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Potential Effects of Prolonged Ultraviolet Radiation Exposure in Plants: Chloroplast DNA Analysis

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Abstract: The present study on the Namaqualand daisy, *Dimorphotheca sinuata* sought to address two main questions:-first whether the natural populations show any evidence of variation in the chloroplast genome and secondly if the changes could be attributed to prior damage by UV-B *i.e.* via the formation of pyrimidine dimers at some stage in their history. Characterization of chloroplast DNA from natural plant populations of *D. sinuata* across a latitudinal gradient was carried out using restriction endonuclease digestion. The enzymes used included *Dra*I (TTTAAA), *Eco*RI (GAATTC) and *Hin*dIII (AAGCTT) whose recognition sequences are possible targets for UV-B radiation and *Bam*HI (GGATCC) and *Eco*RV (GATATC), whose recognition sequences are not obvious UV-B targets. Plants growing at northern latitudes (potentially higher UV-B environments) revealed striking polymorphisms that may be attributed to genome re-arrangements resulting from UV-B stress when compared with plants from southern latitudes (lower UV-B environments). This is the first known attempt at developing a Southern African biological method for predicting the long-term effects of ozone depletion and the resultant rise in UV-B radiation, on our indigenous flora.

Key words: Chloroplast DNA . UV-B . DNA damage . stress

INTRODUCTION

Significant latitudinal variation in incident UV-B radiation has been reported [1, 2]. However, a few studies have been carried out in which natural plant performance across natural solar UV-B gradients at different elevations was compared. Variable sensitivities to UV-B radiation have been reported which implies the presence of natural adaptations to UV-B stress [3, 4]. These studies indicate that species and ecotypes from high UV-B irradiance environments are often less sensitive to elevated UV-B radiation than those from low UV-B irradiance locations [2, 4]. This has led to suggestions that genotypic differentiation may have developed among plants along these gradients. Previous studies of plants grown for several generations in the presence of enhanced UV-B radiation showed evidence of UV-B effects on various physiological processes, growth and reproduction, indicating a likelihood of these effects being heritable [5-7]. UV-B radiation has been reported to cause several lesions in DNA including double strand breaks whose induction in turn increases the frequency of homologous recombination, hence genome rearrangements [8, 9]. Studying UV adaptations in natural plant populations could enable us to find novel or unique protective mechanisms that have not been detected in crop plants already exposed to intensive artificial selection. Perhaps the first place to search for UV-B responsiveness in native plants is in regions where natural levels of UV-B are already quite high. Plants that naturally occur in high UV environments would undoubtedly have evolved specific adaptations that protect them from the deleterious effects of UV-B radiation [3]. However, this does not mean that they do not respond to UV-B: Indeed it might suggest quite the opposite in that their anti-UV mechanisms may be permanently induced. Such plants could also show reduced responsiveness mainly because of reduced sensitivity to UV-B radiation and possibly by possessing some adaptive mechanisms against UV-B radiation.

UV-B induced reductions in pollen viability in several South African annual species grown under enhanced UV-B have been reported [10] and it has been suggested that, even under experimental treatments using natural light, damage to the plant genome caused by elevated UV-B may also be inherited by successive generations of the desert annual *D. sinuata* and thus

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accumulate in the genetic material [9]. This form of damage may be extremely important in populations which have rapid turnover of generations such as annual species which are common in high-radiation desert environments. Furthermore, populations which are isolated by habitat fragmentation may be further at risk to this form of damage due to limited out crossing opportunities.

Based on observations that plant exposure to episodic or steadily increasing doses of UV-B damages photosynthetic reaction centres, cross-links cellular proteins and induces mutagenic DNA lesions, it was proposed that *D. sinuata* plants that occur naturally at higher latitudes associated with higher UV-B levels may be physiologically and reproductively less sensitive to UV-B radiation. Plants are unique in their ability to obtain energy directly from sunlight for photosynthesis and, as a result, are subject to continuous exposure to the ultraviolet (UV) radiation that is present in the spectrum of solar radiation. Unlike animals, plants do not sequester a germ line early in development. Thus a stress-induced mutation in any cell that later gives rise to reproductive tissue can be passed onto the next generation. Such heritable stress-induced somatic mutations may play a potential role in the evolutionary process. Genetic changes induced by environmental stress and the potential impact these changes have on organismal evolution are areas of both great interest and controversy. Stress-induced mutations have been documented in many organisms [11]. Unfortunately, the mechanisms that generate these mutations, the type of stress-induced mutations that occur in plants and whether or not these mutations are inherited and thus of evolutionary significance is still unknown.

