Scientific Research and Essay Vol. 5(16), pp. 2119-2127, 18 August 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2010 Academic Journals

Full Length Research paper

Residual effects of prolonged UV-B exposure on photosynthetic gene expression

S. W. Mpoloka

Department of Biological Sciences, University of Botswana, P/Bag 00704, Gaborone, Botswana.
E-mail: mpoloka@mopipi.ub.bw.

Accepted 31 August, 2009

Residual long-term effects of increased UV-B radiation were investigated in an indigenous plant species, *Dimorphotheca sinuata*, by analysing photosynthetic gene expression. Reductions were observed in the amount of the *rbc*L and *psb*A mRNA expressed in progeny of plants that were previously subjected to enhanced UV-B levels. However, observed reductions did not attain statistical significance. This could explain the reduction in net biomass and physiological and biochemical parameters observed by other researchers as a result of UV-B exposure. Results from this study with plants grown in the absence of UV-B point to changes in the regulation of photosynthetic genes and such mutations due to raised UV-B levels could cause permanent changes in plant populations.

Key words: UV-B, DNA-damage, photosynthetic genes, *Dimorphotheca sinuata*.

INTRODUCTION

The impact of an increase in UV-B on the physiological parameters and morphological features of plants, in particular, has been studied extensively (Bornman and Teramura, 1993; Musil and Wand, 1993; Musil, 1995, 1996; Midgley et al., 1998; Ries et al., 2000a, b; Rozema et al., 2002; Mpoloka et al., 2007). Most of these studies were conducted to ascertain whether ambient and enhanced solar UV-B levels retard growth, development and biomass accumulation in plants (Musil and Wand, 1993, 1994; Musil, 1994, 1995; Strid et al., 1994). The effects of elevated UV-B radiation on plants which range from the molecular scale, such as DNA damage, to tissue and whole plant effects, including decrease in photosynthetic activity and changes in plant structure and biomass (Caldwell et al., 1989; Harlow et al., 1994; Jordan 1996; Jordan et al., 1991, 1992; Musil, 1994; Musil et al., 2002) were found to vary widely among species and even among cultivars.

To date, long-term effects of UV-B radiation in plants are still not well understood and the knowledge of the effects of UV-B at the biochemical and molecular levels is limited. However, there are now indications or evidence to suggest that UV-B effects could potentially be heritable (Molinier et al., 2006). The chloroplast is reportedly the major site of damage by UV-B (Bornman, 1989) and studies focussing on the molecular mechanisms under-lying UV-B sensitivity of photosynthesis (Strid et al., 1994; Baker et al., 1997; Mackerness et al., 1999) have pointed to changes in gene expression in response to supplemental UV-B mainly the reduction in expression and synthesis of key photosynthetic genes including Rubisco (*rbc*S and *rbc*L), D1 polypeptide of photosystem II (*psb*A), chlorophyll *a/b*-binding protein (*Lhcb* or *cab*), a decline in total RNA enzyme activity and the ATPase complex. Since photosynthetic genes have been implicated in the past, this study looked at photosynthetic gene expression in plants with a history of UV-B exposure but grown in absence of UV-B radiation.

Findings of UV-B-induced reductions in pollen viability have also been reported in several South African annual species grown under enhanced UV-B (Musil, 1995). Pollen grains form an ecologically critical developmental stage of the plant, and in its natural state, pollen could be exposed to UV-B during the period between anther dehiscence and pollination, and therefore is potentially vulnerable to genetic damage by UV-B. Damage to DNA caused by UV-B exposure during plant development may not be fully repaired, and thus could be inherited by offspring and accumulated over successive generations (Musil, 1996).

Dimorphotheca sinuata is an arid environment winter ephemeral of the family Asteraceae. Its range extends

between 33°56'S, 18°29'E (Cape Town, South Africa) its southerly distribution, and 26°38'S, 16°18'E (Aus, Namibia) the northerly distribution limit where the anticipated stratospheric ozone depletion is 20% (Musil and Wand, 1993; Musil, 1995). Physiological and biochemical effects of cumulative exposure of *D. sinuata* to UV-B radiation have been studied *in vitro* (Musil and Wand, 1993; Musil, 1995, 1996; Midgley et al., 1998). Data from these studies point to the possibility that UV-B effects could be cumulative over the life history of the study plant. The studies also showed that accumulated UV-B effects had a greater impact on plant performance than immediate UV-B effects (Musil, 1994, 1995). The effects of UV-B irradiation on growth and allocation of biomass appeared to accumulate as subsequent generations were exposed to UV-B irradiation. Furthermore, after four generations of UV-B irradiation, the effects persisted in a fifth generation that was not exposed to UV-B treatment, implying that the effects of UV-B irradiation changes could be amplified. The present study used an arid environment winter ephemeral, *D. sinuata* as a model plant to study the residual effects of long term UV-B exposure. Seeds from plant material that had been previously subjected to enhanced UV-B radiation for five generations of the plants (Musil 1994, 1995) were used in this study.

The aim of this study was therefore to look at the genetic effects of cumulative exposure to UV-B exposure in an indigenous species *D. sinuata*. This study was therefore also aimed at establishing whether the observed accumulated effects are genetically based. To achieve this objective, differences in gene regulation in key photosynthetic pathways using mRNA analysis was conducted.

