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**Regulation of Gene Expression in Pea (*Pisum sativum* L.) by
Ultraviolet-B Radiation**

By

Liansen Liu

**Submitted in partial fulfilment of the requirements for the degree of Doctor of
Philosophy**

At

**Dalhousie University
Halifax, Nova Scotia, Canada
August, 1999**

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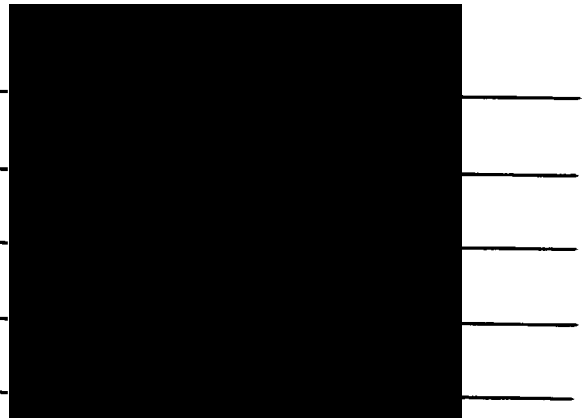
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by Liansen Liu

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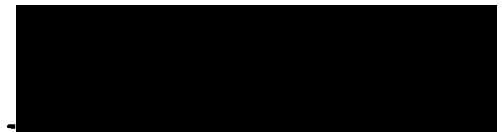
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To My Parents

Liu, Qinxin (刘清鑫)

Yang, Shoulan (杨守兰)

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Abstract

The stratospheric ozone layer depletion causes an increase in solar ultraviolet-B radiation (UV-B, 290-320 nm) reaching the earth's surface. This may adversely affect agricultural and forestry production because enhanced UV-B may inhibit photosynthesis. The nuclear genes encoding light-harvesting complex II proteins (*lhcb*), which are components essential for the first step of photosynthesis, are extremely sensitive to UV-B. It is possible that UV-B down-regulates *lhcb* genes by damaging chloroplasts, for the transcription of these genes requires intact chloroplasts, the intracellular organelles most fragile upon UV-B exposure. In order to test this hypothesis, pea (*Pisum sativum* L.) seedlings were treated with norflurazon, an inhibitor of carotenoid biosynthesis, to increase the sensitivity of chloroplasts to photo-oxidative stress. The norflurazon-treated plants were grown under dim red light to exclude complexity caused by photoreceptors other than phytochromes. Opposite to the hypothesis that damage to chloroplasts may increase the sensitivity of *lhcb* gene expression to UV-B, UV-B exposure increased rather than reduced *lhcb* transcript accumulation under dim red light in seedlings after chloroplasts were partially photobleached by norflurazon and white light exposure. Thus, mature, intact chloroplasts are required for UV-B to exert an inhibitory effect on pea *lhcb* mRNA accumulation. Using reverse transcription polymerase chain reaction, all seven members of the *lhcb* family were found to respond similarly to UV-B; that is, mRNA accumulation for each gene decreased in green leaves but increased in etiolated buds. The UV-B response of these pea *lhcb* genes divided them into the same two subfamilies as defined by their response to red light, suggesting a possible link between the signal transduction pathways mediating UV-B and red light effects on the genes. As determined by examination of *lhcb1*2* and *lhcb1*4* mRNA accumulation, a carotenoid-related component may mediate UV-B inhibition of both subfamilies, while a chlorophyll-related component may only mediate UV-B inhibition of the subfamily represented by *lhcb1*2*. However, a carotenoid-related component only mediated red light effects on the subfamily represented by *lhcb1*2*, while a chlorophyll-related component mediated the red light effects on both subfamilies. Moreover, UV-B protected the red light-linked chloroplast components against white light-induced photodamage, suggesting that the UV-B and red light pathways are not identical. In addition, cDNA clones of six UV-B-responsive genes were identified in pea using rapid amplification of 3' cDNA ends by the polymerase chain reaction. Among them, *psUVzinc*, *psUVRub* and *psUVDeh* were UV-B-repressible, and *psUVaux*, *psUVglu*, and *psUVrib* were UV-B-inducible. The nucleotide sequence of *psUVzinc* was not closely related to any gene registered in GenBank, representing a novel UV-B-responsive gene in pea. The other clones shared sequence similarity with genes encoding Rubisco activase, β -1,3-glucanase, the pea dehydrin cognate B61, some 40S ribosomal proteins and a putative auxin-repressed protein of apricot, respectively. The cloning of these UV-B-responsive genes has generated new ideas concerning how UV-B affects plant growth and development.

Abbreviations and symbols

ATA	aurin tricarboxylic acid
bzip	basic leucine zipper
CG-1	CG-containing cis-element-binding protein-1
Chl a	chlorophyll a
Chl b	chlorophyll b
<i>Chs</i>	chalcone synthase gene
COP1	constitutive photomorphogenic mutant protein 1
CPD	cyclobutane pyrimidine dimer
CPRF	common plant regulatory factor
CRY1	cryptochrome
DEPC	diethylpyrocarbonate
DET1	deetiolated1
EDTA	ethylenediaminetetraacetic acid
<i>gDcPAL1</i>	<i>Daucus carota</i> phenylalanine ammonia-lyase gene
lhcb	light-harvesting complex II proteins
<i>lhcb</i>	genes encoding lhcb proteins
MED	minimal erythematol dose
NCBI	National Center of Biological Information
NER	Nucleotide excision repair
RT-PCR	reverse transcription polymerase chain reaction
P_{fr}	far-red light absorbing form of phytochrome
PCR	polymerase chain reaction
6-4 photoproduct	pyrimidine [6-4] pyrimidone photoproduct
<i>psbA</i>	the gene encoding photosystem II reaction center D1 protein
3' RACE	rapid amplification of 3' cDNA ends
RbcS	rubisco small subunit
SDS	sodium dodecyl sulfate
Tris	Tris-(hydroxymethyl)aminomethane
UV-B	ultraviolet-B (290-320 nm)

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I. Introduction

1.1 Global increase in solar ultraviolet-B radiation reaching the earth's surface

Stratospheric chlorines, including the fabricated chlorofluorocarbons, halons and other halogenated compounds, generate reactive fragments and therein destroy ozone molecules (Hayman, 1997). Although the consumption of these chemicals is restricted world wide, their accumulation in the stratosphere is predicted to continue for at least the next 70 years (Coldiron, 1996). Because chlorines are long lasting (Tevini, 1994), stratospheric ozone depletion will probably proceed for several hundred years (Coldiron, 1996).

Ozone efficiently absorbs all irradiation within the solar spectrum shorter than 290 nm, but its absorption coefficient drops in the ultraviolet-B (UV-B, 290-320 nm) region and it hardly absorbs irradiance longer than 320 nm (Frederick and Lubin, 1994). Therefore, depletion of the stratospheric ozone layer will preferentially increase UV-B transmission of solar irradiance through the atmosphere.

1.2 Effects of UV on plant gene expression

UV affects numerous aspects of plant growth and development (Tevini, 1994; Jordan, 1993; Jenkins et al., 1995; Manning and Tiedemann, 1995). Such effects may result from UV-induced inhibition or stimulation of gene expression. A good example of this is the correlation between UV-B-induced repression of photosynthesis-related genes and photosynthesis efficiency (Jordan, 1996). On the other hand, UV probably increases the resistance of plants to UV and/or pathogen by activation of the genes encoding phenylpropanoid-pathway

enzymes that catalyze biosynthesis of UV-screening pigments, and pathogen-related proteins (references in Table 1). Thus, to understand the mechanisms in this regard, a few studies were carried out before the early 1990's to look at the effects of UV on plant gene expression, using either UV-C (<290 nm), UV-A (320-400 nm) or the combination of UV-A and UV-B (Table 1). In recent years, such studies tended to focus on UV-B (Jordan, 1996) or to compare UV-B with other light qualities (Merkle et al., 1994; Jenkins et al., 1995). Although these three wavebands may affect plant gene expression in similar patterns, signal transduction pathways mediating their effects may, at least partially, differ from one another (Christie and Jenkins, 1996).

UV-responsive plant genes identified to-date either encode transcription factors (Kircher et al., 1998), photosynthesis-related proteins (Jordan, 1996), pathogen-related proteins (Brederode et al., 1991), enzymes for phenylpropanoid metabolism (Douglas et al., 1991) or cell cycle-related proteins (Logemann et al., 1995). Additionally, cloning of two UV-responsive genes encoding molecular chaperones in the cyanobacterium *Synechocystis* sp. suggests that plants may possess other UV-regulated genes (Chitnis and Nelson, 1991). Effects of UV on genes are variable, with some repressed and others induced (Table 1). The UV-repressible genes are mostly photosynthesis- or cell cycle-related, while the inducible genes are normally pathogen- or defense-related. The magnitude of UV-induced repression or induction differs from gene to gene. As one example, transcripts of the nuclear genes encoding light-harvesting complex II proteins (lhcb) are reduced to low levels after only 4 h of UV-B treatment and are

Table 1. UV-responsive genes in plants

Gene	Species	Radiation	Transcription	Encoded protein	Reference
<i>CPRF1</i>	parsley	UV-A and UV-B	no response	transcription factor	Kircher et al., 1998
<i>CPRF2</i>	parsley	UV-A and UV-B	no response	transcription factor	Kircher et al., 1998
<i>CPRF4a</i>	parsley	UV-A and UV-B	no response	transcription factor	Kircher et al., 1998
<i>CG-1</i>	<i>Petroselinum crispum</i>	UV-containing white light	inducible	DNA-binding protein	da Costa, 1994
<i>lhcb</i>	pea	UV-B	repressible	light-harvesting complex II protein	Jordan et al., 1991
<i>psb A</i>	pea	UV-B	repressible	photosystem II center D1 protein	Jordan et al., 1991
<i>H2A</i>	parsley	300 – 400 nm	repressible	Histone	Logemann et al., 1995
<i>H2B</i>	parsley	UV-containing	repressible	Histone	Logemann et al., 1995
<i>H3</i>	parsley	white light	repressible	Histone	Logemann et al., 1995
<i>H4</i>	parsley		repressible	Histone	Logemann et al., 1995
<i>p34cdc2</i>	parsley		repressible	Protein kinase	Logemann et al., 1995
<i>cyclin</i>	parsley		repressible	Mitotic cyclin	Logemann et al., 1995
<i>PAL</i>	parsley		repressible	Phenylalanine ammonia-lyase	Logemann et al., 1995
<i>chs</i>	<i>Antirrhinum majus</i>	UV-B	inducible	Chalcone synthase	Staiger et al., 1989
	soybean	UV-A or UV-B	inducible		Wingender et al., 1989
	pea	NA*	inducible		Ito et al., 1997
	pea	UV-B	inducible		Jordan et al., 1994
<i>PR</i>	tobacco	UV-C	inducible	Pathogen-related protein	Brederode et al., 1991
<i>chi</i>	bean	UV-C	inducible	Chitinase	Margis et al., 1993
<i>chit 4</i>	peanut	UV-C	inducible	Chitinase	Herget et al., 1990
<i>OMT</i>	barley	NA*	inducible	O-methyltransferases	Gregersen et al., 1994
<i>PI-II</i>	potato, tomato, tobacco	NA*	inducible	Type II proteinase inhibitor	Balandin et al., 1995
<i>Adh</i>	potato	UV-C	inducible	Alcohol dehydrogenase	Matton et al., 1990
<i>STS</i>	peanut	UV-B	inducible	Stilbene synthase	Hain et al., 1990
<i>CPR</i>	parsley	UV-containing white light	inducible	NADPH:cytochrome P450 oxidoreductases	Koopmann and Hahlbrock, 1997
<i>APX3</i>	<i>Arabidopsis</i>	UV-A	inducible	Peroxisomal membrane-bound ascorbate peroxidase	Zhang et al., 1997
<i>Cat2</i>	maize	UV-A, UV-B	inducible	Catalase-2	Boldt and Scandalios, 1997
<i>Cat3</i>	maize	UV-A, UV-B	inducible	Catalase-3	
<i>4CL-1</i>	parsley	300 – 400 nm UV-containing white light	inducible	4-coumarate:CoA ligase	Douglas et al., 1991
<i>SOD</i>	pea	UV-B	repressible	superoxide dismutase	Strid, 1993
<i>GR</i>	pea	UV-B	inducible	glutathione reductase	Strid, 1993

NA*, UV wavelengths are unknown.

undetectable after pea seedlings are exposed to UV-B for 3 days. In contrast, mRNA of the chloroplast gene *psbA* that encodes the D1 subunit of the photosystem II reaction center, although reduced in amount, is still present in these plants (Jordan et al., 1991).

Differences in response to UV also extend to individual members of multigene families (Table 2). For example, in the family encoding chitinases, the *chit 3* gene is constitutively expressed at a low level and shows no response to UV-C nor to other stimuli, while *chit 4* gene is induced by UV-C, and both *chit 1* and *chit 2* genes are activated by stimuli other than UV-C (Herget et al., 1990). Additionally, differential UV responses of individual genes have been observed in the multigene families encoding NADPH:cytochrome P450 oxidoreductases (Koopmann and Hahlbrock, 1997), and chalcone synthase (Ito et al., 1997). The biological significance for plants of such selective responses within the multigene families is unknown. However, for cyanobacteria, induction of *psbA* genes by differential transcription in response to UV-B provides these bacterial cells with identical proteins or isoforms to replace UV-B-damaged proteins, thereby increasing the resistance of the organism to UV-B radiation (Campbell et al., 1998; Mate et al., 1998).

The effects of UV-B on gene expression may be developmentally regulated. Upon UV-B irradiation, *lhcb* transcripts decrease in green apical buds and fully expanded leaves (Jordan et al., 1994, 1996), but increase in etiolated tissues. It seems that this developmentally regulated UV-B response correlates with chloroplast maturation (Mackerness et al., 1996). If chlorophyll accumulates

Table 2. Differential UV-B responses of multigene family members

Family	Genes	Radiation	Species	Transcription	Reference
Chit	chit 1 chit 2 chit 3 chit 4	UV-C	Peanut	no response no response no response inducible	Herget et al., 1990
CPR	CPR1 CPR2	UV-containing white light	Parsley	inducible no response	Koopmann and Hahlbrock, 1997
CHS	CHS1, 2, 3, 4, 5, and 8 CHS6, 7	UV-B	Pea	inducible no response	Ito et al., 1997

Refer to Table 1 for the protein that each gene encodes.

to some extent, UV-B reduces *lhcb* mRNA, otherwise transcription is increased.

