



Changes in Chlorophyll *a* fluorescence and DNA as a plant response to UV-B radiation in *Gnaphalium vira-vira*

Pedro CUADRA^{1*} , Víctor FAJARDO¹ , Paula PIMENTEL² ,
M. Alejandra MOYA-LEON³ and Raúl HERRERA³

¹ *Universidad de Magallanes, Facultad de Ciencias, Avenida Bulnes 01890, Punta Arenas 620000, Chile*

² *Centro Estudios Avanzado de Fruticultura (CEAF), Avenida Salamanca s/n, Los Choapinos, Rengo 2940000, Chile*

³ *Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad de Talca, Avenida Lircay s/n, Talca 3465548, Chile*

* corresponding author <pedro.cuadra@umag.cl>

Abstract: Ozone depletion at southern latitudes has recently increased the fluence of ultraviolet-B (UV-B) radiation striking the ground. This phenomenon has sparked much interest in unravelling the effects of this harmful radiation on living systems. UV-B radiation triggers several responses that affect plant physiology, morphology and biochemistry. In this study, the effect of supplemental UV-B radiation on DNA profile and chlorophyll *a* (CHL *a*) fluorescence characteristics were analyzed. An increase in the genetic variability of irradiated plants was observed in the Inter Sequence Simple Repeats products. The effect on photosynthesis was studied through fluorescence emissions. The obtained data showed that photochemical quenching (qP) decreased in irradiated plants. This effect may be attributed to a decrease in the number of open reaction centers of photosystem II (PSII) as suggested by the decreased values of minimal and maximal fluorescence. Likewise, non-photochemical quenching (NPQ) increased in both control and irradiated groups, but treated plants presented lower NPQ values than controls. The heat dissipation mechanism was also altered, probably due to a decrease in the yield of the maximal fluorescence in light-adapted leaves (Fm'). According to these findings, UV-B radiation affects the CHL *a* fluorescence mechanisms and modifies DNA profile.



Consequently, these changes influence the yield and growth of plants, which is an important consideration given the current climate change situation.

Keywords: Antarctic, SII reaction center, DNA damage, photochemical and non-photochemical quenching.

Introduction

In recent decades, the level of UV-B radiation striking the earth surface has increased significantly in the southern hemisphere as a consequence of ozone depletion during the Antarctic springtime (Barnes *et al.* 2019). The thinning of the ozone layer at this time of the year allows the entrance of UV-B rays in an inverse relationship. For instance, a small reduction in ozone concentration can almost double the flux of radiation received in the Earth ecosystems. At local level (53.2° S 70.9°W), a decrease of 30% in the ozone column, *e.g.*, 233 Dobson Units, DU, resulted in an increase up to 4947 Jm⁻² of UV-B radiation at ground level, the highest amount of UV-B radiation recorded in the last eight spring seasons until 2002. There was an increase in the number of sunburn cases in the local population (Abarca and Casiccia 2002) in particular on these days with low ozone concentration (November 21 and December 5 1999). Similarly, Chiodo *et al.* (2017) measured an increase of up to 3.8 Wm⁻² of UV radiation due to ozone reductions in the Antarctic stratosphere. This effect is attributed to the exacerbated thinning of the ozone layer caused by humankind activities which release chlorofluorocarbons (CFCs) into the atmosphere (Bais *et al.* 2018). Despite UV-B radiation (280–320 nm) being a small part of the solar spectrum it constitutes a remarkable environmental threat which affects different physiological, morphological and biochemical plant processes. This has led to major concerns about the effects of UV radiation in plant ecosystems. Damaging effects of this radiation have been demonstrated by many studies (Ries *et al.* 2000; Vass, 2012; Manova and Gruszka 2015; Jansen *et al.* 2017). Similarly, the impacts on the local flora and seaweeds due to enhanced UV-B radiation in Southern Patagonia have been reported (Cuadra *et al.* 2004, 2020; Navarro *et al.* 2021). In these studies, a wide range of biochemical, physiological and morphological responses to cope with high levels of this biological active radiation were described. Additionally, various deleterious effects on antioxidant and enzyme activity (Krywult *et al.* 2013; Köhler *et al.* 2017), biomass reduction (Chen *et al.* 2016), changes in some reproductive structures such as pollen and flowers (Peng *et al.* 2016), photomorphogenic effects (Jansen *et al.* 2017), morphological and epidermal modifications of leaves (Fina *et al.* 2017) were found in UV-B sensitive plant species. Conversely, high levels of UV-B absorbing compounds (Middleton and