MATERIALS AND METHODS

Plant material

The seeds that were used to generate plants used in this study were generously supplied by Dr. Charles Musil of the Stress Ecology Unit, Kirstenbosch Botanical Gardens, Cape Town, South Africa. Seeds were soaked for five minutes in a 5% solution of sodium hypochlorite, and rinsed five times in distilled water. They were then placed on five layers of moistened Whatman filter paper on Petri dishes, which were then sealed with paraffin-wax film to minimise evaporation. Seeds were germinated in the dark for a week before being potted. After six weeks, leaf samples were taken for mRNA isolation and analysis. Leaves of approximately the same age were used to reduce variations due to various stages of development.

Nucleic acid purification

Total RNA was isolated from leaves collected from mid-adaxial positions of six-week-old plants using the Trizol Reagent (Gibco BRL – Life Technologies) according to the manufacturer's protocol. Total RNA was quantified using a spectrophotometer and equal amounts were resolved by electrophoresis on formaldehyde formamide agarose gels to verify quantification. Once quantification was completed, total RNA stocks were diluted to 50 ng/µl and aliquoted into different tubes. The samples were stored at –70°C. Each tube was thawed once and used for slot blot analysis.

Northern hybridization

A 48-well slot blot apparatus (Hoefer Scientific Instruments) was used for analysis. Three layers of pre-wetted No.3 Whatman paper were placed onto the slot blot apparatus and a 0.45 µm microporous, positively-charged nylon membrane (Roche Diagnostics GmBH, Mannheim, Germany) that had been cut to the size of the slot blot apparatus was placed over the filter papers. The assembly was tightly clamped together and the apparatus was attached to a vacuum pump. Moisture was removed from the filter paper by applying a vacuum for a minute.

Equal amounts of RNA (500 ng) were then loaded directly onto the nylon membrane in duplicate for each sample and the wells were flushed once with 50 µl of RNAse-free milliQ H_2O to ensure complete loading of RNA onto the membrane. Two membranes were prepared each time and one was used for the probe of interest, and the other with the internal standard (*18*S rDNA). The vacuum was turned on for five minutes, leaving RNA bound onto the nylon membrane. The RNA was then fixed on the membrane using an Amersham UV-Crosslinker (RPN 2500/2501) at a pre-set UV exposure of 70 000 µJ/cm^2 for 10 to 15 s. The cross-linked membrane was incubated in 2X SSC (0.3 M NaCl, 30 mM sodium citrate) for 2 min, and then placed in a hybridization bag containing 20 ml pre-warmed Eazy Hyb solution (Roche Diagnostics GmBH, Mannheim, Germany) per 100 cm^2 of membrane surface. The bag was sealed and then prehybridized at 42°C for 30 min.

Preparation of probes and hybridization

The *psb*A gene probe was supplied by S. A-H Mackerness as an 850-bp fragment containing the 3' 60% of the gene from spinach cloned into the *Hind*III site of the plasmid pBR322, selected on ampicillin resistance (Mackerness et al., 1997a, b). The probe was made by restriction endonuclease digestion of the construct with *Hin*dIII to release the insert, which was excised out of a normal 0.8% agarose gel and purified with the Roche Biochemicals High Pure PCR product purification kit according to the manufacturer's protocol. The purified insert was then Digoxigenin- (DIG) labelled using the random-primed labelling method. The *18*S rDNA and *rbc*L probes were generated by the polymerase chain reaction (Mpoloka, 2008) from seedling genomic DNA of *D. sinuata* plants from Generation 0 (the parental stock from which all experimental material was derived).

Oligonucleotide primers designed to complement an internal fragment of the *18*S rDNA gene sequence from *Arabidopsis thaliana* (Unfried et al., 1989) were used to produce a 1.6-kb fragment of the *18*S rDNA gene. The sequence of the forward primer (primer 1) was 5'- GTG TAA GTA TGA ACG AAT TC–3', and that of the reverse primer (primer 2) was 5'– GGAATT CTT CGT TGA AGA CC–3'. The PCR conditions were as follows: 70 ng template DNA; 5 µl of 10 µM primer 1; 5 µl of 10 µM primer 2; 4.0 µl of 5 mM dNTPs; 8 µl of 25 mM MgCl2; 10 µl 10X PCR buffer; 0.5 µl Taq polymerase (5 U/µl). Roche Diagnostics GmBH, Mannheim, Germany, supplied reagents used for PCR. The total reaction volume was made up to 100 µl with distilled water, and then overlaid with mineral oil to eliminate evaporation. The PCR cycle profile was as follows: initial denaturation at 94°C for 60 s, followed by 30 cycles of denaturation at 94°C for 60 s, primer annealing at 50°C for 30 s, and PCR product extension at 72°C for 90 s, followed by a final extension at 72°C for 5 min. PCR products were visualized

by running 2 µl of the reaction mixture on a 1% agarose gel.

The *rbc*L gene probe was generated by amplifying a 1.1-kb fragment of the *D. sinuata rbc*L gene from genomic DNA by PCR. Oligonucleotide primers designed to complement an internal fragment of the *rbc*L sequence from the grain amaranth, *Amaranthus hypochondriacus* (Michalowiski et al., 1990) were used. The sequence of the forward *rbc*L primer (primer 1) was 5'-GAT ATC TTG GCA GCA TTC CG–3', and that for the reverse primer (primer 2) was 5'–TGT CCT AAA GTT CCT CCA CC–3'. The PCR conditions and visualization were as for the *18S* rDNA probe. The probes used for detection were DIG-labelled (Hoeltke et al., 1995) either by the random-priming method or through PCR incorporation according to the supplier's protocol (Roche Diagnostics GmBH, Mannheim, Germany).