Interactions between UV and other stresses have been documented for many biological and biochemical processes in plants (Jordan, 1996). This may be due to the fact that UV-responsive genes also respond to stimuli such as heat shock, oxidative stress (Chitnis and Nelson, 1991), pathogens and wounding (Douglas et al., 1991) (Table 3). The responses of the genes to these stimuli are more lasting and stronger than to UV (Logemann et al., 1995; Margis et al., 1993), however, UV may elicit a much faster reaction, as demonstrated by transfection of a groundnut (*Arachis hypogaea*) stilbene synthase gene into tobacco cells (*Nicotiana tabacum*). The stilbene synthase mRNA was detectable 30 minutes after the onset of chemical elicitor treatments and 10 minutes after UV irradiation (Hain et al., 1990).

UV affects mainly transcription. In cyanobacteria, fusion of the promoter regions of *psbA2* and *psbA3* genes to firefly luciferase (*luc*) reporter gene indicates that transcription of *psbA2/luc* and *psbA3/luc* transgenes is elevated by UV-B, similar to that of endogenous *psbA* genes (Mate et al., 1998). Transcription experiments carried out in isolated nuclei demonstrated that UV-B-induced regulation of *lhcb* mRNA accumulation occurs transcriptionally (Soheila A.-H. Mackerness, personal communication). However, the transcription of some UV-responsive genes is unaffected by UV irradiation, and their expression is regulated in translation. The best examples are the CPRF (common plant regulatory factor) transcription factor genes. CPRF4a binds specifically as a homodimer to the ACGT-containing cis-element in the promoter region of the

Table 3. Other stimuli that regulate UV-B-responsive genes

Gene	Encoded protein	UV	pathogen	wound	Reference
<i>4CL-1</i>	4-coumarate:CoA ligase	+	+	+	Douglas et al., 1991
<i>P3-ch</i>	chitinase	+	+	NE	Margis et al., 1993
<i>P4-ch</i>	chitinase	+	+	NE	
<i>P5-chi</i>	chitinase	+	+	+	Melchers et al., 1994
<i>OMTs</i>	O-methyltransferases	+	+	NE	Gregersen et al., 1994
<i>PI-I</i>	proteinase inhibitor I	+	NE	+	Linthorst et al., 1993
<i>PI-II</i>	proteinase inhibitor II	+	+	+	Balandin et al., 1995
<i>H2A</i>	histone	-	-	NE	Logemann et al., 1995
<i>H2B</i>	histone	-	-	NE	Logemann et al., 1995
<i>H3</i>	histone	-	-	NE	Logemann et al., 1995
<i>H4</i>	histone	-	-	NE	Logemann et al., 1995
<i>p34cdc2</i>	protein kinase	-	-	NE	Logemann et al., 1995
<i>cyclin</i>	mitotic cyclin	-	-	NE	Logemann et al., 1995
<i>chs</i>	chalcone synthase	+	NE	+	Wingender et al., 1989
<i>STS</i>	stilbene synthase	+	+	NE	Schubert et al., 1997
<i>PR</i>	pathogen-related protein	+	+	NE	Brederode et al., 1991

+, induced; -, repressed; NE, not examined.

parsley *chs II* gene and forms heterodimers with CPRF1 but not with CPRF2. In protoplasts from suspension culture cells, UV irradiation did not increase the amount of CPRF1, CPRF2 or CPRF4a mRNA, whereas the corresponding CPRF proteins accumulated within 15 min of light treatment. Furthermore, the rapid light-mediated increase in CPRF proteins was insensitive to transcriptional inhibitors, suggesting that a post-transcriptional mechanism controls CPRF accumulation, at least in the short term (Kircher et al., 1998).

1.3 Mechanisms by which UV affects expression of plant genes

1.3.1 DNA damage

UV radiation damages DNA molecules by inducing two major classes of pyrimidine dimers, the pyrimidine [6-4] pyrimidone photoproduct (6-4 photoproduct) and the cyclobutane pyrimidine dimer (CPD). Therefore, it is possible that UV-induced repression of gene expression is due to DNA damage. However, several observations suggest this is not the case. For example, the effects of UV-B are gene-specific (Jordan et al., 1991; Strid, 1993; Kubasek et al., 1992), and white light induces recovery of UV-B-reduced pea *lhcb* mRNA via a photosynthesis-dependent rather than a DNA repair-dependent mechanism (Mackerness et al., 1996).

Additionally, plants have at least two mechanisms, known as light repair and dark repair that remove UV-induced DNA damage rapidly. Many plants produce photolyases that specifically bind to CPD and 6-4 product dimers, splitting them in a UV-A/blue light-dependent manner and reversing damage (Nakajima et al., 1998). Photolyase also stimulates dark repair processes in

Chlamydomonas reinhardtii via pathway(s) exclusive of nucleotide excision repair (NER) that normally functions in darkness (Vlcek et al., 1995). By employing enzymes other than photolyases, NER removes DNA damage including that induced by UV radiation and chemical mutagens. Several NER enzymes have been identified in different plant species (Sturm and Lienhard-S, 1998; Xu et al., 1998; Machado et al., 1996). Of these, RAD23 proteins are putative assembly factors of the NER complex required for transcription-coupled repair as well as efficient overall genome repair (Sturm and Lienhard-S, 1998). Two isoforms of RAD23 from carrot complement the UV-sensitive phenotype of a *rad23* deletion mutant of yeast, indicating that homologous polypeptides may catalyse NER in both plants and yeast (Sturm and Lienhard-S, 1998). Such sequence and function similarities of other identified NER proteins further suggest that the NER mechanism is conserved in yeast, animals and higher plants (Xu et al., 1998). In addition to these proteins, enzymes for the biosynthesis of thiamine are involved in these processes, although the actual mechanisms are not clear (Machado et al., 1996). In further support of the idea that DNA damage is not a cause for UV-induced modulation of gene expression, both light and dark repair are active in correcting UV-C-induced DNA damage in the nuclear-encoded *RbcS* gene. In contrast, only a light-dependent repair process functions for the chloroplast-encoded *psbA* gene and it is much slower than the photolyase-mediated light repair of *RbcS* damage (Cannon et al., 1995). UV-induced damage can, therefore, be removed from *RbcS* rapidly, but remains at high level in *psbA*. However, as mentioned above, in response to UV-B, *RbcS* mRNA decreases in

amount much more quickly than does *psbA* mRNA (Jordan et al., 1991; Mackerness et al., 1996), suggesting that DNA damage has a minor effect on UV-regulated transcription.

1.3.2 Perception and transduction of UV signals

For light to exert its biological effect in plants, the first event is believed to be perception of the photons by wavelength-specific photo-receptors. It has been well established that phytochromes, via conversion between their red- and far-red-absorbing forms, function as red and far-red photoreceptors triggering numerous important biochemical processes in plants. Phytochromes also absorb UV-B (Pratt and Butler, 1970), UV-A and blue light (Batschauer et al., 1996), undergoing photo-conversion between their red- and far-red-absorbing forms (Pratt and Butler, 1970). However, it is evident that UV-A and blue light-regulated biological events in plants are initiated by UV-A/blue light photoreceptors (cryptochrome) other than phytochromes (Batschauer et al., 1996). It is unclear how plants perceive UV-C signal, and the role of phytochromes as UV-B photoreceptors remains controversial (Beggs and Wellmann, 1994; Ballaré et al., 1991, 1995). Investigations using wild-type, UV-A/blue light photoreceptor- and phytochrome-deficient *Arabidopsis* seedlings suggest that either phytochrome A or phytochrome B is required for UV-B-induced hypocotyl growth inhibition and cotyledon expansion (Kim et al., 1998). In contrast, in cucumber and tomato seedlings, UV-B-induced inhibition of hypocotyl growth is mediated by UV-B-specific photoreceptors other than phytochromes (Ballaré et al., 1991; Ballaré et

al., 1995), probably requiring flavin as a chromophore (Ballaré et al., 1995). Furthermore, the UV-B-induced transcription of *chs* in *Arabidopsis* seems to be mediated by a UV-B-specific photoreceptor instead of the UV-A/blue light photoreceptor CRY1 cryptochrome (Fuglevand et al., 1996).

Following photon perception, UV-triggered signals are transduced through intermediates to their final targets. Current information on such intermediates is mainly derived from surveys on the potential involvement of pathways or signaling components known to mediate responses of plants to other stimuli. For example, UV-C induces the expression of several plant defensive genes in tomato leaves that are normally activated by wounding through the octadecanoid pathway. As shown either by an inhibitor or a tomato mutant, UV-C must trigger the octadecanoid pathway to activate the genes (Conconi et al., 1996), demonstrating that UV-C and wounding share the octadecanoid pathway. Similarly, red light and UV-B share the photomorphogenesis repressor proteins, COP1 and DET1, which work downstream of phytochromes, to inhibit hypocotyl growth and induce cotyledon expansion in *Arabidopsis* (Kim et al., 1998). In other cases, however, UV signal transduction pathways differ, at least partially, from those mediating other stimuli. UV-B induces the *chs* gene via a pathway that is different from those either linked to the *Arabidopsis* UV-A/blue light photoreceptor CRY1 (Fuglevand et al., 1996) or phytochromes as proposed in other species (Christie and Jenkins, 1996). Moreover, the signal transduction pathway mediating UV-B-regulated *chs* and *PAL* gene expression in pea differs from the one requiring nicotinamide or trigonelline as signaling compounds (Kalbin et al.,

1997).

UV-B radiation may influence gene expression by generating active oxygen species through stimulation of membrane-localized NADPH-oxidase activity and inhibition of catalase activity (Rao et al., 1996). UV-B-induced increase in active oxygen species is correlated with the induction of pathogen-related proteins (Green and Fluhr, 1995) and repression of *lhcb* genes (Henkowitz et al., 1996), suggesting that plant oxidative systems are components of UV signal transduction pathways. However, this may not be the case, at least for UV-B induction of the pea *chs* gene, since non-oxidative low UV-B doses activate its transcription (Kalbin et al., 1997). In summary, most of the details concerning signal transduction mechanisms that mediate UV responses remain elusive, and more extensive investigation is needed.

1.3.3 Involvement of UV-responsive cis- and trans-acting factors

Interaction between cis- and trans-acting factors may be the ultimate event in UV signal transduction pathways regulating gene expression. In this regard, cis-acting elements have been identified for some UV-inducible defense-related genes, especially *chs*, from several plant species (Table 4), but no such information is available for UV-repressible genes. Although divergent light qualities regulate gene expression via distinct photoreceptors and signal transduction pathways, these pathways may, at least for *chs* genes, merge prior to transcription (Schulze-Lefert, 1989; Merkle et al., 1994; Hartmann et al., 1998). This is supported by the evidence that UV-B and UV-A/blue light share cis-acting

Table 4. Plant genes with UV-responsive cis-elements identified

Gene*	Species	Reference
<i>chs</i>	parsley	Schulze-Lefert, 1989 Weisshaar et al., 1991 Merkle et al., 1994
<i>chs1</i>	soybean	Wingender et al., 1990
<i>chs</i>	<i>Arabidopsis thaliana</i>	Hartmann et al., 1998
<i>chs</i>	<i>Antirrhinum majus</i>	Staiger et al., 1989
<i>PAL</i>	parsley	Takeda et al., 1997
<i>PAL1</i>	carrot	Takeda et al., 1997
<i>PAL1</i>	pea	Yamada et al., 1994
<i>PAL2</i>	pea	Yamada et al., 1994
<i>4CL-1</i>	parsley	Lois et al., 1989 Douglas et al., 1991

*Refer to Tables 1 and 3 for proteins encoded by the genes.

elements and cause the same pattern of protein-DNA interaction on *chs* promoters. UV-induced genes also share common cis-acting elements with pathogenic elicitors, which may be separated from what are essential for tissue-specific transcription of the genes (Douglas et al., 1991; van der Meer et al., 1990). Thus, in addition to its minimal promoter, sequences located in the encoding region are required for both elicitor and UV activation of 4CL-1, a plant defense gene encoding 4-coumarate:CoA ligase, but the minimal promoter is sufficient for tissue-specific expression (Douglas et al., 1991). Besides the cis-acting elements that are common to different stimuli, others may specifically respond to UV. This may be why, in the soybean *chs1* promoter, the same sequences affect pathogen elicitor and UV-light inducibility, but in contrast to the pathogen elicitor induction, UV light loses its inductive capacity when sequences downstream of -75 are replaced by a heterologous minimal promoter (Wingender et al., 1990).

The minimal promoter regions essential for UV-induced transcription are fairly short, and they include basic leucine zipper- (ACGTGGC) (Weisshaar et al., 1991; Hartmann et al., 1998) and MYB transcription factor-binding sites (Hartmann et al., 1998), and sequences like CTCCAACAAACCCCTTC and ATTCTCACCTACCA (Lois et al., 1989). These elements are located near transcription initiation sites (Schulze-Lefert, 1989; Wingender et al., 1990; Mate et al., 1998; Yamada et al., 1994; Takeda et al., 1997), and their orientation is critical for action (Weisshaar et al., 1991). Other short cis-acting elements located either in the upstream vicinity of (Schulze-Lefert, 1989; Weisshaar et al.,

1991; Takeda et al., 1997) or regions distal to the transcription initiation sites (Hartmann et al., 1998; Yamada et al., 1994) enhance transcription. The upstream distal sequences may functionally resemble cis-acting elements in the proximal regions, and this is exemplified by the observation that two transcription factor-binding sites within the minimal promoter of the *Arabidopsis thaliana chs* gene are partially complemented by upstream elements (Hartmann et al., 1998). Additionally, as mentioned above, sequences located in the encoding regions may be required for both pathogen elicitor and UV activation (Douglas et al., 1991).

The overall UV response of a full-length promoter usually results from cooperation between dispersed regulatory components (Takeda et al., 1997), and sometimes this type of cooperation is essential for UV-induced transcription (Schulze-Lefert, 1989; Weisshaar et al., 1991). One example involves the DNA-binding sites, Box I and Box II, within the minimal promoter region of parsley *chs* gene. These elements act as one cis-acting unit and both are necessary for the minimal, UV-responsive *chs* gene promoter. Replacement of 10 bp within either of these sequences leads to a complete loss of UV responsiveness (Schulze-Lefert, 1989; Weisshaar et al., 1991).