Teramura 1993; Cuadra *et al.* 1997) and changes in surface flavonoids (Beggs *et al.* 1986; Cuadra and Harborne 1996) have been reported in more tolerant plant species. In addition to these reactions, UV-B irradiation results in phenotype alterations (Jansen *et al.* 2017) and gene expression (Dinamarca *et al.* 2013; Cuadra *et al.* 2020). Photochemical reactions at DNA level which produce pyrimidine (6–4) pyrimidone photoproducts are frequently reported (Sancar and Sancar 1988; Sinha and Häder 2002). In fact, these cyclobutane-type pyrimidine dimmers may cause inhibition of DNA replication and transcription (Manova and Gruszka 2015). Similarly, UV-B radiation exposure induces changes in the photosynthetic machinery, including damage to photosystem II (PS II), reduction in photosynthetic activity, pigment content and core proteins (Gadi 2018). Moreover, UV-B radiation causes numerous detrimental effects on the photosynthetic machinery: from Rubisco activity, gas exchange (Kataria *et al.* 2013), through to other indirect effects which have also been described, such as leaf and canopy morphology and stomatal conductance (Zhao *et al.* 2004). Damaging effects of UV-B radiation on photosynthesis include harm to the oxygen evolving system (Vass 2012) and Mn²⁺ cluster of water oxidation complex (Tyystjärvi 2008). D1/D2 reaction center proteins of PSII and quinone carriers are frequently identified as one of the targets of this radiation (Kataria *et al.* 2014). The effects on photochemistry of PSII, the electron transport system and fluorescence parameters are the other main impacts on photosynthesis (Barbato 2020). Fluorescence emission is one of three mechanisms by which plants can dissipate excitation energy. Once a Chl *a* molecule reaches the lowest vibration level of the excited state, it can emit a photon of a lower energy than it absorbed (photochemical quenching, qP). Another way is to decay to the ground state by emission of heat (non-photochemical quenching, NPQ). All of these are complementary processes of photosynthesis, where energy is used in order to undergo a photochemical reaction. Chl *a* fluorescence is a very useful tool in plant ecophysiological studies and for analyzing photosynthesis (Maxwell and Johnson 2000). The increase in fluorescence is due to PSII reaction centers (if they are closed or in a reduced state, they are unable to accept further electrons and reduce the overall photochemical process) so the efficiency of PS II can be estimated by measuring the photochemical quenching efficiency (Zhu *et al.* 2005). Chl *a* fluorescence measurement can be used as an indicator of stress as they affect the PS II functioning (Kataria *et al.* 2014). Minimal (F_o) and maximal fluorescence (F_m) parameters are measured directly using fresh leaves. According to the standard protocols used in several studies (Björkman and Demmig 1987; Demmig and Björkman 1987; Genty *et al.* 1989; Maxwell and Johnson 2000; Surabhi *et al.* 2009), a period of adaptation to the dark is given before F_o is measured (usually by using a low modulated light so it does not induce any changes in fluorescence). Sometimes a short far-red pulse is used to produce maximal oxidation of PS II. The F_m is then measured at saturating flash light (3000–10000 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

There are several reports about the effect of UV-B radiation on the electron transport system of PS II (Renger *et al.* 1986; Bornman 1989; Kataria *et al.* 2014). In these studies, the oxidizing side of PS II was identified as one of the primary targets. In addition to the reaction centers, other sensitive sites of PS II, such as thylakoid membranes (Lidon *et al.* 2011), the electron transport in the donor side (tyrosines, Tyr Z and Tyr D, and Mn cluster) have also been proposed as prime targets (Vass *et al.* 1999; Hakala *et al.* 2005). According to these studies, UV-B radiation affects the PS II itself, dissipating the excitation energy, and also acts on the reducing site of PS II (Dobrikova *et al.* 2013).

