) the rate and duration of cell expansion; (2) the rate and duration of cell division; (3) the number of cells in the leaf primordium. The third factor may be ignored in the present context as since the leaf primordial tissue is well protected from sunlight and therefore the UV-B radiation does not affect it. However, UV radiation seems to affect cell division processes, as was observed on germinated seeds exposed to UV-B radiation which generated dwarf plants with small leaves. Seemingly, a change in the photoreceptors can induce this condition and the significance of several photoreceptors and signal transduction pathways as a response mechanism has been demonstrated in Arabidopsis and various other plant species [36–38].

Chlorophyll contents were severely affected and may be the cause of the small size observed in irradiated plants. A similar deleterious effect on plant height has also been found in alfalfa [39], cucumber [26] and, Gnaphalium spp [12,13]. According to the literature, this negative effect on plant growth may be a consequence of changes in plant hormones [26], or be a consequence of direct DNA damage caused by the damaging action of UV-B radiation [39,40], as we have found here.

Previous analyses of flavonoids and associated phenolics in plants subjected to UV-B stress have either been carried out on total leaf content or else on the internal leaf constituents. In the present study on J. magellanica attention has focused on internal phenolics collected by grinding leaves in acidic aqueous methanol. Our results show that significant increases occur in these internal components after UV-B irradiance. Apparently, plants activate other mechanisms – different to those involved in the light harvesting complexes – to deal with enhanced levels of UV-B radiation. These findings tally with those reported in the literature showing that plants accumulate unidentified UV-B absorbing compounds (reported as absorbance at 300 nm) after irradiation with UV-B light [11,13,19,41–46]. In this way, plants respond to UV-B stress by synthesizing greater amounts of vacuolar metabolites which can efficiently dissipate high levels of this radiation. The results also agreed with Cooper and Bhattacharya [47] who have suggested that hydroxycinnamic acid is significant in plant UV-B protection. In a manner similar to the beginning of the evolution of land-plants (470×10⁵ years BP), when the atmospheric ozone was reduce [48] and plants were confronted with high levels of UV-B radiation [49], cinnamic acids and their derivatives play an important role in plant defense against UV-B radiation.

It is thus clear that the results observed in this study, such as increase in the phenylpropanoids content and the reduction in both chlorophyll content and biomass of irradiated plants, are in agreement with those in reported literature, for example in Gnaphalium spp, bean, Colobanthus sp, soyabean and barley [13,41,47,50,51].

When the genetic polymorphisms were analyzed a small degree of similarity was detected. In general, primers are used in diversity studies to demonstrate the differences between individuals from a plant population [22]. The bulking of samples are used to reduce the natural genetic variation of the individuals from the population and emphasize particular differences. In our samples, obtained from the same species, a high level of similarity is expected (between 80% and 90%). However, statistical analysis showed only a 40% similarity between plants after treatment and
the control, indicating effects at DNA level due to UV-B radiation and changes at nucleotide sequences which should be complementary to the primer sequences (see Table 4).

UV-B irradiated plants showed morphological, biochemical and genetic differences compared to non-irradiated plants. These findings support the idea that plants respond to UV-B radiation, using different strategies. The molecular response is oriented to minimize the external disturbances to which they are exposed in their natural habitat. However, when these disruptions exceed the natural levels to which they are exposed during the initial periods of growth, plants must use a complex mechanism integrating different pathways to survive [52]. More attention should be paid to in the changes at genetic level, particularly those involved in metabolic pathways. Even if UV-B effects are difficult to detect in natural conditions, they play a role in natural and managed ecosystems. The integration of molecular, chemical, physiological and biochemical information could provide some details about the actual responses to this radiation.

Acknowledgements

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References


Table 4
List of primer sequences used in this study

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<th>Primer</th>
<th>Primer sequence (5’–3’)</th>
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<tr>
<td>P3</td>
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<tr>
<td>P4</td>
<td>AGCCACGCGAA</td>
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<td>P5</td>
<td>GAAACGGGTG</td>
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<td>GGTTAAGGCC</td>
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<tr>
<td>888</td>
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</table>

Where B: (C,G,T); D: (A,G,T but not C); R: (A,G); Y: (C,T); V: (A,C,G).