The transcription machinery mediating UV responses is conserved across plant species, as suggested by the fact that the soybean *chs1* promoter directs UV radiation inducibility in parsley protoplasts (Wingender et al., 1990). Additionally, two distinct CG-1 proteins have been identified as transcription factors bound to UV-responsive CG-containing cis-elements. The CG-1 found in

several dicotyledonous plant species, including *Nicotiana tabacum*, *Antirrhinum majus*, *Petunia hybrida*, *Arabidopsis thaliana*, and *Glycine max*, specifically recognizes a hexameric motif with internal dyad symmetry, CACGTG. This motif is required for UV light-induced expression of the *chs* gene of *A. majus*. Binding of CG-1 is influenced by C-methylation of the CpG dinucleotide in the recognition sequence (Staiger et al., 1989). The parsley CG-1, in contrast, binds to a UV-responsive CGCG element. The mRNA encoding this CG-1 accumulated rapidly and transiently in cultured parsley cells after treatment with UV-containing white light (da Costa, 1994).

1.4 Light-harvesting complex II protein (Lhcb) genes

1.4.1 *lhcb* multigene family in pea

Among UV-B-responsive genes, those encoding Lhcb proteins are of great interest because their products are critical components of plant photosystem II and transcription of these genes is severely repressed upon UV-B irradiation (Jordan, 1996; Soheila A.-H. Mackerness, personal communication). The Lhcb proteins capture light and the trapped energy is transferred to the reaction center of photosystem II. Lhcb polypeptides are the most abundant members of light-harvesting chlorophyll a/b proteins which include 4 types of the Lhca protein, 6 types of Lhcb proteins and early light-induced proteins (Green et al., 1991; Jansson, 1994; White and Green, 1987). In all plant species studied to date, Lhcb proteins are encoded by multigene families located in the nucleus. There are seven known *lhcb* genes in pea (White et al., 1992). Of them, five are classified as type I (*AB96*, *AB80*, *AB66*, *Cab-8*, *Cab-9* = *lhcb1*1*, *lhcb1*2*, *lhcb1*3*, *lhcb1*4*, *lhcb1*5* in

the nomenclature of Jansson et al., 1992, respectively); one as type II (*Cab-215* = *lhcb2*1*) and one as type III (*Cab-315* = *lhcb3*1*).

1.4.2 Expression of *lhcb* genes

The expression of *lhcb* genes is stimulated by light in a process mediated by phytochromes and a UV-A/blue light receptor (Marrs and Kaufman, 1991; Karlin-Neumann et al., 1988; Gao and Kaufman, 1994; reviewed in Briggs and Liscum, 1997). The responses of individual members of the *lhcb* gene family to light vary and they are dependent on the developmental stage of the plant (Karlin-Neumann et al., 1988; White et al., 1992; 1995). For example, *Cab 1* but neither *Cab 2* nor *Cab 3* in etiolated *Arabidopsis* plants is stimulated by either a single red (Karlin-Neumann et al., 1988) or blue (Gao and Kaufman, 1994) light pulse, and the *lhcb* genes expressed during de-etiolation are different from those expressed in 5-week-old mature green leaves (Karlin-Neumann et al., 1988). In pea, the seven *lhcb* genes are divided into two groups according to their response to light pulses, with *lhcb1*1*, *lhcb1*4*, *lhcb2*1*, and *lhcb3*1* more sensitive to red and blue light than *lhcb1*2*, *lhcb1*3* and *lhcb1*5* (White et al., 1992; 1995).

1.4.3 Signal transduction pathways mediating *lhcb* gene expression

Phytochromes are currently the best characterized photoreceptors in plants, and they are the first components in signal transduction pathways for regulation of certain genes by red and far-red light (Kaufman et al., 1984; Karlin-Neumann et al., 1988; Bowler and Chua, 1994; Deng, 1994). Two phytochrome-dependent signal

transduction pathways have been proposed in tomato and soybean cells, with one requiring calcium/calmodulin and the other cGMP. *lhcb* genes are activated by the calcium/calmodulin-dependent pathway and repressed by the one dependent on cGMP (Bowler and Chua, 1994). *lhcb* genes are also regulated by blue light via a UV-A/blue light photoreceptor (Warpeha and Kaufman, 1990). The first gene encoding a UV-A/blue light photoreceptor, namely CRY1, has been characterized in detail (Fuglevand et al., 1996; Christie and Jenkins, 1996). However, it is not known if *lhcb* genes are regulated by UV-A/blue light through CRY1 and the related signaling process.

Chloroplasts possess components of the signal transduction pathways that regulate the expression of *lhcb* genes and other nuclear genes encoding chloroplast proteins, but not those for the expression of most nuclear genes encoding cytosolic proteins (Blackbourn, 1997; Bolle et al., 1994; Taylor, 1989). If chloroplasts are photo-damaged or transcription in the chloroplasts is inhibited, transcription of *lhcb* genes stops (Bolle et al., 1994; Taylor, 1989; Hess et al., 1994). G-proteins, protein kinase C (Blackbourn, 1997), plastid RNA and intermediates of the chlorophyll biosynthesis pathway (Hess et al., 1994) may be intermediates of the chloroplast-dependent signal transduction pathway. Additionally, chloroplast factor-responsive cis-acting elements have been identified in the 5'-flanking regions of several genes in spinach (Bolle et al., 1994) and *Arabidopsis* (Vorst et al., 1993), and three loci coupling the transcription of nuclear genes encoding chloroplast proteins to intact chloroplasts occur in the nuclear genome of *Arabidopsis* (Susek et al., 1993).

1.5 Role of chloroplasts in the regulation of *lhcb* genes by UV-B

UV-B damages chloroplast components and functions, including thylakoid membranes, photosystems, chloroplast pigments, electron transport, photophosphorylation, and photosynthetic enzymes (Chow et al., 1992; Melis et al., 1992; Jordan, 1996; Teramura, 1983). Among them, rupture of chloroplast thylakoid membranes is the earliest detectable effect of UV-B on plants (Jordan et al., 1994). Transcription of *lhcb* genes is extremely susceptible to both chloroplast photodamage (Sagar et al., 1988; Taylor, 1989; Bolle et al., 1994) and UV-B irradiation (Jordan, 1996; Soheila A.-H. Mackerness, personal communication). Therefore, it is possible that UV-B represses nuclear genes encoding chloroplast proteins by damaging the chloroplast factor(s) essential for their expression.

To test this hypothesis, chloroplasts were modified by inhibiting carotenoid biosynthesis and then photobleaching the carotenoid-free seedlings with white light. Pea, as one of the plant species most sensitive to UV-B (Jordan et al., 1994), was used to examine the relationship between the chloroplast modifications and UV-B effects on *lhcb* mRNA accumulation under dim red light. Contrary to our expectations, the experiments indicated that UV-B inhibition of *lhcb* mRNA accumulation under dim red light is not dependent on damage to the putative red light-responsive chloroplast components. Instead, intact chloroplasts are required for UV-B-induced repression of *lhcb* genes. Moreover, individual *lhcb* genes respond differentially to UV-B irradiation and chloroplast alterations. UV-B- and red light-induced signal transduction pathways are different from one another at least in terms of their dependence on chloroplast components. In addition to these

findings, cDNA fragments of six UV-B-responsive genes were cloned. UV-B increased the amount of mRNA from three of the genes and reduced the mRNA from the other three, suggesting explanations at the molecular level for stress-related phenomena observed by other researchers.

II. Materials and Methods

2.1 Plant materials and growth conditions

Three pea (*Pisum sativum* L.) cultivars, Feltham First, Extra Early Alaska and Alaska were used in this study. When detecting UV-B responses of individual *lhcb* genes, Feltham First seedlings were grown in a Weiss controlled environment cabinet, with 12 h light (22 °C), 12 h dark (16 °C) cycles, at 70% humidity for 17 days prior to UV-B treatment. Incident radiation was provided by Philips warm white fluorescent tubes giving an irradiance of 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active radiation (PAR). For experiments using etiolated buds, pea seedlings were grown in the dark (22 °C) for 6 days prior to UV-B treatment.

In other experiments, Alaska and Extra Early Alaska seedlings were grown in a Weiss growth chamber, at 22 °C under 12 h light (110 $\mu\text{mol m}^{-2}\text{sec}^{-1}$), 12 h dark cycles or continuous dim red light (0.2 $\mu\text{mol m}^{-2}\text{sec}^{-1}$) for 6-9 days before treatment with UV-B. Dim red light was obtained by filtering white light from two 40-W incandescent bulbs (Litemor, Canada) through 2 sheets of laser jet paper and a sheet of red acrylic filter (Plexiglas MC, AtoHaas, PA, USA). The two incandescent bulbs were installed 52 cm apart and 30 cm above the red filter, with the red filter 35 cm above the top of the seedlings. The space above the red filter was wrapped with cardboard and black plastic to block light-leakage. Aluminium foil was applied to the chamber walls to ensure even irradiation.

The planting procedures were previously described (Liu et. al, 1998). Briefly, 20 seeds were surface sterilized with 10% commercial Javex for 10 min, rinsed with distilled water 3 times and then sown in a tray (13 x 11 x 6 cm) containing

vermiculite or Pre-mix potting soil. The vermiculite and potting soil in each tray were wetted with 100 and 200 ml of distilled water, respectively, prior to planting. The plants were irrigated with 100 ml distilled water immediately after sowing and once every 48 h.

2.2 Generation of carotenoid-free seedlings

Carotenoid-free seedlings were generated basically as described by Sagar et al. (1988). Norflurazon (Zorial Rapid 80, Sandoz Agro. Inc., Greenville, MS, USA) was dissolved in distilled water according to the manufacturer's instructions. After surface sterilization with Javex and soaking in 100 μM of norflurazon solution for 3 h in darkness, 20 seeds of Extra Early Alaska or Alaska were planted in trays (13 x 11 x 6 cm) that contained vermiculite pre-wetted with 100 ml of the norflurazon solution. Each tray was irrigated with 100 ml of 100 μM norflurazon immediately after sowing and once every 48 h. The containers were maintained in the growth chamber for continuous exposure to dim red light. All steps were conducted with the laboratory lights off to prevent photodamage to embryos.

2.3 Partial photobleaching of carotenoid-free seedlings

Six- or seven-day-old carotenoid-free seedlings were irradiated for 3 h with white light ($110 \mu\text{mol m}^{-2}\text{s}^{-1}$) before or after UV-B treatment to partially photobleach the plants. The white light was generated by two 100-W incandescent bulbs (Phillips, Canada) and two 40-W warm white fluorescent tubes (Sylvania, Canada). The vertical distance between the plants and the incandescent bulbs was 20 cm. The white light intensities were measured with a Li-Cor LI-170 quantum/radiometer/photometer (Li-Cor, Lincoln, Nebr., USA), and the recorded

intensities were the averages of six or more measurements taken at random locations at the height of plant terminal buds.

2.4 UV-B Treatment

In the experiments detecting UV-B responses of individual *lhcb* genes, after growing for 17 days, plants were exposed to UV-B radiation for the entire light period of the 12 h light/12 h dark cycle (Mackerness et al., 1998). UV-B irradiation was generated from two UV-B lamps (Philips TL 12, 40 W) wrapped with one sheet of cellulose acetate to remove wavelengths shorter than 290 nm. The control plants were treated in the same way as the UV-B-irradiated plants except that the UV-B lamps were off.

In other experiments, UV-B treatments of 3 h were carried out on the 6th or 7th day after sowing. The UV-B radiation was obtained from one FS20T12/UVB tube (Light Sources, Milford, CT, USA) wrapped with one sheet of cellulose acetate (0.05 mm). The UV-B lamp was placed between two fluorescent tubes when treating plants grown under light/dark cycles. Alternatively, it was located 21 cm below the red filter and aligned parallel with the light fixture when exposing the continuous dim red light-grown plants to UV-B. The control plants were treated the same way except that UV-B lamp was off. The plants were placed 15-20 cm below the UV-B tube and UV-B intensity was adjusted by varying the distance between the plants and the lamp. UV-B and UV-A intensities were measured using an Erythema UV & UVA Intensity Meter (Model 3 D V2.0, Solar Light CO., Philadelphia, USA). The UV-B fluence used was ~2.9 minimal erythemal dose (MED) h⁻¹, which was ~85% of the outdoors solar UV-B fluence measured in

Halifax at noon of a clear summer day in 1996. The background UV-A in the growth chambers was always less than 0.01 mW cm^{-2} . Control plants were kept under the same conditions except that the UV-B lamp was not turned on.

2.5 Plant sample collection

When using Feltham First to detect UV-B responses of individual *lhcb* genes, either apical buds from etiolated plants or the third leaflets from the base of green seedlings were collected at the time points indicated in the text. In all other experiments, either the apical buds or the first two leaflets of the top leaf were harvested from 8-40 plants/treatment, 5 or 21 h after UV-B exposure. Samples were placed in a 50-ml Falcon tube on ice, frozen in ethanol at $-90 \text{ }^{\circ}\text{C}$ and stored at the same temperature until RNA extraction. All experiments were independently duplicated or triplicated as indicated in the text, and the samples were taken at random from different parts of the cabinet and from separate plants.

2.6 Quantification of chlorophylls, carotenoids and UV-B-absorbing pigments

To quantitate photosynthetic and UV-B-absorbing pigments, either terminal buds or the first two leaflets from the first leaf, harvested from 10 plants for each treatment, were placed in a pre-weighed 1.5 ml microcentrifuge tube on ice. After the samples were weighed, plant materials were ground in $400 \text{ }\mu\text{l}$ of 95% ice-cold ethanol for two min with a mortar and pestle. The homogenate was transferred to a microcentrifuge tube, and the mortar and pestle were rinsed with $200 \text{ }\mu\text{l}$ of ethanol three times, with each rinse transferred to the microcentrifuge tube. After storage at $-20 \text{ }^{\circ}\text{C}$ overnight, the homogenates were centrifuged in a microcentrifuge (3200

Eppendorf, Germany) at 4 °C for 10 min in darkness. The supernatant was transferred to a fresh 1.5-ml tube and its volume was adjusted to 1 ml with 95% ethanol. The tubes were wrapped with aluminium foil and stored on ice until optical densities were determined with a Hewlett Packard 8452A Diode Array spectrophotometer (Hewlett-Packard Canada Ltd., Orangeville, Ontario, Canada). Duplicate measurements were done for each sample, and 4 replicate samples were analysed for each treatment. All of these procedures were carried out with all lab lights off and the curtains closed. The concentrations of photosynthetic pigments were calculated by use of the Lichtenthaler formula (1987):

$$\text{Chl}_a = 13.36A_{664.2} - 5.19A_{648.6} ,$$

$$\text{Chl}_b = 27.43A_{648.6} - 8.12A_{664.2} ,$$

$$C_{x+c} = (1000A_{470} - 2.13C_a - 97.64C_b) / 209 ,$$

where Chl_a , Chl_b and C_{x+c} are the concentrations, in $\mu\text{g ml}^{-1}$ plant extract solution, of chlorophyll a, chlorophyll b and carotenoids, respectively; A is absorbance of the extract measured at the wavelength (nm) as indicated in subscript. Absorbance at 295 nm of 1 ml ethanol extract containing 1 g of fresh leaf or bud samples was taken as the concentration of UV-B-absorbing pigments. Multiple comparisons of the pigment levels between treatments were conducted using the Tukey test (Zar, 1996).