In previous work, Cuadra and Harborne (1996) analyzed the effects of UV-B radiation on the epidermal flavonoids of *Gnaphalium vira-vira* Molina which grows in the highlands of Ultima Esperanza province under the Antarctic springtime hole in the ozone layer. In the present study, carried out in the Southern sub-Antarctic hemisphere (Fig. 1), the impact of this radiation on PSII fluorescence (qP and NPQ) and DNA damage is reported.

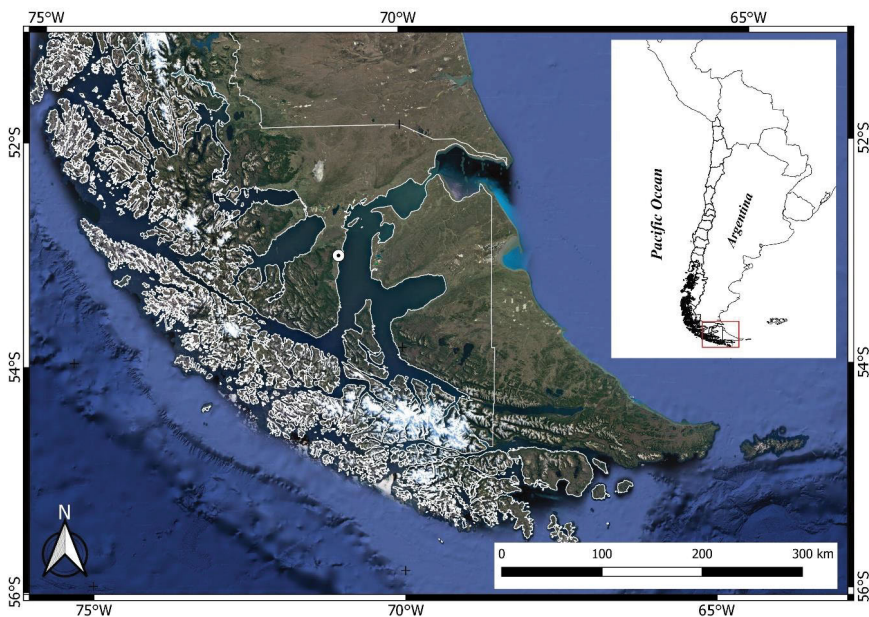


Fig. 1. Satellite map of the study area with marked the city of Punta Arenas (53.2°S 70.9°W) and surroundings.

Methods

Plant material. — Seeds of *G. vira-vira* (collected from plants growing in the University of Magallanes's greenhouses) were sown using the same compost mixture indicated in Cuadra *et al.* (2004). After germination (*c.* 20 days), nine

25-day old plants were separated into two groups (3 in the control group and 6 in the irradiated group) for the irradiation experiments.

Light Sources. — UV-B and PAR radiation doses given to plants were provided by using the same lamps and filter systems reported by Cuadra *et al.* (2004). Previously, lamps and filters were treated as indicated in Adamse and Britz (1992).

UV Treatments. — Two independent irradiated sample sets (I and II, 3 plants each) were used for CHl *a* fluorescence measurements. Plants were irradiated for 3 days (18.5 h d^{-1}). This time of exposure to radiation resembles the sunlight period on summer days at Southern latitudes (18–20 h). For DNA analysis plants received 9 h d^{-1} of UV-B radiation and samples were collected after 3 days of treatment. Plant-to-lamp distance was kept at 0.5 m by adjusting a mobile rack. This distance provides a UV-B fluence rate of 1.5 W m^{-2} , similar to average values measured in Punta Arenas during occurrences of ozone depletion.

