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Reduction in *cab* and *psb* A RNA transcripts in response to supplementary ultraviolet-B radiation

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The *cab* and *psb* A RNA transcript levels have been determined in *Pisum sativum* leaves exposed to supplementary ultraviolet-B radiation. The nuclear-encoded *cab* transcripts are reduced to low levels after only 4 h of UV-B treatment and are undetectable after 3 days exposure. In contrast, the chloroplast-encoded *psb* A transcript levels, although reduced, are present for at least 3 days. After short periods of UV-B exposure (4 h or 8 h), followed by recovery under control conditions, *cab* RNA transcript levels had not recovered after 1 day, but were re-established to ca. 60% of control levels after 2 more days. Increased irradiance during exposure to UV-B reduced the effect upon *cab* transcripts, although the decrease was still substantial. These results indicate rapid changes in the cellular regulation of gene expression in response to supplementary UV-B and suggest increased UV-B radiation may have profound consequences for future productivity of sensitive crop species.

cab RNA; *psb* A RNA; UV-B stress; Pea

1. INTRODUCTION

The depletion of the stratospheric ozone layer is predicted to increase the solar ultraviolet-B radiation (UV-B: 280–320 nm) with potentially deleterious consequences for plant growth and development [1–3]. A major effect of increased UV-B is a change in the biochemical composition of the chloroplast and impairment of photosynthetic function [4]. In a recent study [5] using supplementary UV-B radiation, the activities of a number of chloroplast proteins (e.g. chloroplast coupling factor 1; Rubisco) were progressively reduced in pea leaves over a period of days. In addition, there was a loss of photosynthetic quantum efficiency (~90% reduction over 7 days), mainly caused by alterations in photosystem II function. Although these and similar effects on photosynthesis are established, there is no information on the molecular mechanisms that lead to these changes. In this study, the steady state RNA transcript levels have been determined for two important thylakoid membrane proteins (the nuclear-encoded chlorophyll *a/b*-binding protein and the chloroplast-encoded D1 protein of the photosystem II reaction centre complex) in pea plants subjected to moderate levels of supplementary UV-B radiation.

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2. MATERIALS AND METHODS

2.1.

Pea (*Pisum sativum* L., cv. Greenfeast) seedlings were grown in a controlled environment with a 12 h light (22°C), 12 h dark (16°C) cycle. Incident irradiation was provided by 7 Philips TL 40W 33RS fluorescent tubes, approximately 150 μmol photons m^{-2} s^{-1} (400–700 nm) for 17 days. Half of the plants were then transferred into another cabinet where fluorescent lamps (4 Philips TL 40W 33RS and 4 Philips SL35 Prismatic supplying equal photosynthetically active radiation) were supplemented by 3 UV-B lamps (Philips TL 40W/12 UV) during the 12 h period. The levels of UV-B were 50 and 220 mW m^{-2} nm^{-1} at 297 nm and 313 nm respectively, as determined by using an IL1700 Research Radiometer with calibrated photodetector/filters (International Light, Newburyport, USA). The UV-B level at 297 nm is approximately 2.5 times that detected in Canberra in mid-summer and at 313 nm the UV-B level is equivalent to mid-summer in Canberra (further details of the UV-B treatment can be found in [5]). In experiments with increased photosynthetically active radiation, a Siemens (Wotan) Power Star HQIT WD 400 W lamp was used in addition to the fluorescent lamps, giving an approximate irradiance of 400 μmol photons m^{-2} s^{-1} (400–700 nm).

2.2. *Purification of RNA*

RNA was purified by a modification of the methods described in [6]. Samples (ca. 1.0 g fresh weight of pea leaf tissue) were homogenised in a buffer containing 50 mM Tris-HCl; pH 8.0, 4% (w/v) *p*-aminosalicylate, 1% (w/v) tri-isopropylnaphthalene sulphonate (sodium salt) and 2% (v/v) mercaptoethanol using a mortar and pestle. The aqueous phase was then blended with a polytron in an equal volume of 1:1 phenol (containing 0.1% w/v hydroxyquinoline):chloroform. The homogenate was partitioned by cen-