2.7 Isolation of total RNA

Total RNA was purified as described previously (Horwitz et al., 1988; Elliott et al., 1989) with the following modifications. Briefly, about 1 g of sample was homogenised in 8 ml of 1:1 ice-cold RNA extraction buffer:phenol reagent

(Appendix I) for 1.5 min on ice. The homogenates were centrifuged at 4 °C for 10 min at 7,000 rpm in an HS3 clinical centrifuge. After adding 12 M LiCl to a final concentration of 2.7 M in 3 ml of the aqueous fraction, RNA was precipitated on ice overnight. Following centrifugation for 10 min at 7,000 rpm in the HS3 clinical centrifuge at 4 °C, the supernatant was discarded and pellet was dissolved in 1 ml of resuspension buffer. The RNA was re-precipitated on ice overnight by adding 12 M LiCl to a concentration of 3.04 M, and spun for 5 min in a microcentrifuge at 4 °C. After two washes in 2.5-3 volumes of 0.2 M sodium acetate in 76% ethanol and air-drying, the pellet was dissolved in 50 µM ATA (Appendix I). The quantity and quality of RNA were monitored by measuring the absorbance at 230, 260, 280 and 340 nm and it was then stored at -70 °C.

2.8 Removal of Genomic DNA from total RNA preparations

Genomic DNA was removed from the RNA preparations as described by Liang and Pardee (1994). A 100 µl reaction mixture containing 40 mM Tris-Cl (pH 7.6), 6 mM MgCl₂, 0.2 unit µl⁻¹ human placental RNase inhibitor (Pharmacia), 0.2 unit µl⁻¹ RNase-free DNase I (Pharmacia), and 200 µg of RNA was incubated at 37 °C for 30 min. The mixture was extracted with 1 volume of phenol/chloroform (3:1). The RNA was precipitated overnight with 0.1 volume of 3 M sodium acetate and 2.5 volumes of absolute ethanol at -70 °C. The pellet was washed with 70% ethanol, air dried and dissolved in 50 µM ATA.

2.9 Isolation of mRNA

mRNA was purified using an mRNA Purification Kit (Pharmacia Biotech, Canada) following the manufacturer's instructions. Briefly, about 1.25 mg of RNA,

isolated as just described, was heated at 65 °C for 5 min to remove secondary structure, then applied to a pre-treated oligo(dT)-cellulose spun column. The column was centrifuged at 350 x g, washed with high- and low-salt buffer, and the mRNA was eluted. This procedure was repeated with another oligo(dT)-cellulose spun column after which the mRNA was concentrated by ethanol precipitation in the presence of glycogen and stored in 50 µM ATA at -80 °C.

2.10 Synthesis of full-length first strand cDNA

2.10.1 cDNA used for reverse transcription-polymerase chain reaction (RT-PCR)

Full-length cDNA was synthesized using oligo dT₁₂₋₁₈ (Sigma, St Louis, MO, USA) and MMLV H⁻ reverse transcriptase (GIBCO BRL, Burlington, Ontario, Canada) following the manufacturer's instructions. Reverse transcription was carried out at 42 °C for 1 h in a 20 µl reaction mixture containing 500 ng of oligo dT₁₂₋₁₈, 1 µg of total RNA, 1x reverse transcription buffer (Gibco BRL), 10 mM DTT, 0.5 mM dNTP mixture and 10 units of RNasin (Promega). After 20 µl stop buffer (100 mM EDTA, pH 8.0) was added, first strand cDNA was separated from mRNA templates by heating the reaction at 95 °C for 5 min and precipitation with 2 volumes of ethanol and 0.83 M (final concentration) ammonium acetate. The pellet was dissolved in 200 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

2.10.2 cDNA used for rapid amplification of 3' cDNA ends (3' RACE) PCR

Full-length cDNA used for 3' RACE PCR (polymerase chain reaction) was synthesized essentially as just described with the following modifications. An oligo dT₁₇-adaptor primer (5'-GACTCGTGACAACATAGCGTTTTTTTTTTTTTTTTTT-3')

was substituted for the oligodT₁₂₋₁₈ primer. In the 20 µl reaction mixture, 1000 ng of oligo dT₁₇-adaptor primer, and either 1 µg of total RNA or 0.1 µg of mRNA were included with the other components as described above. The synthesized cDNA pellet was dissolved in 50 µl of TE to give a concentration of 2 ng/µl and stored at –80 °C.

2.11 PCR amplification and labelling of cDNA for individual *lhcb* genes

To quantitate the mRNA for individual members of the *lhcb* multigene family, the cDNA for each of the seven genes was individually amplified using PCR (White et al., 1992) with the following modifications. The cDNA synthesised from 5 ng of total RNA was added to each 50-µl PCR reaction mixture. The amount of internal standard DNA (White et al., 1992) added to each reaction mixture was 0.25 pg for *lhcb1*4*, 0.025 pg for *lhcb1*2*, 0.01 pg for *lhcb1*3*, 0.08 pg for *lhcb1*1*, 0.1 pg for *lhcb3*1* and *lhcb2*1*, and 0.005 pg for *lhcb1*5*. The mixtures for PCR contained, as final concentrations, 200 nM of primers, 100 µM each of dTTP, dCTP, dGTP, 87.5 µM dATP (Promega, USA) and 12.5 µM biotin-14-dATP (GIBCO BRL). Amplification was done with cycles of 1 min at 94 °C, 1.5 min at 42 °C and 2 min at 72 °C using a PHC-3 Dri-block thermal cycler with a heating lid attached (Techne, New Jersey, USA). Sixteen cycles were used for *lhcb1*4* and *lhcb1*1*, 18 for *lhcb2*1* and *lhcb3*1*, and 20 for *lhcb1*2*, *lhcb1*3* and *lhcb1*5*.

2.12 Southern blotting of RT-PCR products

The amplified internal standard and the cDNA were separated by electrophoresis in 1% (w/v) agarose gels in 1 x TAE (Appendix I). The gels were denatured in 0.2 M NaOH, 0.6 M NaCl for 15 min, neutralized in 25 mM sodium

phosphate buffer (pH 6.5) for 45 min, then pressure-blotted onto positively charged nylon membranes (Tropilon-Plus™, Tropix, Bedford, USA) using a PosiBlot 30-30 Pressure Blotter and Pressure Control Station (Stratagene, La Jolla, CA, USA) at 75 mm Hg for 1 h following the manufacturer's instructions. The membranes were exposed to 120 mJ cm⁻² of UV radiation ($\lambda_{\text{max}} = 254$ nm) in a FB-UVXL-1000 UV Crosslinker (FisherBiotech, Ottawa, ON, Canada).

2.13 Chemiluminescent detection of biotinylated RT-PCR products

The biotinylated PCR products blotted onto the membranes were detected with the Chemiluminescent Detection System for Biotin-labelled Probes with CDP-Star™ substrate (Tropix, Bedford, USA) following the manufacturer's instructions, or with CDP™ substrate with the following modifications. The membranes were immersed, for 1 h in a 1:30,000 dilution of streptavidin-alkaline phosphatase in 0.5% (w/v) I-block buffer. Following three washes (5 min each) in 0.2% (w/v) I-block buffer, three washes (5 min each) in 1 x phosphate-buffered saline containing 0.5% (w/v) SDS, and two washes (5 min each) in assay buffer (0.96% diethanolamine, pH 10, 0.02% (w/v) MgCl₂), membranes were incubated for 5 min in 125 μM CDP™ substrate (Tropix, Bedford, USA) which was diluted with the assay buffer. Moist membranes were sealed in an airtight bag and kept at room temperature for 16-18 h. The membranes were exposed to Kodak BioMax MR film for 15-20 min, which was processed in Kodak GBX developer and fixer. The blots shown in this thesis are representatives of two or more experimental replicates. The quantitative data were obtained by scanning the films with a GS-670 densitometer and analysis with Molecular Analyst Software (Bio-Rad, CA, USA).

2.14 3' RACE PCR

The oligonucleotide, bzip (5'-AAYMGIGARTSNGCNAG-3') (Y=C+T; M=A+C; I=Inosine; R=A+G; S=C+G; N=A+C+G+T), based on the peptide NRESAR (Chem et al., 1996), and a second oligonucleotide, zinc (CAAGCWYTNGGWGGNCA) (W=A+T), designed on the basis of the peptide QALGGH conserved in C₂H₂ zinc finger proteins (Meissner and Michael, 1997), were used as sense primers. The antisense primer was the oligonucleotide named adapter (GACTCGTGACAACATAGCG). The 3' RACE PCR was performed in a 50- μ l reaction containing 1x PCR buffer, 200 μ M dNTP mix, 1x Q-solution (Qiagen), 0.2 μ M adapter primer, 1 μ M bzip or zinc primer, 1.25 units hot-start Taq DNA polymerase (Qiagen) and 8 ng of first-strand cDNA. The mixture was covered with 46 μ l of mineral oil. The reaction was performed in a PTC-100 thermal controller (MJ Research, Inc., Watertown, Mass, USA). The conditions were one cycle at 95 °C, 15 min; 35 cycles at 95 °C, 50 seconds; 40 °C (for bzip) or 45 °C (for zinc), 2 min; 72 °C, 2 min; and one cycle at 72 °C, 10 min.

2.15 Cloning of UV-B-responsive PCR products

PCR reaction mixtures were electrophoresed in 1% agarose gels in 1x TAE (Appendix I) and selected PCR fragments were purified using the GFXTM PCR DNA and Gel Band Purification Kit from Pharmacia Biotech. The purified fragments were cloned into pUC18 using the SureCloningTM ligation kit (Pharmacia Biotech) following the manufacturer's instructions. In brief, the PCR fragments were blunt-ended by removing the 3' d(A) overhangs, phosphorylated at the 3' end, and ligated into the *Sma* I site of blunt-ended, dephosphorylated pUC18 vector using T4 DNA

ligase. DH5 α *Escherichia coli* cells were transformed with the recombinant DNA by standard protocols (Sambrook et al., 1989). White colonies from the X-gal/IPTG plates were propagated for plasmid mini-preparation by incubation in 2 ml of LB/ampicillin broth (Appendix I) at 37 °C overnight. The cells were collected by centrifugation (Eppendorf 5414, Brinkmann, Westbury, NY, USA) for 30 s. The cell pellet was resuspended in 100 μ l of ZE lysis buffer (Appendix I) and incubated at room temperature for 3 min. After incubation in boiling water for 1 min, the mixture was cooled on ice for 3 min, and cell wall debris was pelleted by centrifugation at 4 °C for 15 min in the Eppendorf 5414 centrifuge. The purified plasmid DNA in the supernatants was stored at -20 °C.

2.16 Probe preparations

2.16.1 Preparation of double-stranded probes by PCR:

The purified plasmids were used in probe production and labelling with PCR, which was done essentially as described for 3' RACE PCR with the following modifications. In each 12.5- μ l reaction, 100 μ M each of dTTP, dCTP, dGTP, 87.5 μ M dATP (Promega, USA) and 12.5 μ M biotin-14-dATP were included. Deionized formamide (2.5% v/v) and 0.3 units of recombinant *Taq* DNA polymerase (Pharmacia) were substituted for the 1 x Q-solution (Qiagen) and hot-start *Taq* DNA polymerase (Qiagen), respectively, while 0.01 μ l of purified plasmid DNA was used as template. The mixture was covered with 20 μ l of mineral oil.

2.16.2 Asymmetrical PCR for single-stranded probes:

In order to determine which of the two strands in a clone hybridized to a UV-B-responsive transcript on northern blots, asymmetrical PCR was performed to

obtain individual single-stranded probes from each strand of the plasmid. Each 25- μ l PCR reaction contained 1x PCR buffer, 75 μ M each of dTTP, dCTP, dGTP, 65.6 μ M dATP and 9.4 μ M biotin-14-dATP, 2.5% (v/v) deionized formamide, 0.6 units of recombinant *Taq* DNA polymerase and 5 ng of plasmid DNA. In one of the two reactions for each plasmid, 0.2 μ M adapter primer and 0.1 μ M of either bzip or zinc primer were included, while in the other reaction 0.02 μ M adapter primer and 1 μ M of either bzip or zinc primer were included. The mixture was covered with 30 μ l of mineral oil, and the reaction was performed in the PTC-100 thermal controller with the cycling conditions as described for 3' RACE PCR.

2.16.3 Nick translation for double-stranded probes:

When conducting northern hybridization with *psb A*, probes were prepared with the Nick Translation System (GIBCO BRL) following the manufacturer's instruction. The *psb A* sequence is an 850 bp *Hind* III fragment at the 3'-end containing 60% of the spinach gene (Jordan et al., 1991). After enzymatic release from plasmids, fragments were purified by electrophoresis in 1% agarose gel and extraction with the GFX™ PCR DNA and Gel Band Purification Kit. Nick translation was at 15 °C for 1 h in a 50- μ l mixture containing 5 μ l 0.2 mM dNTP Mix (minus dATP), 2.5 μ l 0.4 mM biotin-14-dATP, 37.5-112.5 ng of the purified DNA fragment and 5 μ l Pol I/DNase I Mix. The labeled probes were precipitated with 0.5 volumes of 7.5 M ammonium acetate and 2 volumes of ethanol at -70 °C for 30 min. After washing in 70% ethanol, the pellet was air-dried, dissolved in TE buffer (pH 8.0, Appendix I) and stored at -80°.

2.17 Identification of cDNA clones from UV-B-responsive genes

Five μl (20 or 50 μg) of total RNA extracted from leaves of control and UV-B-treated plants was heated at 65 °C for 10 min in 5 μl of denaturing buffer (Appendix I). The denatured RNA samples were resolved in 1% formaldehyde-denaturing agarose gels with 2x MOPS running buffer (Appendix I) at 64 V for 2 h. A 0.24-9.5 kb RNA ladder (GIBCO BRL) was treated the same way and run on the gel in parallel with the samples. The RNA samples were transferred overnight by capillary action to HybondTM-N⁺ positively charged nylon membranes (Amersham, Amersham, UK) with 20x SSC, following the manufacturer's instructions. After baking at 80 °C for 2 h, the lane containing the RNA ladder was excised and stained with methylene blue as described by Sambrook et al. (1989). The remainder of each blot was stored between two pieces of filter paper wrapped in aluminium foil until hybridization.