Radiation measurements. — The spectral irradiance received by plants under the lamps was measured with the same spectrophotometer used in Cuadra *et al.* (2020).

Chlorophyll fluorescence. — Fluorescence parameters were measured using a modulated fluorimeter (Hansatech FMS1, UK) on three leaves from three different plants for each set of samples. Leaves were dark-adapted for 30 min before irradiation with actinic light to obtain F_0 . F_m in darkness was obtained with a saturating flash of $3500 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Before fluorescence measurements (F_0' , defined as, minimal fluorescence in light-adapted leaves; and F_m' , maximal fluorescence in light-adapted leaves), each level of the actinic light (50, 100, 200, 400, 800, 1200, 2000 μmol) were maintained for 30 s. qP and NPQ were calculated according to Pastenes *et al.* (2005).

Isolation of chromosomal DNA, PCR amplification and reproducibility.

— Total DNA was extracted from three leaves and the Inter Sequence Simple Repeats (ISSR) genetic assay was carried out as described in Cuadra *et al.* (2010) and Herrera *et al.* (2002). The amplification procedure with eleven ISSR primers was carried out twice independently and only reproducible bands were considered.

Experimental Design. — The study was carried out using the same experimental design described in Cuadra *et al.* (2010). Nine plants (25 days old) were distributed in two groups: the control group (–UVB) and the irradiated group (+UVB).

Statistical analysis. — According to the experimental design utilized in this study, the data were analyzed as indicated in Cuadra *et al.* (2010). Statistical assessment (ANOVA, LSD test) was performed for all measurements (qP and NPQ) using the Statgraphics Centurion XVI Statistical Package.

Results

Chlorophyll fluorescence. — Photochemical quenching and non-photochemical quenching were analyzed in *G. vira-vira*. Tables 1 and 2 show the effect of the UV treatment in qP and NPQ at different light levels in both sample sets (I and II). Leaves from control and irradiated plants show a decrease in qP during the experiments. Values of irradiated plants are lower than controls however, and these differences are statistically significant at all PAR levels used in both sample sets (Table 1). After a slight decrease at the first 200 μmol in both plant groups

Table 1.

Effect of UV-B radiation on photochemical quenching (qP)^{a,b}.

Actinic pulse (μmol)	Control	Treatment	F ^c	P ^d
50	0.893 $\pm$ 0.007	*0.856 $\pm$ 0.007 **0.757 $\pm$ 0.030	15.46 10.14	0.0012 0.0058
100	0.854 $\pm$ 0.005	*0.811 $\pm$ 0.005 **0.714 $\pm$ 0.046	36.67 4.71	0.0000 0.0454
200	0.781 $\pm$ 0.009	*0.727 $\pm$ 0.009 **0.651 $\pm$ 0.037	18.43 5.59	0.0006 0.0310
400	0.646 $\pm$ 0.011	*0.540 $\pm$ 0.011 **0.535 $\pm$ 0.033	45.92 5.49	0.0000 0.0324
800	0.595 $\pm$ 0.007	*0.523 $\pm$ 0.007 **0.458 $\pm$ 0.032	54.20 9.38	0.0000 0.0074
1200	0.582 $\pm$ 0.014	*0.485 $\pm$ 0.014 **0.458 $\pm$ 0.032	23.31 7.30	0.0002 0.0157
2000	0.600 $\pm$ 0.015	*0.459 $\pm$ 0.015 **0.457 $\pm$ 0.035	45.77 8.52	0.0000 0.0100

^aMean qP (N=18) and standard error of chlorophylls fluorescence measurements at different PAR doses.