All Northern hybridization steps were done according to standard procedures (Sambrook et al., 1989). The DNA probe was incubated in a boiling water bath for 10 min to denature the DNA and then placed immediately on ice. The prehybridization solution was poured off and the hybridization solution containing the DIG-labelled probe was added at a concentration of 25 ng/ml and hybridization was carried out for 12 h at 42°C. After hybridization, the membrane was washed twice for 5 min in 2X SSC + 0.1% SDS at room temperature, followed by two 15 min washes in 0.1X SSC + 0.1% SDS at 65°C.

Detection of DIG-labelled nucleic acids

Following hybridization, signals were detected using the CSPD chemiluminescent alkaline phosphatase substrate according to the supplier's protocol (Roche Diagnostics GmBH, Mannheim, Germany). The membrane was equilibrated for 2 min in Buffer 1 (100 mM maleic acid, 150 mM NaCl; pH 7.5), then blocked in blocking solution (1% skim milk powder in Buffer 1) for 30 min. The membrane was incubated in anti-DIG solution (Anti-Digoxigenin–AP diluted 1:10 000 in blocking solution) for 30 and then washed twice for 15 min in wash buffer (Buffer 1 + 0.3% Tween 20®) at room temperature. The membrane was then equilibrated in detection buffer (Buffer 3 – 100 mM Tris-HCl, 100 mM NaCl; pH 9.5) for 2 min before incubating in CSPD solution (CSPD diluted 1:100 in detection buffer) for 5 min. The CSPD substrate was allowed to reach steady state by incubating at 37°C for 10 min. The CSPD solution was retained for further use. The membrane was placed in a sealed hybridization bag, placed in an X-ray cassette, and exposed to X-ray film. The blot was developed after an appropriate exposure time, which was determined empirically. All reagents for detection of DIG were supplied by Roche Diagnostics GmBH Mannheim, Germany, unless otherwise stated.

Hybridization with the different probes (*rbc*L, *psb*A and *18S* rDNA) was done in separate bags. Washes and detection were carried out in the same bag with the same solutions. One membrane was probed with either the *rbc*L or *psb*A probes and the other was hybridized with an *18*S rDNA probe. Membranes were exposed on the same X-ray film for the same duration. After appropriate incubation, blots were developed and the intensities of the resulting bands were determined using the Macbeth Trans-mission Densitometer (TD-901). Readings were taken for each slot on the membrane for all the samples, and the corresponding densitometer reading for the same plant was determined for the *18*S DNA.

Statistical analysis

After all the values were compiled, ANOVA was carried out using the Genstat statistical package (Payne, 1998). Variations in loading were accounted for in the analysis by using the *rbc*L/*18S* rDNA and *psb*A/*18S* rDNA ratios that is the *18S* rDNA gene expression was used as an internal standard to normalize the results. The ratios R_r and R_p were defined as R_r = *rbc*L/*18S* rDNA and R_p = *psb*A/*18S* rDNA. The ratios of the observations of each probe (*rbc*L and *psb*A) to their respective *18*S rDNA readings were used in the analysis. A nested mixed effects analysis of variance was used to test the differences between the radiated and control plants and to estimate the variation due to plant, blots and duplicate observations on the blot that is the relative contribution by each factor was investigated (Scheffe, 1959; Hicks, 1982). Missing values present on any of the blots were estimated using an iterative missing value formula, which ensured that the missing values did not contribute to the variation.

Let y denote a single ratio reading identified by the subscripts ijkl which represents treatments, plants, blots and duplicates, then:

$$y_{ijkl} = \mu + \alpha_i + b_{j(i)} + c_{k(ji)} + d_{l(kji)}$$

where b, c, d are random variables representing variation due to plants, blots and duplicate readings on the blots respectively. Subscripts within brackets denote the nesting.

The differences between the irradiated and control plants were assessed using the variation due to plants. The variation due to blots and duplicates measures the technical (that is measurement) errors. These sources were estimated and compared both separately in the radiated and control plants and overall.

RESULTS AND DISCUSSION

Determination of mRNA levels

To determine mRNA levels for the *rbc*L, *psb*A and *18S* rDNA genes, blots were hybridized independently with the corresponding probe for at least 12 h and the densitometer readings were determined. Figure 1 is a representative blot of control and test plants probed with *psb*A and *18S* rDNA probes. The corresponding densitometer readings from these blots (Figure 1) are presented in Table 1.

*rbc*L mRNA levels and *rbc*L:*18*S rDNA ratios

A total of twenty-one control plants and twenty-three radiated plants were analysed with the *rbc*L probe. Mean values for the *rbc*L mRNA levels, and the corresponding standardized ratios computed from the *18S* rDNA internal standard are shown in Table 2. Except for plant T9 which had two observations, at least four observations were made for each plant, from a total of ten blots. The mean values are represented graphically in Figure 2. The overall mean ratios for the control and radiated plants were found to be 0.703±0.050 and 0.669±0.067 respectively (Figure 2), that is treated plants were lower than the control plants.