Biotin-labelled probes, prepared by PCR as described in section 2.15.1, were employed in northern hybridization to detect UV-B-responsive transcripts. Probes obtained from asymmetrical PCR were used without additional treatment, while probes prepared by other methods were denatured at 95 °C for 5 min prior to hybridization. The hybridization procedures described in the manual, Chemiluminescent Detection System for Biotin-labelled Probes (Tropix), were followed. Briefly, the blots were wetted in 0.25 M disodium phosphate buffer (pH 7.2), then prehybridized with hybridization buffer (Appendix I) for 60 min at 65 °C. The blots were incubated in fresh hybridization buffer (50 $\mu\text{l cm}^{-2}$) containing the probes (50 ng ml^{-1}) at 65 °C overnight. The blots were washed twice for 5 min at room temperature with 1x SSC containing 0.1% SDS (w/v), once for 15 min at 65

°C with 0.1x SSC containing 0.1% SDS and twice for 5 min at room temperature with 1x SSC. The mRNA on the blots was detected with the chemiluminescent detection procedures described in section 2.12.

2.18 Sequence analysis of cDNA clones

The 3' RACE PCR-isolated clones that hybridized to UV-B-responsive transcripts were sequenced at NRC (Halifax, Canada) by either the Li-Cor DNA Sequencer Long Readir 4200 (Li-Cor, Nebraska, USA), or the Applied Biosystems 373 DNA sequencer Stretch (CA, USA). The nucleotide and deduced amino acid sequences from all six hypothetical open reading frames of each clone were compared with genes registered in the databases at the National Center of Biological Information (NCBI), by use of the Basic Local Alignment Search Tool (BLASTN and BLASTX, Altschul et al., 1990). The similar gene sequences were retrieved from NCBI databases using the Web browser based Entrez program. Multiple sequence alignments were with ClustalW (Thompson et al., 1994), and the output files of ClustalW were edited in Microsoft™ word. Analyses of DNA sequences, including sequence editing and restriction enzyme mapping, were done with Gene Runner (Hastings Software, Inc.).

III. Results

3.1 Individual *lhcb* mRNAs were differentially modulated by UV-B radiation

3.1.1 UV-B-induced increase in mRNA of etiolated buds divided *lhcb* genes into two groups

Transcript abundance for each of the seven pea *lhcb* genes was separately determined with gene-specific primers and RT-PCR. The genes fell into two groups according to the amount of transcript in etiolated buds of 6-day-old Feltham First pea seedlings. Specifically, *lhcb1*4*, *lhcb2*1*, *lhcb1*1* and *lhcb3*1* produced small amounts of transcripts (Fig. 1), while transcripts of *lhcb1*5*, *lhcb1*3* and *lhcb1*2* were undetectable. These two groups of genes also differed from one another in response to UV-B. Although the transcripts encoded by all of the *lhcb* genes increased during UV-B exposure, transcript accumulation of *lhcb1*5*, *lhcb1*3* and *lhcb1*2* lagged behind that of *lhcb1*4*, *lhcb2*1*, *lhcb1*1* and *lhcb3*1* (Fig. 1).

3.1.2 UV-B-induced reduction of mRNA accumulation in green leaves divided *lhcb* genes into the same two groups

Green leaves (Fig. 2) contained more transcripts of all the *lhcb* genes than etiolated buds (Fig. 1). Exposure to UV-B reduced the amount of transcript for all of these genes in green leaves of 17-d-old Feltham First pea seedlings (Fig. 2). The UV-B-induced transcript reduction varied with genes, and the extent of repression divided them into the same two groups as just described for etiolated buds. Transcripts encoded by *lhcb1*5*, *lhcb1*3* and *lhcb1*2* were reduced by UV-B to a larger extent upon exposure to UV-B, than were those for *lhcb1*4*, *lhcb2*1*, *lhcb1*1* and *lhcb3*1* (Fig. 2).

Figure 1. Exposure to UV-B differentially increased the accumulation of transcripts encoded by pea *lhcb* genes in etiolated buds

The RNA samples used in this experiment were provided by Dr. S. A.-H.-Mackerness of the Horticultural Research International in England. Feltham first pea seedlings were grown in the dark (22°C) for 6 days and then exposed to continuous UV-B during light periods of 12 h light/12 h dark cycles. Apical buds were collected from the seedlings at 0, 8 and 54 h after initiation of the UV-B treatment and total RNA was extracted. The cDNA synthesised from 5 ng of total RNA was amplified by PCR for each sample, and the transcripts for each gene were individually detected as described in Materials and Methods. UV (h), duration of UV-B treatment (hours); art, artefact; st, PCR-amplified internal standard used to monitor PCR amplification efficiency; cDNA, gene-specific PCR amplified cDNA; C, PCR control lacking reverse transcriptase but containing total RNA and internal PCR standard. The PCR standard for *lhcb2*1* was larger than the cDNA, while the standards for all the other genes contained an internal deletion and were smaller than the cDNAs. The name of each *lhcb* gene is shown above each gel. M, biotinylated λ /Hind III size markers (23.1, 9.4, 6.6, 4.4, 2.3, 2.0 and 0.56 kb).

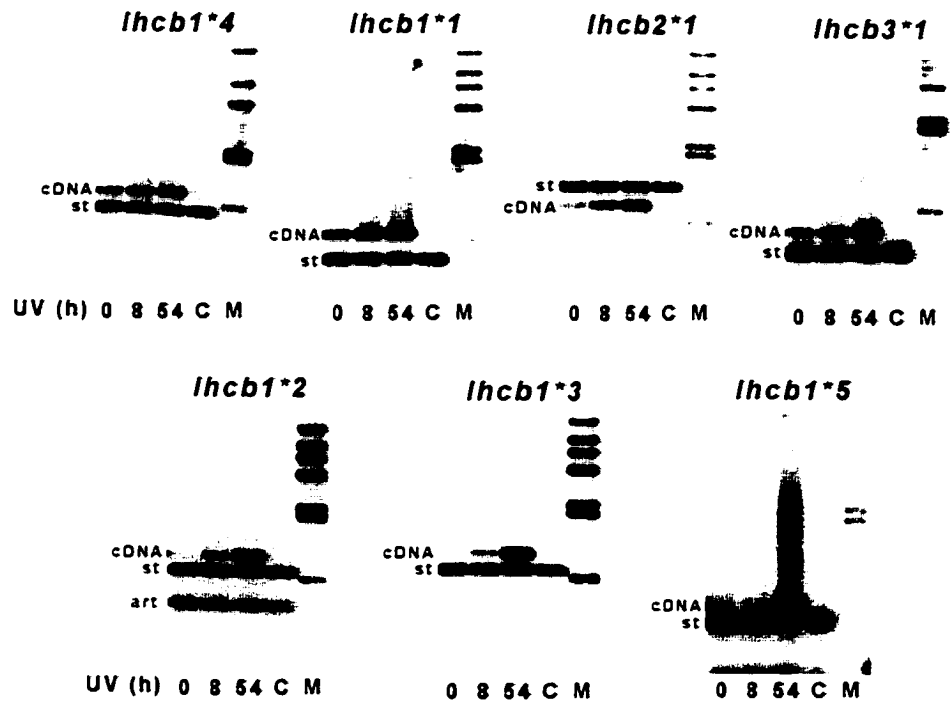


Figure 1

Figure 2. Exposure to UV-B decreased the amount of transcript encoded by pea *lhcb* genes in green leaves to different extents

The RNA samples were provided by Dr. S. A.-H.-Mackerness at the Horticultural Research International in England. Feltham first pea seedlings were grown in a Weiss controlled environment cabinet, with 12 h light (22°C), 12 h dark (16°C) cycles, at 70% humidity for 17 days, then exposed to continuous UV-B during the light portion of the light/dark cycle. The leaves were collected either 0 or 54 h after initiation of the UV-B treatment and total RNA was extracted. Transcripts encoded by each gene were individually detected as described in Materials and Methods. The symbols are the same as those in the legend to Figure 1.

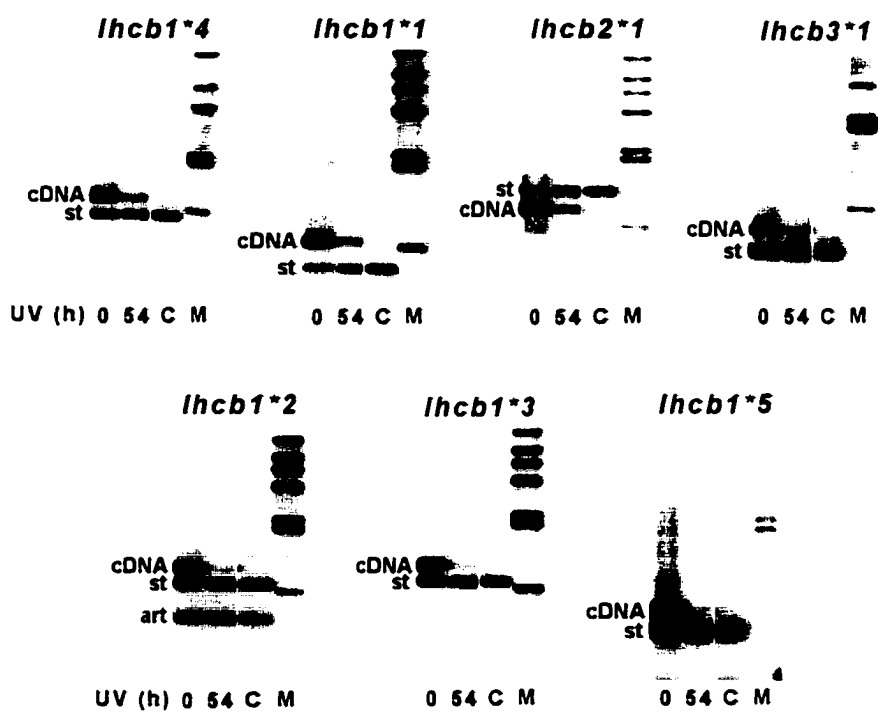


Figure 2

To ensure that these variations were not due to a potential difference between the genes with respect to sensitivity to the assay system, detection thresholds for *lhcb1*4* and *lhcb1*2*, one from each group, were determined. A 2-fold serial dilution of the cDNA from green leaves sampled prior to UV-B treatment was made. In parallel with 5 ng of cDNA from 54 h UV-B-treated green leaves (Fig. 3, lane 6), the diluted cDNA samples (Fig. 3, lanes 1-5) were amplified by PCR using either *lhcb1*4* or *lhcb1*2* primer. When the amount of cDNA included in the PCR reaction mixture was reduced to 0.05 ng, it became undetectable for both *lhcb1*4* and *lhcb1*2* (Fig. 3, lane 5). The amount of *lhcb1*4* mRNA in the 54-h, UV-B-treated green leaflets was between quantities of the mRNA contained in 0.8 and 0.4 ng of cDNA pools of the control leaves. In contrast, *lhcb1*2* mRNA in the same sample was at such a low level that its cDNA product was almost invisible on the blot (Fig. 3, lane 6).

3.2 UV-B modulation of *lhcb* mRNA was affected by chloroplast modifications

3.2.1 Norflurazon completely inhibited carotenoid biosynthesis in chloroplasts

As a prerequisite for determining the role of chloroplasts in UV-B-induced regulation of *lhcb* genes, carotenoid-deficient plants were obtained by growing pea seedlings under continuous dim red light in the presence of 100 μ M norflurazon in vermiculite. Norflurazon-treated plants were pale green, while control plants grown under the same conditions in the absence of norflurazon were yellowish-green (Fig. 4). Plant growth was not substantially affected by the norflurazon treatment until the

Figure 3. Quantitative comparison of transcripts encoded by *lhcb1*4* and *lhcb1*2* in UV-B-treated green leaves

*lhcb1*4* and *lhcb1*2* transcripts were individually amplified by RT-PCR. Each PCR reaction was initiated with either 0.8, 0.4, 0.2, 0.1 and 0.05 ng of first strand cDNA prepared from leaves not exposed to UV-B (lanes 1-5), or 5 ng of first strand cDNA obtained from leaves that were exposed to the UV-B treatment as described in Fig. 2 for 54 h (lane 6). tmp (ng), ng of first strand cDNA template used in each PCR reaction; UV (h), duration (hours) of the UV-B treatment. Other abbreviations are the same as indicated in the legends of previous figures.

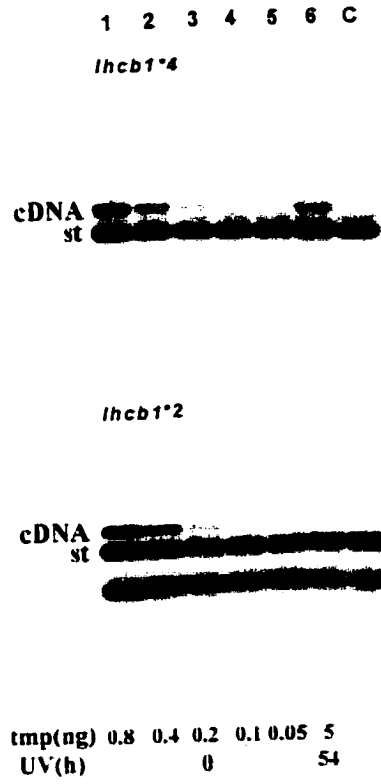


Figure 3

Figure 4. Norflurazon affected growth and pigmentation of pea plants

Extra Early Alaska pea seedlings were grown under continuous dim red light in the presence (a and c) or absence (b and d) of norflurazon as described in Materials and Methods. Pictures were taken on either the 7th (a and b) or 8th day (c and d) after sowing.