^bDegrees of freedom=1; F and P (determined in ANOVA) are given for each qP value by treatment.

^cF-value was determined by dividing the mean square between-groups by the mean square within-groups.

^dConfidence level is 95%.

*= independent experimental measurements of qP.

**= independent experimental measurements of qP.

(19% in set I and 27% in set II; 14% controls), there is a sharp reduction in the qP values at 400 μmol in irradiated plants (40% in set I and 40%, set II in 28% controls). This rate of reduction did not continue, as we might have expected, when the PAR levels were increased (49% in set I and 49% in set II; 33% controls at 2000 μmol ; Fig. 2). The effect of UV-B radiation on the nonradioactive NPQ at different light levels in both sample sets (I and II) is shown in Table 2. Although the capacity to dissipate heat was augmented in both plant groups, this increase was always lower in irradiated plants from both sample sets. These differences are statistically significant from controls at all PAR levels used in samples from

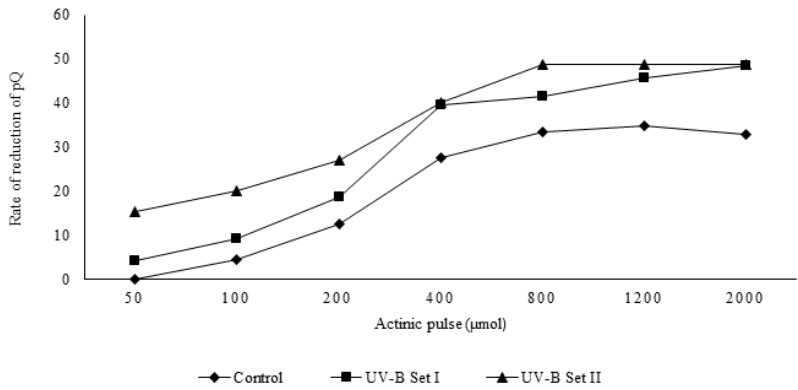


Fig. 2. Comparison of percentage of decrease in qP (photochemical quenching) between control and irradiated plants (set I and II) after 3 days of UV-B treatment by increasing the PAR levels.

Table 2.

Effect of UV-B radiation on non-photochemical quenching (NPQ)^{a,b}.

Actinic pulse (μmol)	Control	Treatment	F	P
50	0.233 ± 0.010	*0.229 ± 0.010 **0.213 ± 0.005	0.08 7.26	0.7837 0.0159
100	0.303 ± 0.015	*0.289 ± 0.015 **0.255 ± 0.015	0.45 5.43	0.5131 0.0332
200	0.393 ± 0.018	*0.343 ± 0.018 **0.332 ± 0.015	3.80 8.49	0.0689 0.0102
400	0.595 ± 0.030	*0.458 ± 0.030 **0.444 ± 0.026	10.37 16.64	0.0053 0.0009
800	0.645 ± 0.032	*0.529 ± 0.032 **0.527 ± 0.023	6.47 12.96	0.0217 0.0024
1200	0.661 ± 0.034	*0.548 ± 0.034 **0.561 ± 0.020	5.40 12.42	0.0337 0.0028
2000	0.674 ± 0.035	*0.537 ± 0.035 **0.567 ± 0.018	7.65 16.90	0.0138 0.0008

^aMean NPQ (N=18) and standard error of chlorophylls fluorescence measurements at different PAR doses.

^bDegrees of freedom=1; F and P (determined in ANOVA) are given for each qP value by treatment.

^cF-value was determined by dividing the mean square between-groups by the mean square within-groups.

^dConfidence level is 95%.

*= independent experimental measurements of NPQ.

**= independent experimental measurements of NPQ.

set II only. By contrast, samples from set I become statistically different at 400 μmol and higher intensities of PAR. Following differences at the beginning of experiments, at 200 μmol the percentage increase intragroup (set I and II) is similar until the end of measurements as the PAR levels increase. A different situation is observed in the control group where the percentage increase rises until it reaches the highest value (189%, Fig. 3).