*psb*A mRNA levels and *psb*A:*18*S rDNA ratios

A total of twenty-one control plants and twenty-five radiated plants were analysed with the *psb*A probe and the mean values for the *psb*A mRNA levels, and the corresponding standardized ratios computed from the *18S* rDNA

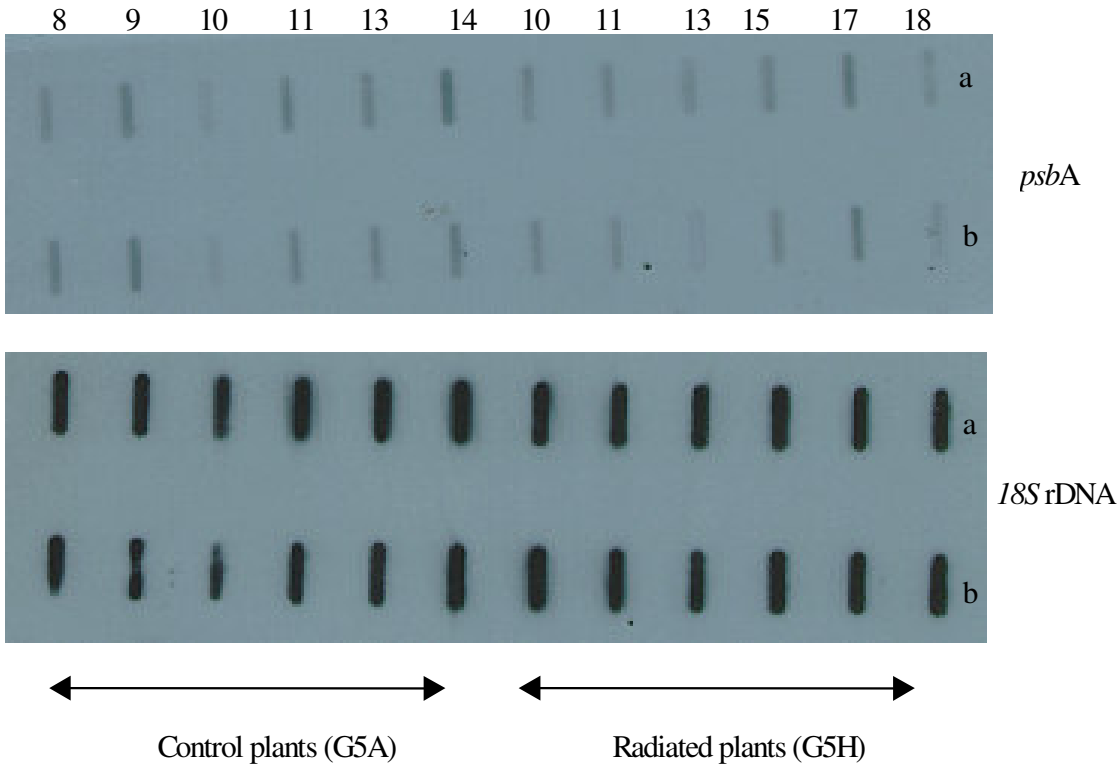

Figure 1. Representative blot of both control and test plants probed with an 18S rDNA probe (lower panel) and a psbA probe (upper panel). Both membranes were exposed for the same duration on the same X-ray film to eliminate errors arising from exposure time. G5A and G5H represent fifth generation control and radiated plants, respectively, grown from seeds taken from the fourth generation. The numbers at the top of the blot represent arbitrary numbers allocated to individual plants. The letters a and b represent duplicate readings for each probe (a = Reading 1, and b = Reading 2).

Table 1. Example of calculation of densitometer readings taken from the blot in Figure 1 for both psbA and 18S rDNA probes and the corresponding psbA: 18S rDNA ratios.

	***psb*A readings**			***18*S rDNA readings**			**Ratio**
Sample	**Reading 1**	**Reading 2**	***psb*A**	**Reading 1**	**Reading 2**	***18*S rDNA**	***psb*A/*18*S**
C*8	0.31	0.34	0.325	1.11	1.11	1.110	0.292793
C9	0.35	0.37	0.360	1.05	1.03	1.040	0.346154
C10	0.27	0.27	0.270	0.78	0.68	0.730	0.369863
C11	0.32	0.32	0.320	1.06	0.95	1.005	0.318408
C13	0.32	0.32	0.320	1.05	0.93	0.990	0.323232
C14	0.39	0.34	0.365	1.20	1.16	1.180	0.309322
T*10	0.30	0.31	0.305	1.24	1.22	1.230	0.247967
T11	0.29	0.29	0.290	1.16	1.14	1.150	0.252174
T13	0.30	0.26	0.280	1.02	0.91	0.965	0.290155
T15	0.31	0.31	0.310	1.15	1.12	1.135	0.273128
T17	0.35	0.34	0.345	1.13	1.15	1.140	0.302632
T18	0.28	0.28	0.280	1.14	1.12	1.130	0.247788

* (C and T before the sample number represent control and treated (radiated) plants, respectively).

Table 2. Mean *rbc*L and *18S* rDNA mRNA levels and their ratios from densitometer readings. The values represent means of at least four independent observations.