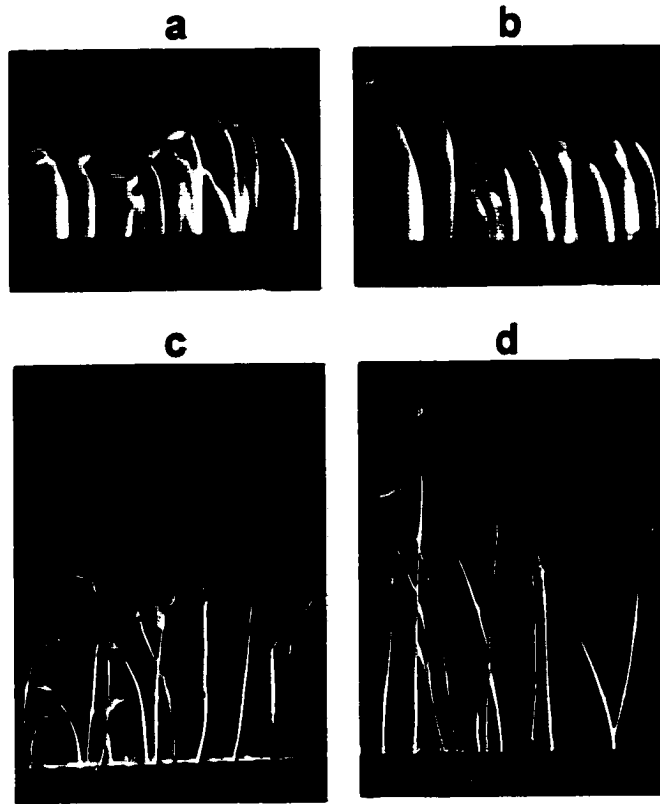


Figure 4

7th day after sowing (Fig. 4a and 4b), and the apical leaflets of the norflurazon-treated plants started to expand when the seedlings were 8 days old while the leaves of the control plants remained unexpanded (Fig. 4c and 4d).

Light absorption spectra of ethanol extracts prepared from either 6-d-old apical buds or 7-d-old leaflets harvested just prior to UV-B irradiation were obtained (Fig. 5). Compared to control seedlings, norflurazon altered the spectra of extracts from both 6-d-old buds and 7-d-old leaflets. Differences between the ethanol extracts of norflurazon-treated and control plants were obvious in two regions of the spectra spanning from 190 to 800 nm. For 6-d-old buds, one of these two regions was at approximately 280 - 330 nm and the other at 400 - 500 nm (Fig. 5a); for 7-d-old leaflets, the two regions were 250 - 310 nm and 380 - 510 nm (Fig. 5b). The absorption of the extracts from norflurazon-treated plants was higher than that of the control plants within the UV region (250-330 nm), and lower in the blue light region (380-510 nm). These data suggest that norflurazon exposure decreased the amount of blue light-absorbing pigments, such as the carotenoids, and increased UV-B-absorbing pigments.

Table 5 shows the quantitative comparison of chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids and UV-B-absorbing pigments between norflurazon-treated and control plants. Because the wavelength showing the highest difference in the UV-B region of the absorption spectra between norflurazon-treated and control plants was 295 nm (Fig. 1), absorbance at 295 nm of a 1 ml extract containing 1 g of fresh leaf or bud samples ($L_{295}/\text{ml}\cdot\text{g}$) was taken as the concentration of UV-B-absorbing pigments. As shown in Table 5, the

Figure 5. Norflurazon inhibited carotenoid biosynthesis in pea plants

Absorption spectra of ethanol extracts were obtained from either terminal buds of 6-d-old seedlings (a) or leaflets of 7-d-old Extra Early Alaska seedlings (b). Duplicate spectra from replicate experiments are shown for each treatment. The spectra were obtained from extracts diluted to 30 mg of fresh sample/ml. Because the UV portion of these spectra was outside the linear range, additional spectra were determined for each extract after dilution to 10 of mg fresh sample/ml (inserts).

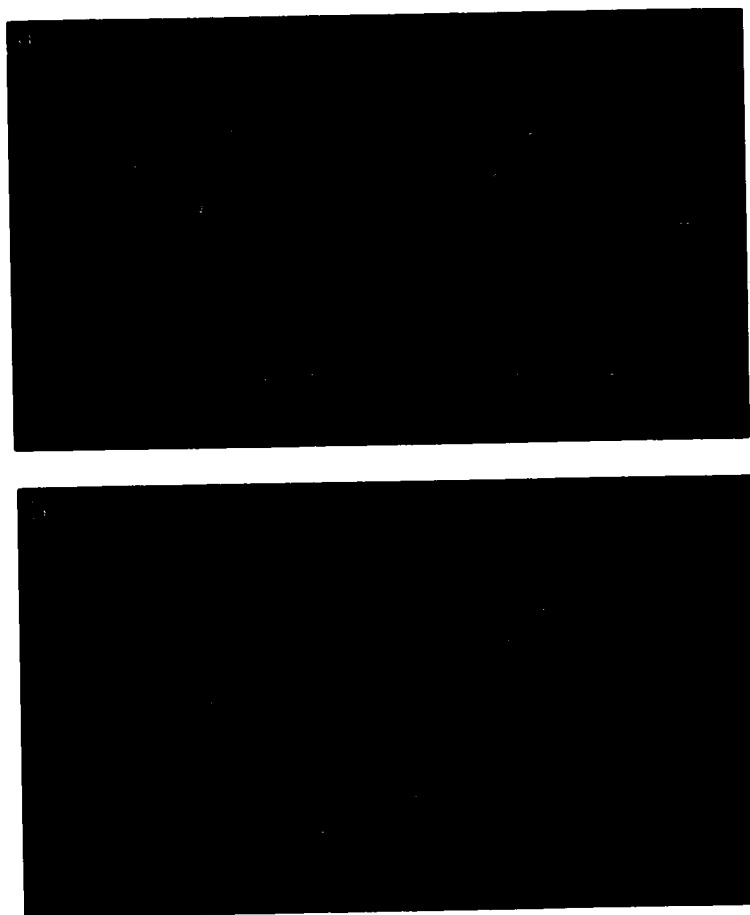


Figure 5

Table 5. Norflurazon inhibited carotenoid synthesis without affecting chlorophylls

Treatment	Sample	Chl a ($\mu\text{g g}^{-1}$)	Chl b ($\mu\text{g g}^{-1}$)	Chl a/b ratio	Carotenoids ($\mu\text{g g}^{-1}$)	UV-B pigments ($L_{295} \text{ ml}^{-1} \cdot \text{g}^{-1}$)
control	bud, 6 d	43 b	27 b	1.6 a	24 b	80 b
norflurazon	bud, 6 d	48 b	33 b	1.5 a	0 c	95 a
control	leaf, 7 d	95 a	53 a	1.8 a	54 a	82 b
norflurazon	leaf, 7 d	96 a	63 a	1.7 a	0 c	92 a

Pigments of 6- and 7-d-old pea seedlings grown in the presence (norflurazon) or absence (control) of norflurazon under continuous dim red light. $L_{295} \text{ ml}^{-1} \cdot \text{g}^{-1}$ is the absorbance of extract of 1 g fresh sample/ml measured in a 1-cm light path quartz cuvette at 295 nm. Mean values in the same column with the same letter are not significantly different as determined by multiple comparisons with Turkey test (Zar, 1996) ($n=4$, $P 0.05$).

norflurazon treatment completely inhibited carotenoid biosynthesis in both 6-d-old buds and 7-d-old leaflets, without significantly affecting Chl a, Chl b contents and Chl a/b ratio. UV-B-absorbing pigments were not significantly affected by the developmental stage, but increased by 19 and 12% in 6-d-old buds and 7-d-old leaflets, respectively. Additionally, the 6-d-old buds contained 50% less Chl a, Chl b and carotenoids than the 7-d-old leaflets.

3.2.2 Short-term white light exposure partially degraded chlorophyll in carotenoid-free chloroplasts

Obtaining plants with chloroplasts that have part of their chlorophyll photo-oxidatively removed is critical for investigating the role of these organelles in UV-B-dependent regulation of *lhcb* genes. However, if all of the chlorophyll is depleted, the investigation of UV-B effects on *lhcb* genes is impossible because their transcripts are absent. When 7-day-old norflurazon-treated pea seedlings were exposed to $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light for 3 h, their leaves became pale (Fig. 6). As detected in the leaflets harvested immediately after white light exposure, the amount of Chl a and b in the norflurazon-treated plants was reduced by 59 and 66%, respectively, while the Chl a/Chl b ratio, carotenoids, and UV-B-absorbing compounds were not affected significantly (Table 6).

3.2.3 Transcripts of chloroplast and nuclear genes were differentially affected by norflurazon, but were both reduced by photobleaching

Both *psbA* and *lhcb* encode photosystem II proteins. *psbA* is located in chloroplasts, while *lhcb* is in the nucleus. When grown under dim red light, norflurazon-induced carotenoid-deficiency increased accumulation of mRNA

Figure 6. White light bleached norflurazon-treated plants

On the 7th day after growth under continuous dim red light in the presence of norflurazon, plants were either retained under continuous dim red light (a) or exposed to white light ($110 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 3 h followed by growth under continuous dim red light for 21 h (b).

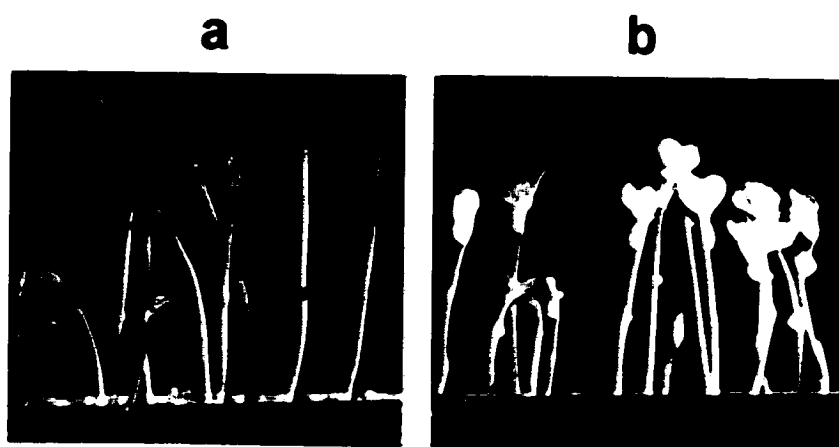


Figure 6

Table 6. White light degraded chlorophyll in carotenoid-free plants

Treatment	Chl a ($\mu\text{g g}^{-1}$)	Chl b ($\mu\text{g g}^{-1}$)	Chl a/b	Carotenoids ($\mu\text{g g}^{-1}$)
norflurazon/red light	85 a	50 a	1.7 a	0
norflurazon/white light	35 b	17 b	2.1 a	0

The data show pigmentation in the leaflets of 7-d-old norflurazon-treated seedlings either exposed to white light ($110 \mu\text{mol m}^{-2} \text{s}^{-1}$) or remained under continuous dim red light for 3 h. Mean values in the same column with the same letter are not significantly different ($n=4$, $P 0.05$).

encoded by *psbA*, but it caused a slight decrease in the accumulation of mRNA transcribed from *lhcb* genes (Fig. 7a). However, if the carotenoid-free plants were photobleached by short term white light exposure, transcripts from both *lhcb* and *psbA* genes were reduced (Fig. 7b).

3.2.4 White light-photobleached carotenoid-free seedlings mimicked etiolated plants in the response of *lhcb* mRNA to UV-B

To determine the effect of chloroplast photobleaching on the UV-B response of *lhcb* genes, 6-d-old carotenoid-containing and carotenoid-free Extra Early Alaska pea seedlings were exposed to white light for 3 h prior to UV-B irradiation (4.2 MED h⁻¹). After growth for 5 h under dim red light following 3 h of UV-B exposure, total *lhcb* mRNA of the carotenoid-containing plants showed little change in buds (Fig. 8a, lanes 1, 2), but it declined in leaves (Fig. 8b, lanes 1, 2). Meanwhile, in the white light-photobleached carotenoid-free plants, the UV-B exposure caused an increase rather than a reduction of *lhcb* transcripts in both buds (Fig. 8a, lanes 3, 4) and leaves (Fig. 8b, lanes 3, 4). Even more surprisingly, the hypocotyls of all plants wilted (data not shown) 21 h after the UV-B exposure regardless of the norflurazon treatment and the increase in *lhcb* mRNA.

3.2.5 Carotenoid depletion reduced *lhcb1*4* and *lhcb1*2* sensitivity to UV-B

Because *lhcb* mRNA was not as sensitive to UV-B in buds as in leaves of dim red light-grown seedlings (Fig. 8), leaves were used in all subsequent experiments. In addition, UV-B intensity was reduced to 67% of that used in the previous experiments to avoid hypocotyl wilting. Exposure to UV-B of lower intensity caused stem curling and reduced plant growth regardless of norflurazon

Figure 7. Carotenoid-deficiency increased the sensitivity of genes encoding chloroplast proteins to photo-oxidative stress

a, Extra Early Alaska pea seedlings were grown under continuous dim red light in either the absence (lanes 1) or the presence (lanes 2) of norflurazon for 7 days; b, Extra Early Alaska pea seedlings were grown under continuous dim red light in the presence of norflurazon for 6 days, and the plants were then either retained under dim red light (lanes 1) or exposed to white light ($110 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 3 h followed by growth under the dim red light condition for 21 h (lanes 2).

RNA was extracted from apical buds collected for all treatments at the same time, and resolved in 1% formaldehyde agarose gel (20 μg of total RNA/lane). Northern blots were hybridized to probes generated from cDNA clones of the genes as indicated.

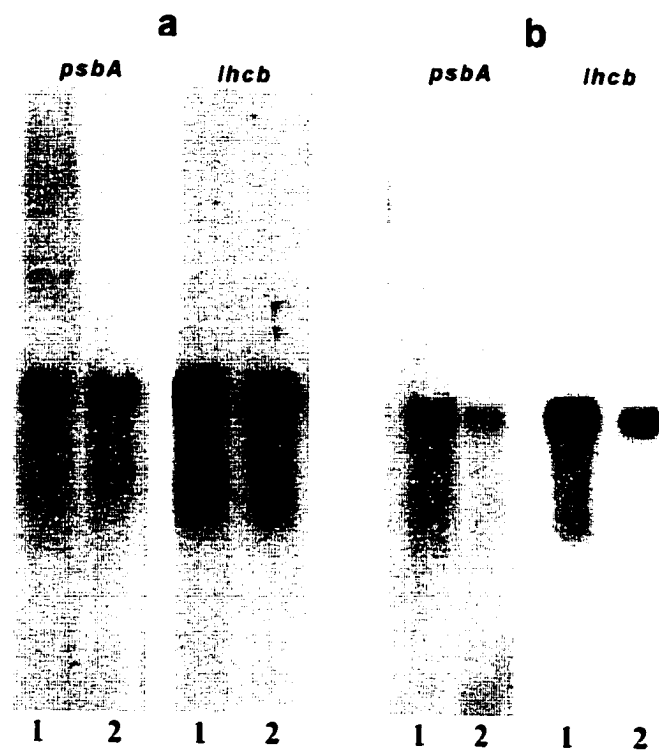


Figure 7

Figure 8. UV-B exposure caused an increase in *lhcb* mRNA accumulation after removal of both carotenoids and chlorophyll

Six day old Extra Early Alaska pea seedlings, grown either in the absence (lanes 1, 2) or the presence (lanes 3, 4) of norflurazon, were exposed to $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light for 3 h. Plants from each group were then either returned to dim red light (lanes 1, 3) or irradiated with UV-B (UV, 4.2 MED h^{-1}) for 3 h followed by growth under dim red light for 5 h (lanes 2, 4). Total RNA was extracted from apical buds (a) or outer leaflets of the apical buds (b) collected at the same time for all the treatments. Northern blots ($20 \mu\text{g}$ total RNA/lane) were hybridized to an *lhcb1*4* cDNA probe for the detection of *lhcb* mRNA.