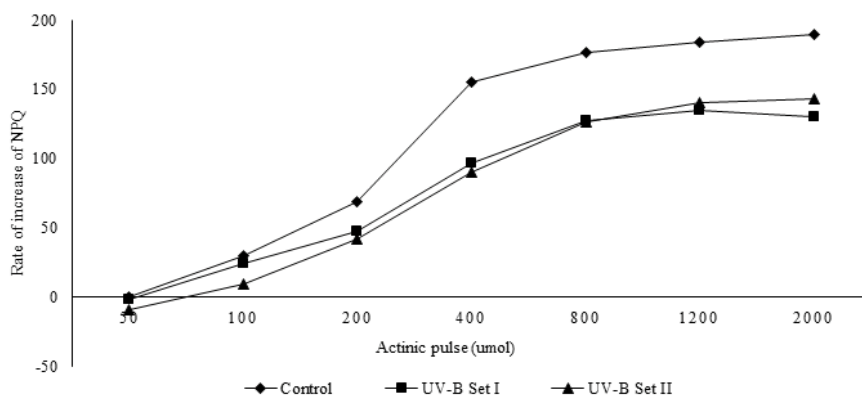


Fig. 3. Comparison of percentage of increase in NPQ (non-photochemical quenching) between control and irradiated plants (set I and II) after 3 days of UV-B treatment by increasing the PAR levels.

Changes in the pattern of chromosomal DNA. — DNA obtained from control and irradiated leaves was analysed as described in Cuadra *et al.* (2020). DNA fingerprinting was obtained and reproducible bands could be visualized. The effect of UV-B radiation on DNA sequence after 3 days of UV-B treatment is shown in Fig. 4. Three of the eleven different primers tested were able to generate amplification products. These three primers generated 21 fragments, four of which were polymorphic. Primers which generated amplification products (811, 826 and 888) were used in the ISSR markers genetic assay. In this way, polymorphisms were detected in treated plants when compared to controls. Two out of three primers (826 and 888) displayed more polymorphic bands. Similarly, some bands observed in controls were not seen in irradiated plants, which presented “new” bands instead. Although genetic variations are observed in both plant groups, these are larger in irradiated samples.

Discussion

Chlorophyll fluorescence. — According to our results shown in Tables 1 and 2, UV-B radiation can affect the energy-quenching mechanisms. One of these photochemical mechanisms is qP, which indicates the amount of excitation energy used in photosynthesis (opened reaction centers of PS II). This mechanism has been addressed as one of the targets for UV-B damage (Renger *et al.* 1986; Vass *et al.* 2005). In the present experiments, qP showed a decrease in both sample sets when compared to controls which may indicate some damage in the PS II complex. Quinone electron acceptor (Q_A and Q_B , specialized plastoquinones attached to polypeptide D1 and D2, respectively) are included