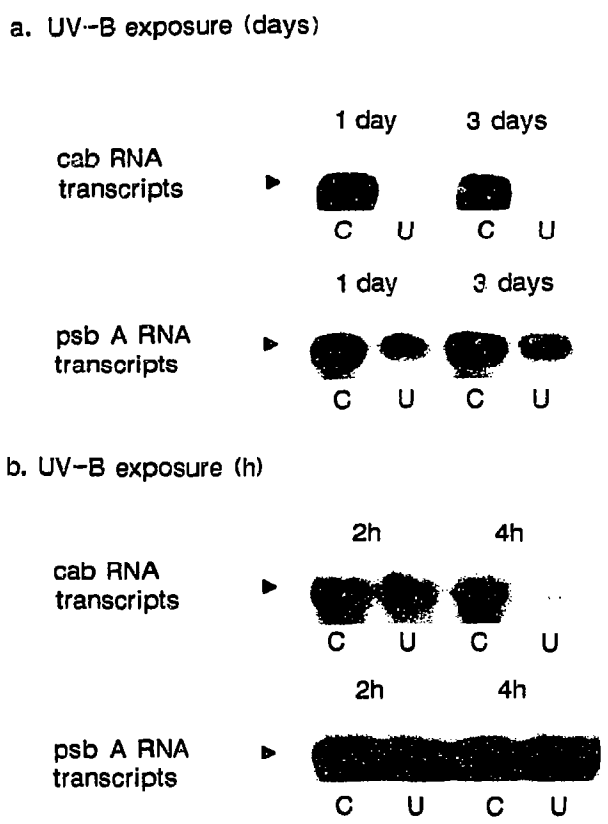

Fig. 1. Autoradiographs of Northern-blot analysis of total RNA (20 μg) from pea leaves. The RNA was isolated from control plants (C) or those exposed to UV-B radiation (U) and then hybridised with either ^{32}P-labelled *cab* or *psb* A sequences. (a) The response to days of UV-B exposure. (b) The response to hours (h) of UV-B exposure.

trifugation at 5000 × *g*, for 10 min in a Sorvall SS-34 rotor. The phenol phase was reextracted with buffer and the total nucleic acids within the combined aqueous phases precipitated by the addition of 0.1 volume of 3.0 M sodium acetate (pH 5.6) and 2.2 volumes of ethanol. After 4 h at −20°C, the nucleic acids were centrifuged at 5000 × *g* for 30 min. The pellet was resuspended in 500 μl of H_2O and LiCl added to a final concentration of 2.7 M. The RNA was selectively precipitated at 4°C overnight and centrifuged in a microfuge for 10 min to collect the pellet. The RNA was washed sequentially in 3.0 M sodium acetate (pH 5.6) and 70% ethanol before drying and resuspending in water. The RNA absorbance was routinely scanned between 320 and 220 nm and quantified by absorbance at 260 nm before storage at −70°C.

2.3. *Electrophoresis, Northern blotting, dot-blotting and hybridisation*

RNA was separated in either 1.2 or 1.5% agarose gels containing 6% (v/v) formaldehyde and transferred to nylon Hybond-N (Amersham International, plc) by capillary blotting. Following transfer, the RNA was cross-linked to the nylon by 5 min UV irradiation. The filters were prehybridised (6–24 h) and then hybridised (24 h) with radiolabelled α-^{32}P CTP (radiolabelling was by Nick translation using a Bresatec Kit) as described previously [6]. Washing of nylon filters was either with 2 × 15 min at room temperature in 1 × SSC; 0.1% (w/v) SDS followed by 2 × 15 min at 55°C in the same solution or 2 × 15 min at room temperature and 2 × 15 min at 65°C in 0.3 × SSC; 0.1% SDS (w/v). Dot-blotting was carried out using a bio-dot microfiltration unit (Biorad Laboratories, Richmond, USA). Autoradiography of the filters was at −70°C using Fuji RX film with a single intensifying screen (further details of these methods can be found in [6,7]). Quantitation of the dots was made using a Hoefer GS300 scanning densitometer.

2.4. *DNA sequences*

The *psb* A sequence for the 32 kDa protein is an 850 bp *Hin*dIII fragment containing the 3′ 60% of the gene from spinach [6]. The *cab* sequence (pAB 96) for the chlorophyll *a/b*-binding protein is a 1.2 kbp *Pst*I cDNA clone from pea [8].