By virtue of being the light energy harvesting machinery of the plant, chloroplasts have a relatively greater potential for acquiring ultraviolet induced genetic damage than other organelles. It was therefore decided that chloroplast DNA (ctDNA) from natural populations would be analysed. The present study describes investigations into the genetics of long term UV-B exposure in Namaqualand daisies from Southern Africa and the aim was to establish if indigenous plants are endangered by prolonged ultra-violet radiation exposure.

MATERIALS AND METHODS

Seeds used to generate study plants were collected from three different sites in the Republic of South Africa. The northern-most site was Augrabies Falls (28°38'S, 20°25'E) and the southern-most was Kirstenbosch Botanical Gardens, Cape Town (35°12'S,

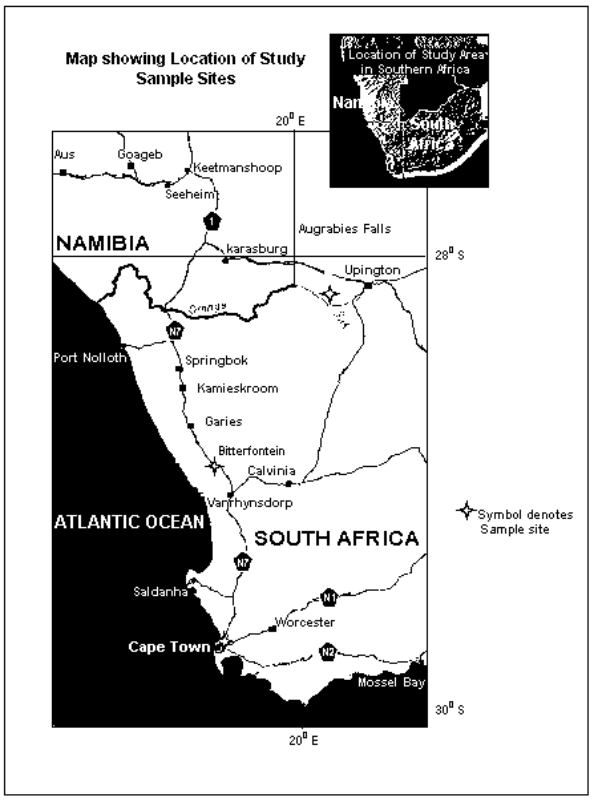

Fig. 1: Location of sites from which samples were collected

18°25'E). Seeds for the Kirstenbosch population were collected from the wild several generations in the past and were propagated in the Kirstenbosch Botanical Gardens. Samples were also collected from Bitterfontein, representing the mid-latitudes (Fig. 1).

Seeds were soaked for five minutes in a 5% solution of sodium hypochlorite and rinsed five times in distilled water. The seeds were then placed on five layers of moistened Whatman filter paper on Petri dishes and these were sealed with paraffin-wax film to minimize evaporation. Seeds were germinated in the dark for three days before being potted in potting medium comprising coarse sand, leaf mould and loam (2:1:1, v/v) and were watered with tap water daily thereafter. The standard conditions in the growth room were as follows: temperature = 22°C, relative humidity = 65%, 16 hours light and 8 hours darkness with an intensity of 100 µmol m^{-2} s^{-1}. After six weeks, leaf samples were taken for chloroplast DNA isolation.

Chloroplast DNA was isolated from fresh plant material by first enriching for the chloroplast fraction in sucrose gradients [12]. 5µg aliquots of ctDNA were cut to completion with the following restriction enzymes (RE): *Bam* HI, *Dra* I, *Eco* RI, *Eco* RV and *Hin*d III (Roche Molecular Diagnostics). The entire digest was then loaded onto a 25 cm-long, 0.8% agarose gel and subjected to electrophoresis for 16 hours at 30V to estimate the ctDNA genome size and to compare the RE digestion patterns of plants from different latitudes. Samples were divided into two and were separated by electrophoresis on 0.6% and 1.5% agarose gels to resolve the high molecular weight and low molecular weight fragments respectively. Alternatively, digests were resolved by electrophoresis on 0.8% agarose gels, blotted onto a positively charged nylon membrane and probed with a DIG-labelled *Bam* HI digest of ctDNA.