Control plants					Radiated plants				
Plant No.	**N[a]**	**Mean *rbc*L**	**Mean *18*S**	**Mean ratio**	**Plant No**	**N**	**Mean *rbc*L**	**Mean *18*S**	**Mean ratio**
C*1	4	1.3850	1.1950	1.1959	T*1	4	0.6575	0.9925	0.6774
C2	8	0.5903	0.9823	0.6545	T2	4	0.5100	1.1800	0.4379
C3	4	1.7950	1.5800	1.1511	T3	4	0.9025	1.1175	0.8258
C4	8	0.7144	0.9413	0.7461	T4	4	1.6275	1.1925	1.4101
C5	4	1.1125	1.1650	0.9756	T5	4	0.6775	1.0700	0.6514
C6	4	0.2763	0.5450	0.5347	T9	2	0.5435	0.3050	1.7768
C8	7	0.7956	1.6370	0.7035	T10	8	0.8120	1.7280	0.6972
C9	8	1.1240	1.8910	0.9432	T11	8	0.6520	1.4450	0.7342
C10	7	0.4450	1.5289	0.4523	T12	4	0.1950	0.4178	0.5013
C11	7	0.8720	1.7222	0.7324	T13	7	0.8700	1.7100	0.6869
C12	4	0.2248	0.2865	0.8163	T14	4	0.1685	0.5483	0.2918
C13	8	0.7180	1.7300	0.6185	T15	8	0.7080	1.7100	0.6800
C14	8	1.0710	1.8950	0.9191	T16	4	0.2925	0.6423	0.4776
C15	4	0.4213	0.5568	0.8071	T17	8	0.3840	1.4140	0.4736
C16	4	0.2633	0.5828	0.4646	T18	8	0.7400	1.6840	0.6835
C21	4	1.1575	2.5350	0.4564	T19	6	0.3353	0.4632	0.6846
C22	4	1.3800	2.4900	0.5542	T20	4	0.4930	0.6370	0.7260
C23	4	1.3050	2.4425	0.5353	T21	4	1.2000	2.4575	0.4904
C24	4	1.2325	2.3975	0.5140	T22	4	1.3800	2.4975	0.5531
C25	4	1.2075	2.4950	0.4838	T23	4	1.2075	2.4750	0.4870
C26	4	1.2475	2.4725	0.5058	T24	4	1.1000	2.4025	0.4577
					T25	4	1.1600	2.4225	0.4790
					T26	4	1.1775	2.3550	0.5010

N[a] = number of observations per plant. * (C and T before the sample number represent control and treated (radiated) plants, respectively).

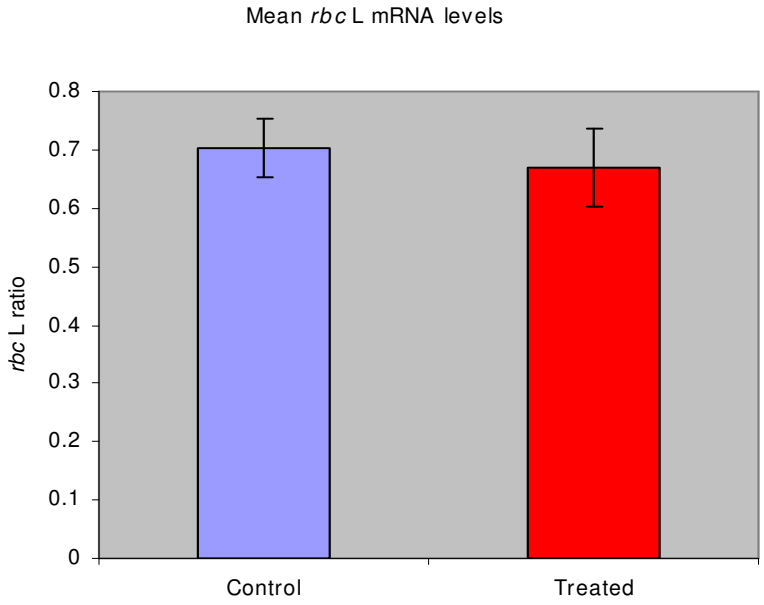

Figure 2. Histogram showing mean *rbc*L: *18*S rDNA ratios. Control = 0.703±0.050, treated = 0.669±0.0676. The error bars represent the standard error.

internal standard are shown in Table 3. Except for plants T5, T8 and T9 which had two observations each (Table 3), at least four observations were made for each plant, from a total of eleven blots. The mean values are represented graphically in Figure 3. The overall mean *psb*A ratios for the control and radiated plants were found to be 1.2346±0.149 and 1.1618±0.148, respectively, that is treated plants were lower than the control plants.

Statistical analysis

The mean ratios for mRNA levels of the two genes were analysed and radiated plants were on the whole found to be lower than control plants. However there was no statistically significant difference between the mean mRNA levels from irradiated and control plants for either gene (F = 1.28; df = 42; p = 0.264) and (F = 0.29; df = 44; p = 0.593). A number of factors contributed to the variation observed in the measurements, so, the stratum variances (error due to each parameter e.g. plant, blot and replicates) of the radiated and control ratios were computed separately and compared with an F-test. The estimated stratum variances for the *rbc*L mRNA levels are indicated in Table 4, and those for *psb*A mRNA are shown in Table 5.

Approximately 50% of the total observed variation in the irradiated plants was due to plants, and the other 50% was due to the blots. For the control plants, 60% of the observed variation was due to blots and 37% was due to plants. The variation due to duplicates was 3 and 1% for the control and radiated plants respectively (Table 4). There was no significant difference in the variation between the control and treated plant standardized rbcL mRNA levels (F = 1.19, p = 0.35).

For the control plants 64% of the total variation was due to plants and 35% of the variation was due to blots. For the irradiated plants, 78% of the total variation was due to plants, while only 22% came from the blots. Only 1.3 and 0.5% of the total variation was caused by errors in the duplicates for the control and irradiated plants, respectively. A comparison of the variances of radiated and control plants was carried out using an F-test and there was no significant difference in the variation between the control and treated plant *psb*A mRNA levels (F = 1.022; p = 0.4748).