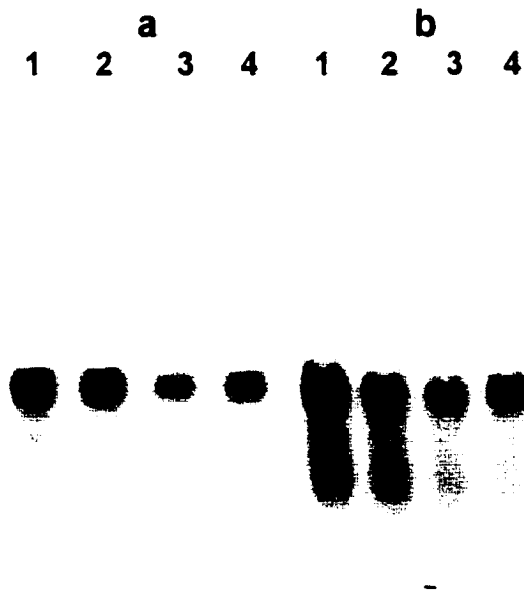


Figure 8

treatment (Fig. 9). As detected after growth under dim red light for 21 h following the UV-B exposure, UV-B reduced mRNA accumulation from both *lhcb1*4* and *lhcb1*2* in the 6-d-old carotenoid-containing seedlings (Fig. 10a, lanes 1, 2). Although the cultivar, growth conditions and UV-B exposure used here were different from those described in Fig. 2, the results obtained in these two experiments were similar; that is, *lhcb1*2* was more sensitive to UV-B than was *lhcb1*4*. The mRNAs for *lhcb1*4* and *lhcb1*2* were reduced by UV-B to 34% and 8% of control values, respectively. Norflurazon treatment reduced the sensitivity of these genes to UV-B. Therefore, when norflurazon was present, the mRNAs for *lhcb1*4* and *lhcb1*2* mRNA were, respectively, decreased to 84% and 62% of the transcripts in control plants by UV-B exposure (Fig. 10a, lanes 3, 4). In addition, 7-d-old seedlings exhibited a pattern similar to that of the 6-d-old plants, except that the former was more sensitive to UV-B (Fig. 10b).

3.2.6 Chloroplast photobleaching abolished the difference between *lhcb1*4* and *lhcb1*2* sensitivity to UV-B

In the following experiments, plants were exposed to UV-B after growth under dim red light for 7 days, due to the higher sensitivity of *lhcb* transcripts to UV-B at this stage (Fig. 10b). In the absence of norflurazon, after the plants were exposed sequentially to white light and UV-B for 3 h, followed by growth under dim red light for 21 h, mRNAs for both *lhcb1*2* and *lhcb1*4* were reduced by UV-B, with *lhcb1*2* mRNA much more sensitive to this treatment (Fig. 11a, lanes 1, 2). However, when norflurazon-grown plants were exposed to the same treatments, UV-B exhibited much less effect on the transcripts of both genes (Fig. 11a, lanes 3,

Figure 9. Exposure of pea plants to lower intensity UV-B caused stem curling and reduced seedling growth regardless of norflurazon treatment

Extra Early Alaska pea seedlings were grown for 7 days in the absence (a ,b) or presence (c, d) of norflurazon under continuous dim red light. Plants were either kept under dim red light (a, c), or irradiated with UV-B for 3 h (b, d), after which they were incubated in continuous dim red light for an additional 21 h, before the photographs were taken.

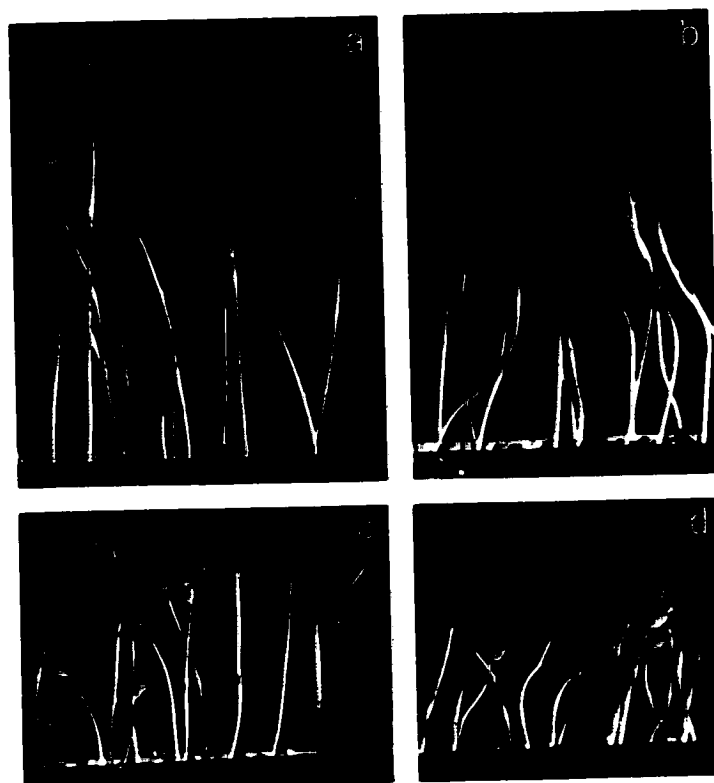


Figure 9

Figure 10. Inhibition of carotenoid biosynthesis reduced the ability of UV-B to affect accumulation of *lhcb1*4* and *lhcb1*2* transcripts

Extra Early Alaska pea seedlings, grown for 6 days (a) or 7 days (b) in the absence (lanes 1, 2) or the presence (lanes 3, 4) of norflurazon, were either retained under dim red light (lanes 1, 3) or irradiated with UV-B (2.88 MED h⁻¹, lanes 2, 4) for 3 h. Leaflets from all treatments were collected 21 h after the UV-B-treated plants were returned to dim red light. Transcripts encoded by *lhcb1*4* and *lhcb1*2* in the leaflets were individually quantitated by the RT-PCR method, as described before. Abbreviations are as described in the legends to previous figures.

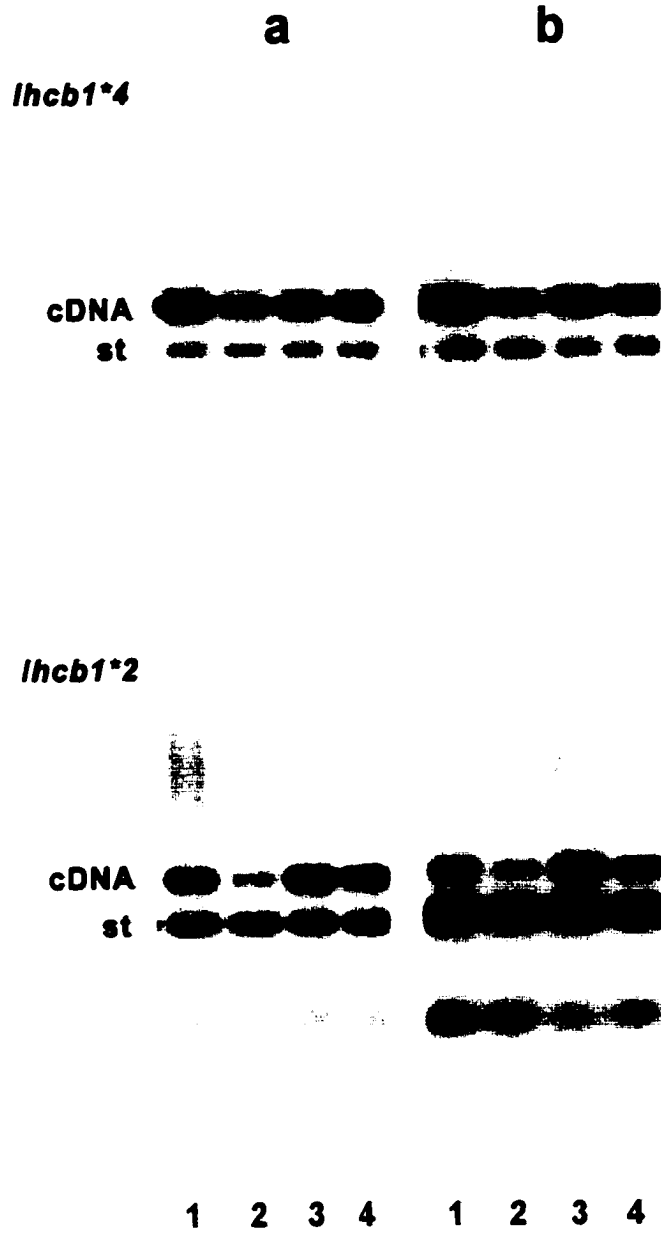


Figure 10

Figure 11. Carotenoid depletion and chlorophyll degradation abolished the difference between *lhcb1*4* and *lhcb1*2*

a, Extra Early Alaska pea seedlings, grown for 7 days under dim red light either in the absence (lanes 1, 2) or the presence (lanes 3, 4) of norflurazon, were exposed to $110 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ white light for 3 h. The plants were returned to dim red light (lanes 1, 3) or irradiated with UV-B (2.8 MED h^{-1} , lanes 2, 4) for 3 h. Leaflets were collected at the same time from all four groups 21 h after the UV-B-irradiated plants were returned to dim red light. The mRNA levels for *lhcb1*4* and *lhcb1*2* in the leaflets were individually determined using the RT-PCR method. Refer to previous figures for abbreviations that are not defined in this legend.

b, Quantitation of UV-B-induced *lhcb* mRNA reduction in photobleached carotenoid-free pea seedlings. The bands on films from three independent experiments as exemplified by those in lanes 3 and 4 of Fig. 11a were scanned with a GS-670 densitometer, and analyzed with the Molecular Analyst Software (Bio-Rad, CA, USA). Each column and the bar represent the mean percentage value and error calculated from the amount of mRNA in UV-B-irradiated plants relative to that in non-irradiated plants.

c, Quantitation of UV-B-induced *lhcb* mRNA reduction in non-photobleached carotenoid-free pea seedlings. The bands on films from three independent experiments as exemplified by those in lanes 3 and 4 of Fig. 10b were scanned with a GS-670 densitometer, and analyzed with the Molecular Analyst Software (Bio-Rad, CA, USA). Each column and the bar represent the mean percentage value and error calculated from the amount of mRNA in UV-B-irradiated plants relative to that in non-irradiated plants.

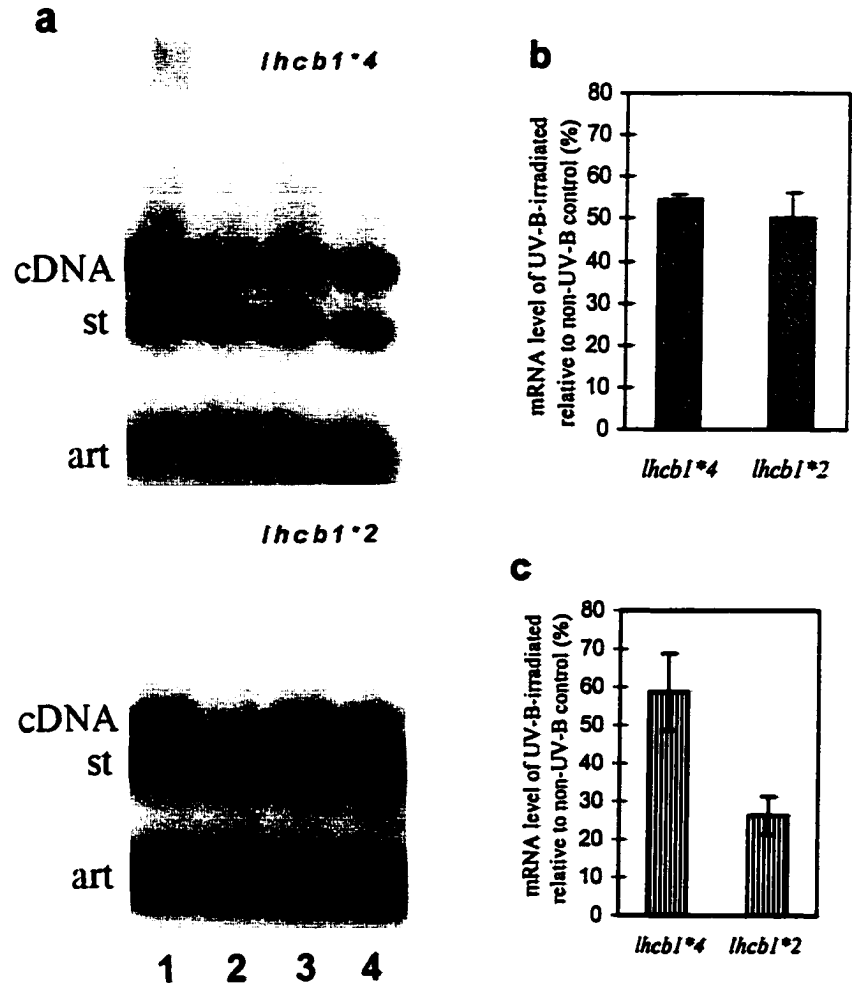


Figure 11

4). The difference in UV-B response between *lhcb1*4* and *lhcb1*2* as seen in the control plants grown in the absence of norflurazon was abolished by photobleaching. Comparison of the transcripts between photobleached (Fig. 11a, lanes 3, 4; Fig. 11b) and non-photobleached (Fig. 10b, lanes 3, 4; Fig. 11c) carotenoid-free plants revealed that photobleaching only protected *lhcb1*2* mRNA but not *lhcb1*4* transcript against UV-B.