RESULTS AND DISCUSSION

The chloroplast DNA digest is shown in Fig. 2. The gel was probed with DIG-labelled chloroplast DNA that had been digested with *Bam* HI. Lanes 1 and 2 = *Dra* I, lanes 3 and 4 = *Bam* HI, lanes 5 and 6 = *Eco*R V. The chloroplast genome size of *D. sinuata* was estimated to be 123.80±11.57 kb and lies within the range reported for chloroplast DNA sizes of higher plants (80-200 kb) [13].

Chloroplast DNA restriction endonuclease analysis was used in this study because T=T sites in the DNA were being proposed as potential candidates for indicating UV-B effects. This is because the chloroplast genome, by virtue of being housed in the light harvesting apparatus, is likely to be targeted by the damaging effects of UV-B radiation. Chloroplast DNA also has a relatively higher likelihood of acquiring UV-induced genetic damage, especially at T=T sites. This

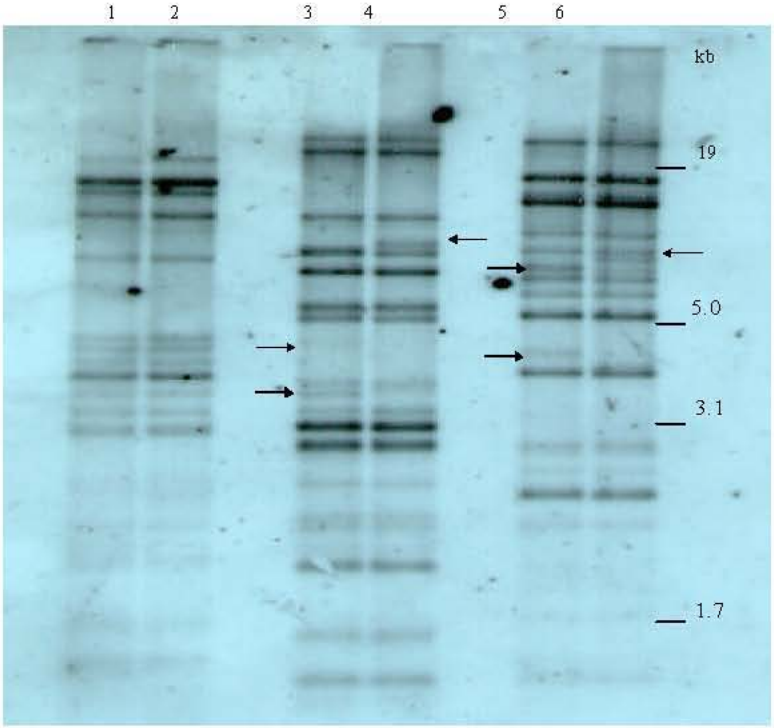

Fig. 2: Southern blot of chloroplast DNA. Samples from Kirstenbosch (lanes 1, 3 and 5) and Augrabies Falls (lanes 2, 4 and 6). The arrowheads indicate the polymorphic bands from the different restriction endonucleases. Fragment sizes are indicated on the right of the figure in kilo bases