DISCUSSION

The results from the experiments that were conducted previously using plants from generations one to four showed reductions in photosynthetic activity, biomass and several other physiological parameters (Musil, 1996). It was hypothesised that the observed changes could possibly be a result of DNA damage. This was tested using the *Dra*I assay (Mpoloka, 2008) but no direct evidence for UV-B-induced mutations was found. It was proposed that a change in the actual genes or in regulation of genes involved in photosynthesis could have occurred at some stage in the history of the plants. The *rbc*L and *psb*A genes were chosen for analysis and these were used as probes to determine the expression of the respective mRNA levels.

The results for the *rbc*L and *psb*A gene probes (Figures 2 and 3) showed that on the whole there was a reduction in the expression of mRNA levels for both genes. Although the pattern was consistent there was no statistically significant difference between the mRNA levels for either gene in the irradiated and control groups (F = 1.28; df = 42; p = 0.264 for *rbc*L and F = 0.29; df = 44; p = 0.593 for *psb*A). A total of twenty-one control plants and twenty-three irradiated plants were used in the *rbc*L analysis. For the *psb*A gene probe, twenty-one control and twenty-five irradiated plants were used. More samples need to be analysed in future in order for the statistical analyses to be more rigorous.

Comparison of the variances of irradiated and control plant *rbc*L mRNA levels were done and it was found that most of the variation was contributed by the blots for control plants (experimental error) whereas the contribution by the plants and the blots were almost the same for the radiated plants (Table 4). However, when the variation in the control and radiated plants was compared with an F-test, there was no difference in the variation in the control and treated plant *rbc*L mRNA levels (F = 1.19, p = 0.35). Analysis of variance for the *psb*A probe showed that most of the observed variance was caused by differences in the expression of mRNA levels in the actual plants used and a smaller percentage was due to experimental error that is due to the blots. A test of significance in the observed values between irradiated and control plants using the F-test showed no significant difference between the variances in the two plant groups (F= 1.022, p = 0.47).

One major limitation of this technique comes from the probe concentration. There is an almost linear relationship between probe concentration and the number of target sites that the probe binds to. As a result, probe concentration might reach saturation earlier for certain samples. What these means is that the probe concentration must be standardised/uniform, but it becomes problematic when one has to assay large samples and has to reuse the same probe more than once. To investigate this, data from blots that contained the same plants that were replicated at least five times (representing a true replication of the experimental technique) were used. Despite the fact that differences were observed in the mRNA levels from the same plants on different blots, the variation was not statistically significant.

Determining the linear range for a given probe, then working with probe concentrations and exposure time within that range could authenticate this observation in future. This was not done in this study as the use of the *18*S rDNA probe as an internal standard has been reported to

Table 3. *psb*A mRNA and *18S* rDNA mRNA levels and the corresponding ratios from densitometer readings.

Control plants					Radiated plants				
Plant No.	**N[a]**	**Mean *psb*A**	**Mean *18*S**	**Mean ratio**	**Plant No.**	**N**	**Mean *psb*A**	**Mean *18*S**	**Mean ratio**
C*1	4	0.2933	0.6118	0.5809	T*1	6	0.2122	0.472	0.4442
C2	10	0.2221	0.4091	0.8057	T2	6	0.3658	0.630	0.6378
C3	6	0.5275	0.5323	0.6347	T3	4	0.4630	0.500	0.9386
C4	10	0.3797	0.4069	1.1166	T4	6	0.3798	0.637	0.6166
C5	6	0.3667	0.5167	0.7577	T5	2	0.4500	0.775	0.5808
C6	10	0.3014	0.4187	0.7011	T7	4	0.3071	0.236	1.0418
C8	4	0.2805	0.6193	1.0650	T8	2	0.2100	0.323	0.6624
C9	4	0.3448	0.5933	1.3073	T9	2	0.1105	0.162	0.6826
C10	4	0.1583	0.4283	0.3699	T10	4	0.2398	0.726	0.5191
C11	8	0.3894	0.5904	0.8396	T11	4	0.1768	0.638	0.3817
C12	8	0.2795	0.1984	1.4849	T12	8	0.1373	0.289	1.7687
C13	4	0.4433	0.6495	1.0791	T13	6	0.1962	0.480	0.5591
C14	4	0.4620	0.8003	0.8209	T14	8	0.1638	0.141	1.3497
C15	6	0.2833	0.4155	0.6333	T15	6	0.2368	0.508	0.7901
C16	4	0.2170	0.2720	1.1967	T16	6	0.2425	0.259	0.9566
C21	6	0.3195	0.2460	1.2950	T17	4	0.3750	0.740	0.7488
C22	4	0.5788	0.2388	2.4319	T18	6	0.2428	0.572	0.7329
C23	4	0.3138	0.2400	1.3159	T19	10	0.3001	0.397	1.0659
C24	4	0.4243	0.1818	2.4450	T20	4	0.3678	0.247	1.5110
C25	4	0.4170	0.1873	2.3209	T21	4	0.4775	0.189	2.6957
C26	4	0.4853	0.1875	2.7242	T22	4	0.3570	0.190	1.9400
					T23	4	0.2415	0.185	1.3001
					T24	4	0.4058	0.195	2.1110
					T25	4	0.4308	0.196	2.2802
					T26	4	0.5780	0.215	2.7311

N[a] = number of observations per plant. * (C and T before the sample number represent control and treated (radiated) plants, respectively).