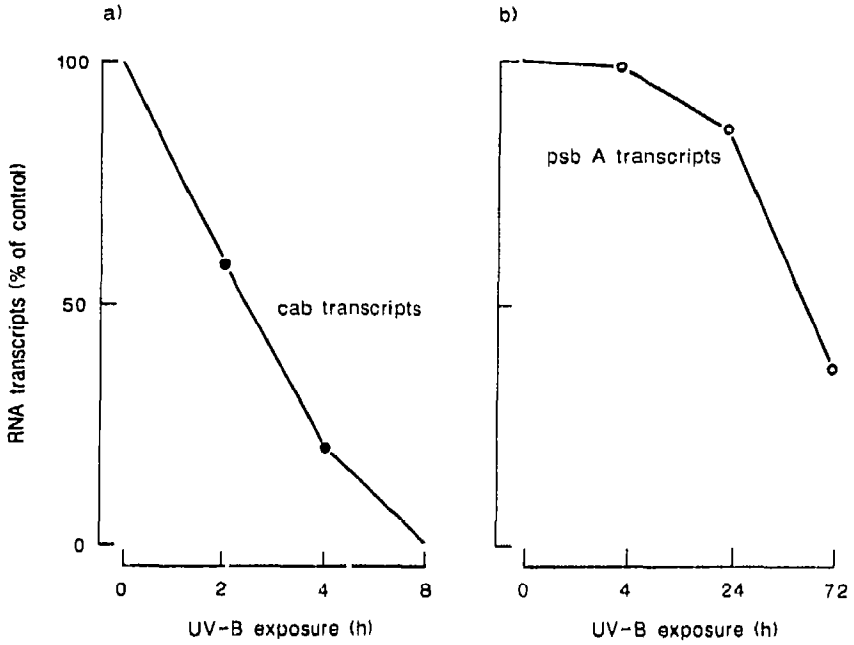

Fig. 2. Quantitative dot-blot analysis of total RNA (10 μg) from pea leaves. RNA was isolated from control plants or those exposed to UV-B radiation for different time periods. Each RNA sample was then spotted onto nylon in triplicate and hybridised with either ^{32}P-labelled *cab* (a) or *psb* A (b) sequences. After autoradiography, the dots were quantitated using a Hoefer GS300 scanning densitometer and the data calculated as a percentage of the controls.

3. RESULTS

RNA was isolated from the third leaf pair from the base of pea seedlings grown in the presence or absence of supplementary UV-B radiation. Aliquots of total RNA were then transferred to nylon and subjected to Northern-blot analysis with either *cab* or *psb* A gene sequences (Fig. 1). The *cab* mRNA transcripts were present in trace amounts after 1 day and not detectable after 3 days of UV-B treatment (Fig. 1a). The *psb* A transcripts were reduced after one day (Fig. 1a), but in contrast to *cab* substantial amounts remained and were still detectable after 3 days of UV-B treatment. To determine the time course of this reduction in *cab* and *psb* A transcripts, RNA isolated from plants subjected to short periods of UV-B radiation was examined by Northern-blot analysis (Fig. 1b). The *cab* mRNA transcripts were found to be severely reduced after 4 h of UV-B treatment. The same RNA samples probed with *psb* A showed no corresponding reduction. At 8 h and 12 h UV-B exposure *cab* transcripts were either undetectable or in trace amounts, whereas *psb* A transcripts showed little change (data not shown). Quantitative dot-blot analysis showed the *cab* transcript level was 58% of the control at 2 h and declined at 4 h to <20% of the control (Fig. 2a). In comparison, the chloroplast encoded *psb* A transcripts were still 35% of the control after 3 days of UV-B radiation (Fig. 2b). The effect of the UV-B radiation on *cab* transcripts was further analysed by Northern and dot-blots after a period of recovery from the UV-B treatment. The plants were subjected to 4 h or 8 h of UV-B radiation and then allowed to recover under the conditions of the control plants. After 24 h (12 h light and 12 h dark) recovery, the *cab* transcripts were not detectable (data not shown), however, after a further 2 days recovery the *cab* transcript level had returned (Fig. 3) to 67% and 52% respectively of the control (as determined by quantitative dot-blot analysis). Increased irradiance may protect plants from UV-B radiation [4]. To study this effect on *cab* transcript levels, plants were subjected to the normal UV-B exposure, but with increased irradiance (400 μmol photons m^{-2} s^{-1} compared with

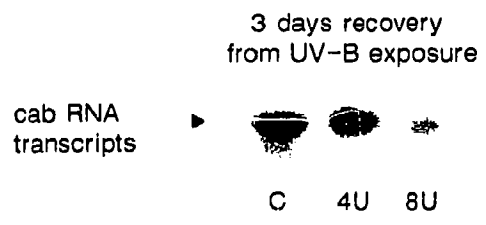

Fig. 3. Autoradiographs of Northern-blot analysis of total RNA (20 μg) from pea leaves. The pea plants were either exposed to 4 h (4U) or 8 h (8U) of UV-B radiation and then allowed to recover for 3 days in control conditions. RNA was then isolated from them and from untreated control plants (C). The RNA was hybridised with a ^{32}P-labelled *cab* sequence.