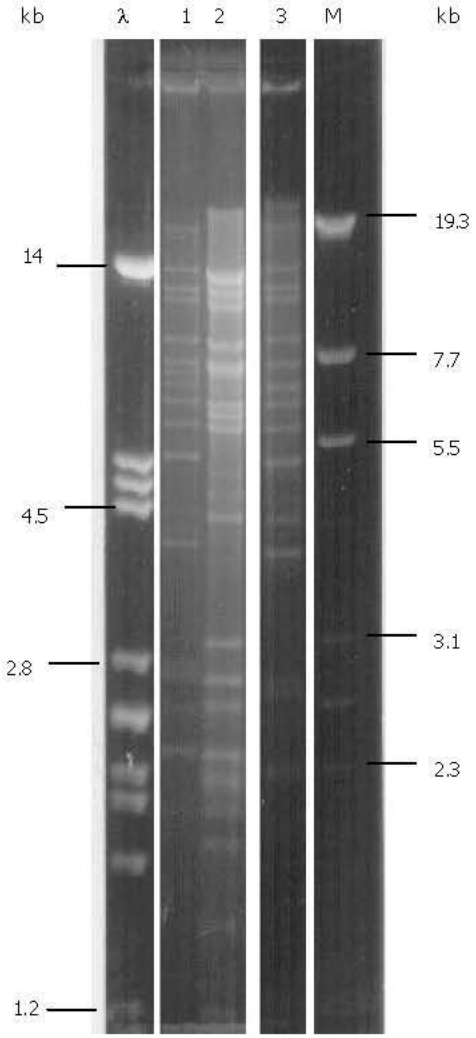

Fig. 3: Chloroplast DNA restriction endonuclease analysis

could be tested using restriction endonucleases which target T=T sites in the DNA such as *Dra*I, *Eco*RI and *Hin*dIII. However, DNA samples showed polymorphisms with enzymes that do not portray obvious UV-B targets in their recognition sequences - *Bam H*I and *Eco R*V, (Fig. 2). Enzymes selected on the basis of being potential UV-B targets did not show any differences (*Dra* I, *Eco* RI and *Hind* III). The observed polymorphisms may be attributed to evolutionary processes acting on this natural population possibly resulting in re-arrangements of the genome. This is in agreement with previous reports that chloroplast genomes usually undergo re-arrangements when subjected to stress [8, 14].

Fig. 3 shows chloroplast DNA from different latitudes digested with the *EcoR* V endonuclease and resolved on a 0.8% agarose gel. This is a composite of ctDNA digests of samples from Augrabies Falls (Lane 1), Bitterfontein (Lane 2) and Kirstenbosch Botanical Gardens (Lane 3). Samples were resolved on the same gel from which a composite was made for comparison purposes and for clarity. λ = λ-*Pst* I molecular weight marker, M = high molecular weight marker IV.

Assessing UV-B radiation sensitivity in plants is not easy. This is because sensitivity differs between species and even varieties and is further influenced by other environmental conditions as well as the developmental history of the plants and geographical origin of the species. It has been hypothesised that species originating from areas that receive high levels of UV-B radiation would be highly resistant to UV-B radiation and there is evidence that species and ecotypes native to low latitudes are inherently more resistant to UV-B irradiation [15]. Since the UV component of sunlight is capable of inducing photo-damage in DNA, plants from areas with high UV-B levels must possess means to prevent DNA damage and repair UV-induced lesions that invariably occur [16]. The absence of any UV-B based differences between the samples in this study could possibly point to the presence of an efficient repair mechanism for UV damaged lesions in *D. sinuata*. It is clear from the results that to fully address this issue, a far more extensive analysis is required. The results do however, indicate the potential for this approach and provide some useful insight into the complexities involved in stress responses in plants. In addition, UV-irradiation is probably a major contributor to plastome damage, but since the chloroplast contains DNA that is used as a transcriptional template for gene products essential for photosynthesis and, therefore plant growth and productivity, it is reasonable to assume that there is an efficient mode of DNA damage repair in the organelles of the Namaqualand daisy, *D. sinuata* at all sites The structure and expression of the chloroplast genome has been studied in a number of plants [16] and the gene content and the sequence of many genes in the chloroplast have been found to be relatively conserved among land plants. However, an analysis of the entire chloroplast genome of *D. sinuata* has revealed that this is not always the case as evidenced by the polymorphic bands in Fig. 2.

To understand the process of chloroplast genome evolution, information on repeated sequences, intergenic regions and pseudo genes in chloroplast DNA is extremely helpful. Knowledge of the plants' UV-B sensitivity would also shed light on the genetic structure of the different populations and the likely existence of heterogeneity of plants in the different populations and the existence of distinct geographic

patterning of the populations. Future studies could also focus on determining the level of polymorphism between ecotypes of other species growing in the same environments and this would shed some light on the sensitivities of those particular plants to UV-B.