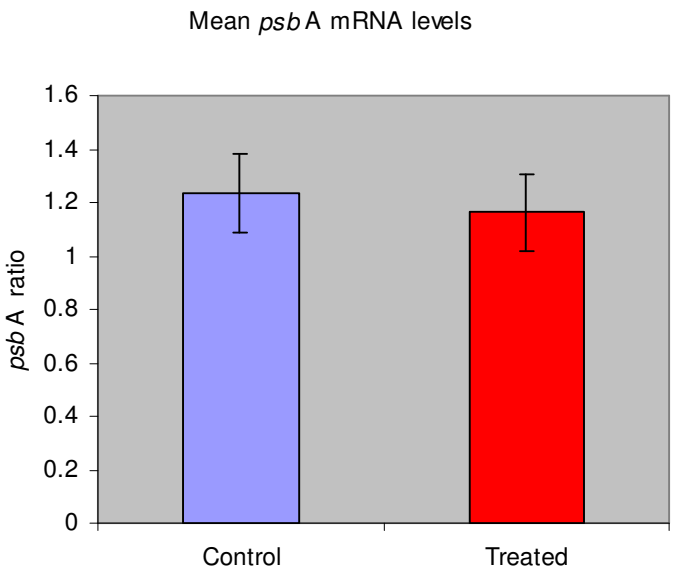

Figure 3. Histogram showing the mean *psb*A: *18*S rDNA ratios. Control = 1.2346±0.149, treated = 1.1618±0.148. The error bars represent the standard error.

to serve the same purpose in previous studies (Surplus et al., 1998). In addition analysis of differences observed from repeated measures showed that they were statistically insignificant. However, a number of different strategies can be applied if it is desired to do single exposure. Possibilities are to reduce the specific activity of the *18*S rDNA probe by reducing the level of labelled nucleotide in the labelling mix, diluting the labelled probe with unlabelled probe or reducing the amount of labelled probe in the hybridization mix. However, the last strategy introduces an additional problem in terms of being sure that the label bound is a true reflection of the homologous RNA on the membrane. It should be noted that the initial rate of reaction in the hybridization is dependent on the concentration of probe, and since there is a great deal of *18*S rDNA on the filters, the probe will be diluted rapidly and the reaction may not go to completion. To get around this problem, a time course of hybridization giving the initial rates of reaction could be carried out in future. In conclusion, one fascinating aspect of this study that should develop into a significant contribution to plant molecular biology in relation to UV-B effects is that, for a long time, it has been a well known fact that increased

Table 4. Estimated stratum variances for the *rbc*L ratio (R_r). The percentage column gives the percentage of the total variation that can be attributed to each source. d.f = degrees of freedom and rep = duplicate samples.

	Control plants			Radiated plants		
Stratum	**Variance**	**Effective df.**	**Percentage**	**Variance**	**Effective df.**	**Percentage**
Plant	0.2566	20	36.6	0.30606	22	49.3
Blot	0.4216	37	60.0	0.30131	34	48.5
Rep	0.0212	55	3.00	0.0693	56	1.00

Table 5. Estimated stratum variances for the *psb*A ratio (R_p). The percentage column gives the percentage of the total variation that can be attributed to each source. df = degrees of freedom and rep = duplicate samples.

	Control plants			Radiated Plants		
Stratum	**Variance**	**Effective d.f.**	**Percentage**	**Variance**	**Effective df.**	**Percentage**
Plant	2.0325	20	63.9	1.98933	24	77.5
Blot	1.1048	38	34.8	0.55738	35	21.7
Rep	0.0410	59	1.30	0.01368	60	0.5

exposure to UV-B caused a variety of physiological and morphological responses in plants but the effect on plant genomic stability was not well established.

ACKNOWLEDGEMENTS

Many thanks go to Dr. Charles Musil of the National Botanic Institute, Kirstenbosch, Cape Town for generously providing seed material and invaluable technical assistance. I also thank Professor June Juritz of the Department of Statistics, University of Cape Town for the statistical analysis. This publication was made possible by grants from the Carnegie Corporation of New York, the Rockerfeller Foundation, the Ridgefield Foundation and the Coca Cola Foundation through the USHEPiA programme at the University of Cape Town. I greatly acknowledge financial assistance from the University of Botswana.

REFERENCES

Baker NP, Nogues S, Allen DJ (1997). Photosynthesis and photoinhibition. *In* Lumsden P (ed.). Plants and UV-B: Responses to Environmental Change, Cambridge University Press pp 95-111.

Bornman JF (1989). Target sites of UV-B radiation in photosynthesis of higher plants. J. Photochem. Photobiol. 4: 145- 158.

Bornman JF, Teramura AH (1993). Effects of UV-B radiation on terrestrial plants. In Young AR, Bjorn LO, Moan J and Nultsch W, eds. Environmental UV Photobiology. Plenum Publishers Co, New York. pp 427-471

Caldwell MM, Teramura AH, Tevini M (1989). The changing solar ultraviolet climate and the ecological consequences for higher plants. TREE 4: 363-367.

Harlow GR, Jenkins ME, Pittalwala TS, Mount DW, (1994). Isolation of *uvh1*, an *Arabidopsis* mutant hypersensitive to ultraviolet light and ionizing radiation. The Plant Cell 6: 227-235.

Hicks CR (1982). Fundamental concepts in the Design of Experiments. 3rd Edition. Holt Saunders International Editions.