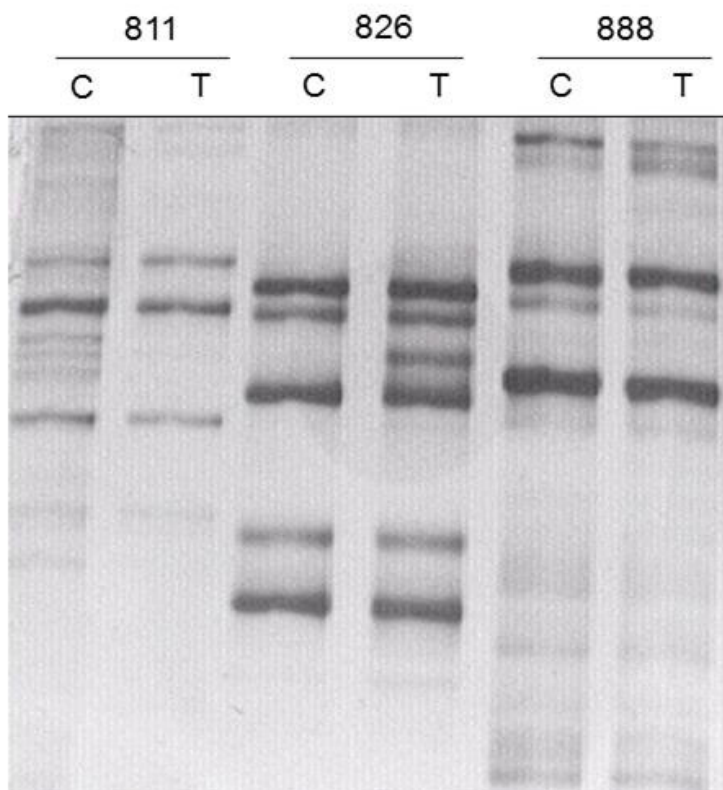


Fig. 4. Amplification products of extracted DNA using 3 different ISSR primers run on agarose gels. Bands showed differences in the DNA profile after 3 days of UV-B treatment. C: control samples. T: treated samples.

among the UV-B sensitive sites (Vass 2012). In this sense, it has been suggested that PS II fluorescence (Fm, Fo) decreased due to this induced “trap closure” and as an effect of Q_A reduction (Dau 1994) because the reduced Q_A cannot quench excited Chl *a* molecules. In fact, this reaction usually occurs in plants exposed to light intensities above 100 W m^{-2} (e.g., $215 \mu\text{mol}$). The sharp decrease in qP observed at $400 \mu\text{mol}$ in irradiated plants confirm this. In this context, Rodrigues *et al.* (2006) reported that UV-B radiation increased the concentration of the reduced primary quinone electron (Q_A) acceptor of PS II. Similarly, Dobrikova *et al.* (2013) found that the acceptor side of PS II is more sensitive to UV-B radiation. These findings also matched those reported by Quan *et al.* (2018) in *Scutellaria baicalensis* Georgi. Similar effects on PSII fluorescence induced by UV-B radiation were reported by Surabhi *et al.* (2009) in three cowpea cultivars (*Vigna unguiculata* (L.) Valp.), in soybean cultivars (Choudary and Agrawal 2015) and in *Thellungiella salsuginea* (Pall.) O.E. Schulz leaves (Barbato 2020). All of this evidence supports the hypothesis that plastoquinones (Q_A and Q_B) are photosensitizers for UV-B radiation preceding damage to PS II.

In addition to fluorescence emission, the release of heat is another energy-quenching mechanism. CHL *a* in plants dissipates part of its excitation energy through this process, and the remaining energy is split between photochemistry (e.g., photosynthesis) and fluorescence emission. When plants are exposed to an intense amount of radiation they are unable to use all of it and the portion that is not used is dissipated to the surroundings as heat. Our findings show that in UV-B irradiated (e.g., stressed) plants the heat dissipation process (NPQ) is affected under such conditions. This decreased capacity to release heat may be attributed to restrictions in the rate of photochemistry and to a decrease in the Fm' yield (Dau 1994; Logan *et al.* 2014). Seemingly, plants activate this flow energy mechanism as soon as the PS II is unable to use this energy to perform photochemical reactions. The lack of quenching by oxidized PQ may also be involved in this effect on NPQ (Dau 1994; Miller *et al.* 2001). Coincidentally, Xue *et al.* (2022) indicated that NPQ can be used by *Neoporphyrha haitanensis* (Chang *et al.* Zheng) Brodie *et al.* Yang, 2020 as an efficient photo-protective tool. In this regard, as the NPQ has been affected by the administered UV-B treatment, the irradiated plants could not use this excess energy dissipation mechanism. All of these results indicate that UV-B radiation induces such responses that prevent plants from developing their natural biochemical processes in order to optimize the effect of light on photosynthesis and growth.