To further enhance the relevance/significance of the results obtained, future studies should look at differences in the sensitivity to UV-B between the samples and identifying a few plants of different sensitivities and limiting studies to those. From the results, it was concluded that there is a difference between the various samples which could be attributed to isolation due to the physical environment. Bitterfontein is physically isolated by the Gamiesberg mountain range and experiences a maritime climate that is foggy most of the time and hence experiences less UV-B levels. On the other hand, Augrabies Falls is in the heart of the Karoo with very clear skies leading to more UV-B radiation. These may be true but unless the differences in sensitivity are very great, it would always be difficult to try and elucidate the mechanism behind the difference based on a limited analysis of chloroplast DNA.

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REFERENCES

1. Green AES, 1983. The penetration of ultraviolet radiation to the ground. Physiologia Plantarum., 58: 351-359.
2. Musil, C.F., M.C. Rutherford, L.W. Powrie, L.F. Bjorn and D.J. McDonald, 1999. Spatial and temporal changes in South African solar ultraviolet-B exposure: Implications for threatened taxa. Ambio, 28: 450-456.
3. Caldwell, M., R. Robberecht and W.D. Billings, 1980. Steep latitudinal gradient of solar UV-B radiation in the arctic-alpine life zone. Ecology, 61: 00-611.
4. Ziska, L.H., A.H. Teramura and J.H. Sullivan, 1992. Physiological sensitivities of plants along an elevational gradient to UV-B radiation. Am. J. Bot., 79: 863-871.
5. Cullis, C.A., S. Swami and Y. Song, 1999. RAPD polymorphisms detected among the flax genotrophs. Plant Mol. Biol., 41:795-800.
6. Hoffer, P.H. and D.A. Christopher, 1997. Structure and blue-light responsive transcription of a chloroplast psbD promoter from Arabidopsis thaliana. Plant Physiol., 115: 213-222.
7. Lidholm, J. and Gustafsson, 1991. The chloroplast genome of the gymnosperm Pinus contorta: A physical map and a complete collection of overlapping clones. Current Genet., 20: 161-166.
8. Midgley, G.F., S.J.E. Wand and C.F. Musil, 1998. Repeated exposure to enhanced UV-B radiation in successive generations increases developmental instability (fluctuating asymmetry) in a desert annual. Plant, Cell and Environment, 21: 437-442.
9. Musil, C.F., 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.
10. Musil, C.F., 1995. Differential effects of elevated ultraviolet-B radiation on the photochemical and reproductive performances of dicotyledonous and monocotyledonous arid-environment ephemerals. Plant, Cell and Environment, 18: 844-854.
11. Waters, E.R. and B.A. Schaal, 1996. Heat shock induces a loss of rRNA-encoding DNA repeats in Brassica nigra. Proc. Natl. Acad. Sci., USA, 93: 1449-1452.
12. Palmer, J.D., 1986. Isolation and structural analysis of chloroplast DNA. Methods in Enzymology 118: 167-186.
13. Musil, C.F. and S.J.E. Wand, 1994. Differential stimulation of an arid-environment winter ephemeral *Dimorphotheca pluvialis* (L.) Moench by ultraviolet-B radiation under nutrient limitation. Plant, Cell and Environment, 17: 245-255.
14. Ries, G., W. Heller, H. Puchta, H. Sandermann, H.K. Seidlitz and B. Hohn, 2000. Elevated UV-B radiation reduces genome stability in plants. Nature, 406: 98-101.
15. Caldwell, M.M., A.H. Teramura and M. Tevini, 1989. The changing solar ultraviolet climate and the ecological consequences for higher plants. Tree, 4: 363-367.
16. Cannon, G.C., L.A. Hedrick and S. Heinhorst, 1995. Repair mechanisms of UV-induced DNA damage in soybean chloroplasts. Plant Mol. Biol., 29: 1267-1277.
17. Wakasugi, T., T. Nagai, M. Kapoor, M. Sugita, M. Ito, S. Ito, J. Tsudzuki, K. Nakashima, T. Tsudzuki, Y. Suzuki, A. Hamada, T. Ohta, A. Inamura, K. Yoshinaga and M. Sugiura, 1997. Complete nucleotide sequence of the chloroplast genome from the green alga Chlorella vulgaris: The existence of genes possibly involved in chloroplast division. Proc. Natl. Acad. Sci., USA, 94: 5967-5972.