Hoeltke HJ, Ankenbauer W, Muhlegger K, Rein R, Sagner G, Seibel R, Walter T (1995). The Digoxigenin (DIG) system for non-radioactive labelling and detection of nucleic acids – an overview. Cell. Mol. Biol. 41: 883-905.

Jordan BR (1996). The effects of ultraviolet radiation on plants: a molecular perspective. Advances in Bot. Res. 22: 97-162.

Jordan BR, Chow WS, Strid A and Anderson JM (1991). Reduction in *cab* and *psb*A RNA transcripts in response to supplementary ultraviolet-B radiation. FEBS Lett. 284: 5-8.

Jordan BR, He J, Chow WS, Anderson JM (1992). Changes in mRNA levels and polypeptide subunits of ribulose 1,5-bisphosphate carboxylase in response to supplementary ultraviolet radiation. Plant Cell and Environ. 15: 91-98.

Mackerness SAH, Jordan BR,Thomas B (1997a). UV-B effects on the expression of genes encoding proteins involved in photosynthesis. In Lumsden P (ed.). Plants and UV-B: Responses to Environmental Change, Cambridge University Press. (pp 113-134).

Mackerness SAH, Jordan BR,Thomas B (1997b). The effects of supplementary ultraviolet-B radiation on mRNA transcripts, translation and stability of chloroplast proteins and pigment formation in *Pisum sativum* L. J. Exp. Botany 48: 729-738.

Mackerness SAH, Surplus SL, Blake P, John CF, Buchana-Wollaston V, Jordan BR, Thomas B (1999). Ultraviolet-B-induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signalling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. Plant, Cell and Environ. 22: 1413-1423.

Michalowiski CB, Bohnert HJ, Klessig DF, Berry JO (1990). Nucleotide sequence of *rbcL* from *Amaranthus hypochondriachus* chloroplasts. Nucleic Acids Res. 18: 2187.

Midgley GF, Wand SJE, Musil CF (1998). Repeated exposure to enhanced UV-B radiation in successive generations increases developmental instability (fluctuating asymmetry) in a desert annual. Plant, Cell and Environ. 21: 437-442.

Molinier J, Ries G, Zipfel C, Hohn B (2006). Transgeneration memory of stress in plants. Nature 442: 1046-1049.

Mpoloka SW (2008). Investigating evidence for UV-B induced mutagenesis in *Dimophotheca sinuata* using the *18S* rDNA and *rbc*L gene sequences. Advances in Environ. Biol. 2(2): 81-88.

Mpoloka SW, Abratt VA, Mundree SG, Thomson JA, Musil CF (2007). Potential effects of prolonged ultraviolet radiation exposure in plants: chloroplast DNA analysis. Am.-Eurasian J. Agric. Environ. Sci. 2(4): 437-441.

Musil CF (1994). Ultraviolet-B irradiation of seeds affects photo-chemical and reproductive performance of the arid-environment ephemeral *Dimorphotheca pluvialis*. Environ. Exp. Bot. 34: 371-378.

Musil CF (1995). Differential effects of elevated ultraviolet-B radiation on the photochemical and reproductive performances of dicotyledonous

and monocotyledonous arid-environment ephemerals. Plant, Cell and Environ. 18: 844-854.
Musil CF (1996). Cumulative effect of elevated ultraviolet-B radiation over three generations of the arid environment ephemeral *Dimorphotheca sinuata* DC (Asteraceae). Plant Cell and Environment 19: 1017-1027.
Musil CF, Chimphango SBM, Dakora FD (2002). Effects of elevated ultraviolet- B radiation on native and cultivated plants of Southern Africa. Ann. Bot. 90: 127-137.
Musil CF, Wand SJE (1993). Responses of *Sclerophyllous* Ericaceae to enhanced levels of ultraviolet-B radiation. Environ. Exp. Bot. 33: 233-242.
Musil CF, Wand SJE (1994). Differential stimulation of an arid-environment winter ephemeral *Dimorphotheca pluvialis* (L.) Moench by ultraviolet-B radiation under nutrient limitation. Plant, Cell and Environ. 17: 245-255.
Payne RW (1998). Genstat 5 Release 4.1, Lawes Agricultural Trust (Rothamsted Experimental Station, UK).
Ries G, Buchholz G, Frohnmeyer H, Hohn B (2000b). UV-B-mediated induction of homologous recombination in *Arabidopsis* is dependent on photosynthetically active radiation. Proc. Natl. Acad. Sci., 97: 13425-13429.
Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B (2000a). Elevated UV-B radiation reduces genome stability in plants. Nature 406: 98-101.
Rozema J, Bjorn OL, Bornman JF (2002). The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-B absorbing compounds. J. Photochem. Photobiol. B: Biol., 66: 2-12.
Sambrook J, Fritsch EE, Maniatis T (1989). Molecular cloning: a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Scheffe H (1959). The Analysis of Variance. John Wiley and Sons Inc.
Strid A, Chow WS, Anderson JM (1994). UV-B damage and protection at the molecular level in plants. Photosyn. Res., 39: 475-489.
Surplus SL, Jordan BR, Murphy AM, Carr JP, Thomas B, Mackerness SAH (1998). Ultraviolet-B-induced responses in Arabidopsis thaliana: role of allicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis-related proteins. Plant, Cell and Environ. 21: 685-694.
Unfried I, Stocker U, Gruendler P (1989). Nucleotide sequence of the *18S* rRNA gene from *Arabidopsis thaliana* co10. Nucleic Acids Res. 17: 7513.