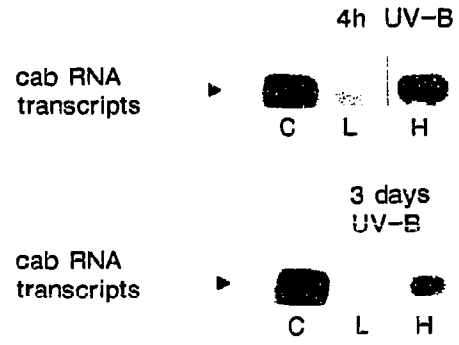

Fig. 4. Autoradiographs of Northern-blot analysis of total RNA (20 μg) from pea leaves. Plants were exposed to UV-B radiation in the presence of low (L: 150 μmol photons m^{-2} s^{-1}) or high (H: 400 μmol photons m^{-2} s^{-1}) irradiance for either 4 h or 3 days. RNA was then isolated from them and from untreated controls (C). The RNA was hybridised with a ^{32}P-labelled *cab* sequence.

150 μmol photons m^{-2} s^{-1}). Although the *cab* transcripts were still reduced, they were maintained at a higher level than under low irradiance, both after 4 h and 3 days of UV-B exposure (Fig. 4).

4. DISCUSSION

To our knowledge this is the first study of the effect of supplementary UV-B radiation on the gene expression for chloroplast proteins. The results show a profound reduction in the steady-state mRNA transcript levels for the *cab* sequence. The *psb* A RNA transcripts are also reduced, but neither as rapidly nor to the same extent as those for *cab*. The results also show that the supplementary UV-B treatment has not caused an irreversible change to the regulatory control involved in maintaining the normal RNA transcript levels (Fig. 3). In addition, increased irradiance during UV-B exposure 'protects' the *cab* transcript levels to some extent (Fig. 4).

The specific molecular mechanisms involved in the rapid reduction in *cab* transcripts (i.e. transcription, translation, RNA stability, etc.) cannot be established from the present study. However, the RNA transcripts for Rubisco (*rbc* S) show the same response as *cab*, suggesting a common UV-B effect upon nuclear encoded biosynthesis of chloroplast proteins. It is also well known that the levels of RNA transcripts for nuclear-encoded proteins such as *cab*, are frequently regulated at transcription, involving *cis*-elements and *trans*-acting factors [9,10]. It is therefore likely that the perception of the UV-B radiation leads to the transduction of a signal that rapidly inhibits the transcription of the *cab* gene and subsequently to the reduction in the RNA transcript level. Furthermore, the rapid increase in gene expression for enzymes of the flavonoid biosynthetic pathway in response to UV-B is known to be regulated at the level of transcription [11]. In the present study,

an increase in UV-B absorbing pigments (flavonoids, etc.) was detected in the leaf tissue. The increase could be initiated by short periods (4 h) of UV-B treatment and continued with the time of UV-B exposure. This response suggests that enzymes required for flavonoid biosynthesis are rapidly produced in pea, presumably by increased transcription. The evidence that transcription can be switched on or off for different nuclear-encoded genes therefore suggests that the response to UV-B is not due purely to physical damage to the DNA.

Greenberg et al. [12] showed that the synthesis of the D1 protein in *Spirodella oligorrhiza* is not affected by UV-B radiation, although there is a significant increase in the rate of its degradation. In the present study the *psb* A transcripts are reduced to some extent, but this decline correlates with the relatively small decline over 3 days in functional photosystem II activity, suggesting an absolute priority to maintain the biosynthesis of the D1 protein [5]. Another chloroplast-encoded gene (*rbc* L) shows a more dramatic reduction in transcript levels under these UV-B treatments (after 8 h the *rbc* L is reduced by 60%). This suggests that the stability of the chloroplast encoded RNA transcripts may determine their steady state levels after UV-B exposure. In contrast to nuclear-encoded genes, chloroplast genes are frequently controlled at the level of post-transcriptional mechanisms [13] and it is therefore likely that a complex array of regulatory mechanisms are involved in adapting the level of gene expression for chloroplast proteins under UV-B stress conditions. Furthermore, similar rapid down-regulation of Rubisco has been shown in plants infected with pathogens [14] and this response may also reflect a similar regulation to that initiated by UV-B.

This study suggests that even short, repeated periods of moderate supplementary UV-B radiation can have a profound effect upon gene expression, particularly if the recovery kinetics are slow. Thus any future increase in the UV-B radiation level may have a significant impact upon crop productivity and the ecology of plant communities. However, increased irradiance does reduce the UV-B effect, either by increasing the efficiency of photo-repair mechanisms or by the provision of more energy. This response could be important in moderating the UV-B effect under natural daylight conditions.

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