Changes in the pattern of chromosomal DNA. — The DNA polymorphism observed in treated samples indicates that some changes in nucleotide sequences may be related to morphological changes (plant height, epidermal tissues) observed in a previous study on the same plant species (Cuadra and Harborne 1996). If this is the case, the UVR8 photoreceptor might be involved in these plant responses. These findings are consistent with those reported in another *Gnaphalium* species. By using ISSR markers, Cuadra *et al.* (2010) described a high ratio of polymorphic DNA bands and this was related to DNA damage caused by the different UV-B doses. Similarly, an increase in the frequency of somatic homologous DNA rearrangements, which might be involved in repairing UV-B induced DNA damage in plants, was reported in *Arabidopsis* and tobacco plants (Ries *et al.* 2000). In this connection, Jenkins (2009) suggested that UV-B radiation initiates several effects in plants; some of them are related to differential regulation of gene expression. A transcriptomic analysis was recently reported by Peng *et al.* (2021) in relation to this. Two cultivars of *Angelica sinensis* (Oliv.) Diels were UV-B irradiated and differentially expressed genes analyzed. Interestingly, the two irradiated plants presented differences in the expression of several genes, particularly those related to photosynthetic systems. All of these findings indicate that UV-B radiation induces changes at DNA level, promoting differences in gene expression when plants from the same species are compared.

These types of studies relating to changes at DNA level induced by UV-B radiation may help to identify UV-B resistant species which can produce food, fiber and fruits under high levels of this type of radiation. Plants which have been

exposed to intense solar radiation for long periods during the course of evolution have genetically adapted and developed the indispensable attributes required in order to survive in highly irradiated environments. Although *G. vira-vira* is a shrub, its behavior under this abiotic stress may be considered as a guideline for stress tolerance responses with potential use in different strategies directed at growing important agronomical plant cultivars which can be employed in crop breeding programs. In the same way, as high levels of UV-B radiation will continue to strike the ground in the Magallanes Region, many components of the large number of forest ecosystems (deciduous and perennial forests) may also be impacted by UV-B.

UV-B effects on plants may also have important consequences in ecological communities. Besides alterations in photosynthesis and plant architecture, the effect on gene transcription and enzyme activity can modify the secondary metabolite content. Most of these compounds participate in several ecological interactions so changes in their concentration could have consequences in natural ecosystems because they may affect plant resistance to herbivores.

Although the damaging effects of UV-B radiation on the environment have decreased, due in part to the Montreal protocol (Barnes *et al.* 2019), it is expected that UV-B levels will remain high for a long time as a complete recovery of the stratospheric ozone layer has not been achieved. This fact means that research studies on the effects of UV-B radiation on living organisms will continue to remain crucial in the future due to the current climate situation. In this context, the large amount research carried out in recent years on the role of UV-B radiation in terrestrial ecosystems has expanded our understanding beyond its adverse effects on living systems and highlights its role as a plant regulator and its influence on the functioning of ecosystems.

Conclusions

According to these findings, UV-B radiation affects the photosynthesis process and induces mutations at DNA level. By using the CHL *a* fluorescence technique, we have found that the efficiency of PS II decreased as a consequence of UV-B radiation. We suggest that in *G. vira-vira* the electron acceptor side of PS II has been damaged by UV-B radiation. This effect on the electron transport chain may be a consequence of accumulation of reduced Q_A in leaves because the photosystem cannot process more excitation energy issuing from the antennae. UV-B radiation can also modify the energy-quenching mechanisms such as the non-radioactive NPQ used by plants to dissipate the excitation energy from the PSII.

Genetic analysis has showed differences in the DNA profile of irradiated plants when compared to controls (Fig. 4). In this sense, we consider the ISSR assessment as a very useful molecular technique for studying genetic variations

and polymorphisms in plants by using DNA markers. It seems that UV-B radiation enhances the genetic differences between plants.

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