3.3 UV-B exposure and chloroplast photobleaching by white light differently affected *lhcb* mRNA accumulation under dim red light in carotenoid-free plants

Without either UV-B or white light exposure, norflurazon differentially affected *lhcb1*4* and *lhcb1*2* accumulation under dim red light, with the *lhcb1*4* transcript slightly decreased, while *lhcb1*2* mRNA was substantially increased (Fig. 12a). To compare the effects of UV-B and white light on *lhcb1*4* and *lhcb1*2* mRNA accumulation in carotenoid-free plants, 7-d-old norflurazon-treated seedlings grown under dim red light were exposed to either UV-B or white light for 3 h, followed by growth under dim red light for 21 h. The UV-B exposure differentially affected mRNA accumulation of *lhcb1*2* and *lhcb1*4* under red light, with the former repressed to a larger extent (Fig. 12b). However, the white light exposure repressed the mRNA accumulation of these two genes to approximately the same extent (Fig. 12c).

3.4 UV-B prevented the white light-induced reduction of *lhcb* mRNA in carotenoid-free plants

Pea seedlings were irradiated with UV-B for 3 h immediately before

Figure 12. Effect of norflurazon, UV-B and photobleaching on *lhcb1*4* and *lhcb1*2* mRNA accumulation under dim red light

Extra Early Alaska pea seedlings were grown in either the absence (WA) or presence (NF) of norflurazon under continuous dim red light for 7 days. The plants were then exposed to either $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light (WL) or 2.88 MED h^{-1} UV-B (UV) for 3 h followed by growth under dim red light for an additional 21 h. Transcripts encoded by *lhcb1*4* and *lhcb1*2* were quantitated by the RT-PCR method as described before. Refer to the legends to previous figures for other abbreviations.

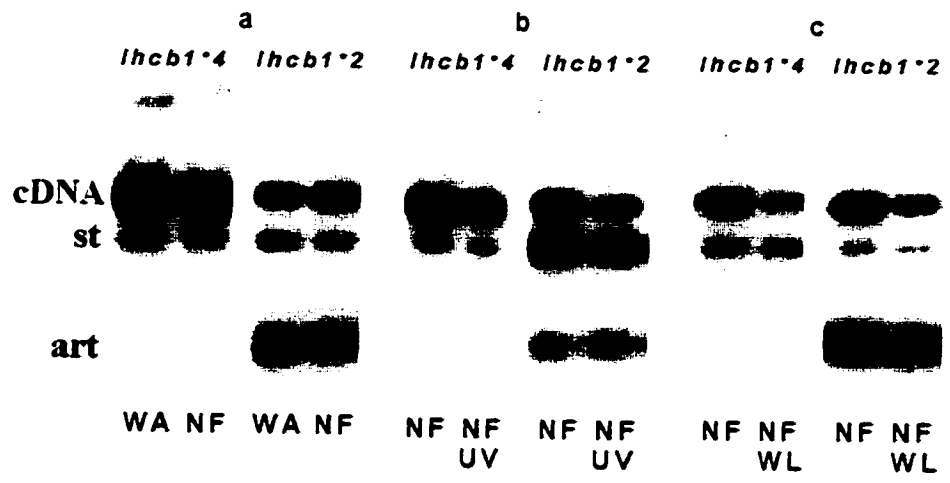


Figure 12

exposure to the white light for 3 h, in order to determine if UV-B influenced how white light-induced photobleaching affected *lhcb* mRNA accumulation. In the absence of norflurazon, the transcripts for both *lhcb1*4* and *lhcb1*2* were reduced by UV-B (Fig. 13, lanes 1, 2). However, in the presence of norflurazon, UV-B increased these transcripts (Fig. 13, lanes 3, 4), demonstrating that UV-B protected the mRNAs from the effects of white light-induced photobleaching (Fig. 12c).

3.5 UV-B irradiation affects other genes

3.5.1 Cloning of UV-B-responsive genes

Using 3' RACE PCR with the degenerate primer, bzip (see Materials and Methods), a 560 bp fragment that was slightly induced by UV-B, and a 480 bp fragment that was only present in UV-B-treated leaves were obtained (Fig. 14a). With the degenerate primer, zinc, PCR amplification produced a 440 bp fragment mainly present in the UV-B-treated seedlings (Fig. 14b). These DNA fragments were cloned in pUC18, from which probes were generated and labeled with biotin by 3' RACE PCR (data not shown). The probes were hybridized to northern blots of total RNA from leaves of UV-B-irradiated and non-irradiated Alaska pea seedlings were hybridized to these probes, revealing two types of UV-B-responsive transcripts, one induced (Fig. 15) and the other repressed by UV-B (Fig. 16).

3.5.2 UV-B-inducible genes:

Clone *psUVRib*, 371 bp in length, was obtained from the 3' RACE PCR using the bzip primer. It was highly related to sequences that encode ribosomal protein S23 of strawberry and *Rattus. norvegicus*, and ribosomal protein S28 of yeast and its human homologue (Fig. 17a). The 5' portions of these genes from

Figure 13. Exposure to UV-B protected *lhcb1*4* and *lhcb1*2* mRNAs from the effect of white light-induced chloroplast photobleaching

On the 7th day after growth in the absence (lanes 1, 2) or presence (lanes 3, 4) of norflurazon, Extra Early Alaska pea plants were either irradiated with UV-B (2.8 MED h⁻¹, lanes 2, 4) for 3 h or retained under dim red light (lanes 1, 3). All of the plants were then exposed to 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light for 3 h. *lhcb1*4* and *lhcb1*2* mRNAs were detected with the RT-PCR method, in the leaflets that were collected after the plants were grown under dim red light for an additional 18 h.

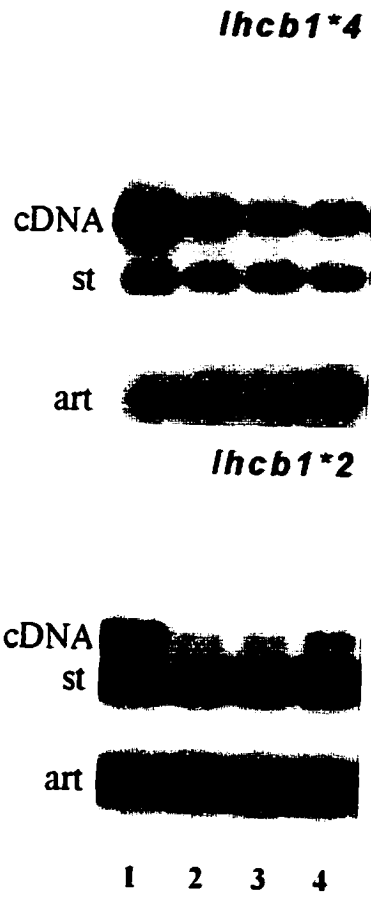


Figure 13

Figure 14. Agarose gel electrophoresis-purified 3' RACE PCR products

Alaska pea seedlings, grown under 12-h light/12-h dark cycles at 22 °C for 9 days, were either exposed to white light or irradiated with UV-B (2.89 MED h⁻¹) under white light for 3 h. Poly(A) mRNA was purified from leaves collected 21 h after UV-B exposure. Products of hot-start 3' RACE PCR, using either a bZIP (a) or a zinc finger primer (b) were resolved by electrophoresis in 2% low-melting temperature agarose gels. Lanes 1 and 2, PCR products amplified from mRNA of non-treated leaves; lanes 3 and 4, PCR products amplified from mRNA of the UV-B-irradiated leaves; M, 100-bp ladder. Sizes (bp) of the PCR products cloned into pUC18 are indicated by the labelled arrowheads.

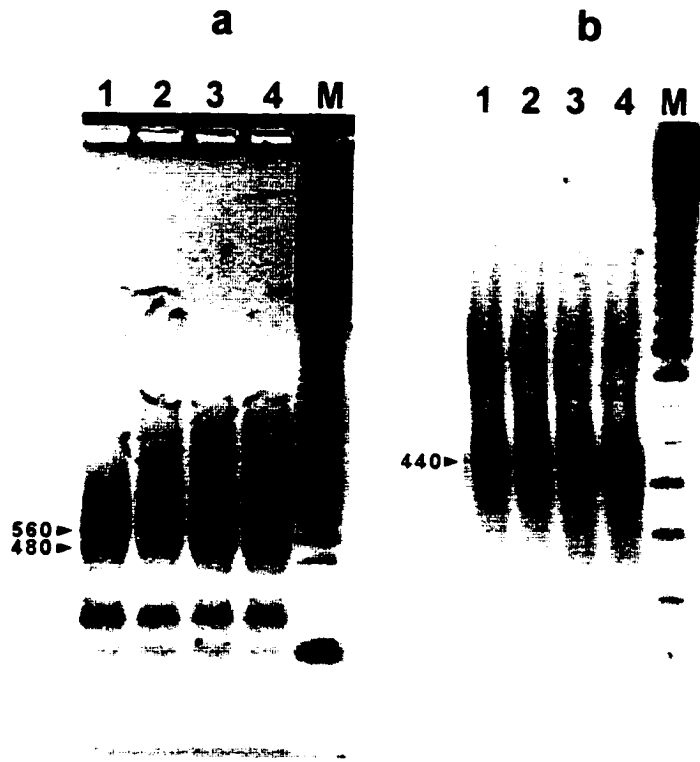


Figure 14

Figure 15. Northern blots of transcripts of cloned UV-B-inducible genes

Twenty μg of total RNA prepared as described in Figure 14 was resolved in each lane of a 1% formaldehyde agarose gel and blotted onto an N⁺ nylon membrane. Cloned cDNA fragments obtained by 3' RACE PCR were labelled by PCR amplification. The resulting products were used as probes for hybridization to the northern blots. C, non-irradiated; UV, UV-B-irradiated; 1, *psUVRib*; 2, *psUVGlu*; 3, *psUVaux*. The sizes (kb) of hybridized mRNAs are indicated by the labelled arrowheads.

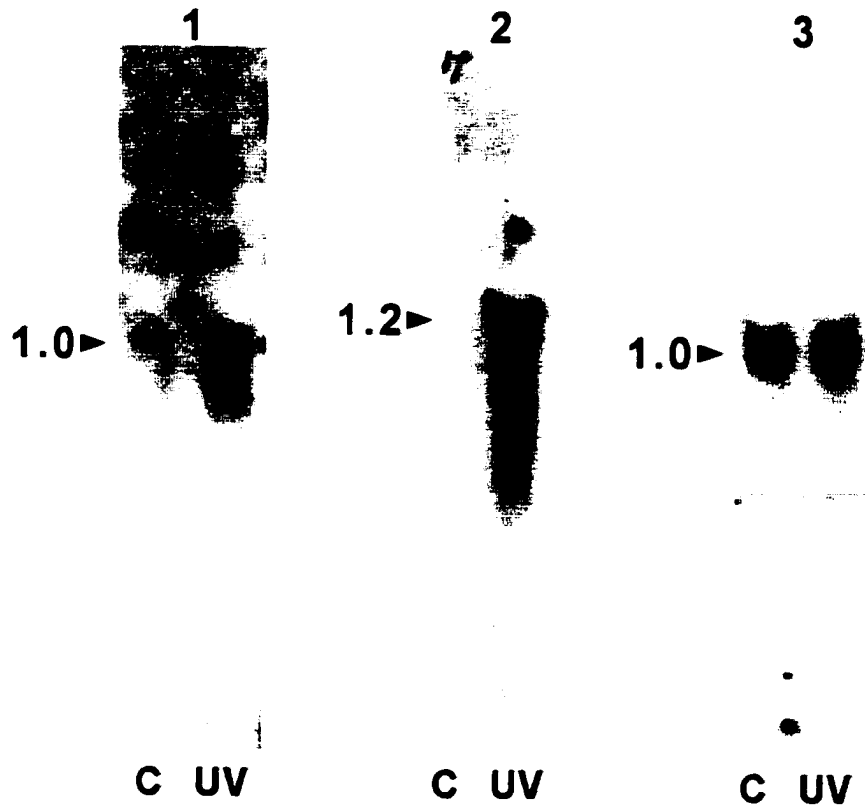


Figure 15

Figure 16. Northern blots of transcripts of cloned UV-B-repressible genes

Probe preparation and northern blotting were performed as described in the legend to Figure 15. C, non-irradiated; UV, UV-B-irradiated; 1, *psUVRub*; 2, *psUVDeh*; 3, *psUVzinc*. The sizes (kb) of the hybridized mRNA are indicated by the labelled arrowheads.

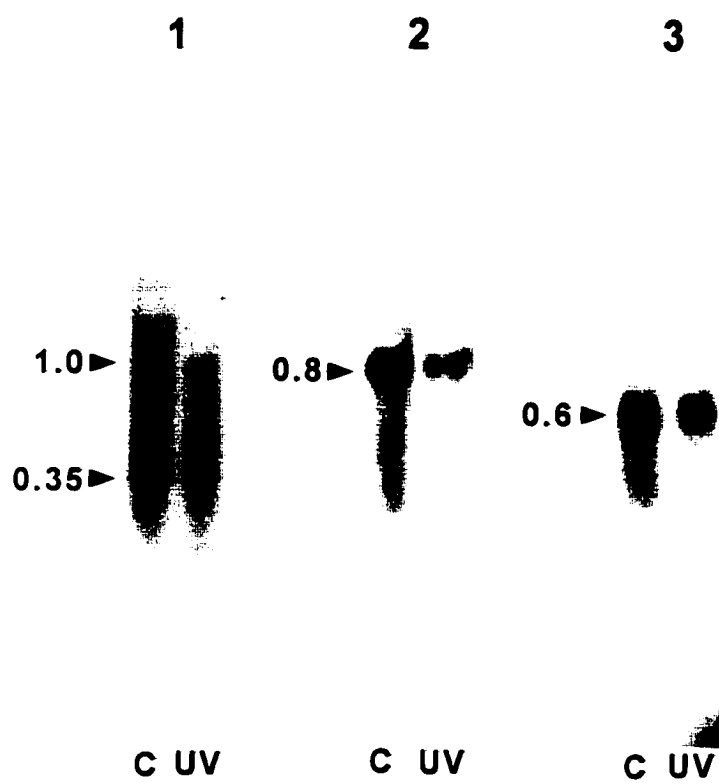


Figure 16

Figure 17. Sequence analysis of *psUVRib* and its deduced peptide

GenBank was searched for homologues using both *blastn* and *blastx*. Nucleotide (a) and encoded amino acid (b) sequences of *psUVRib* were compared by the *ClustalW* program to those for strawberry ribosomal protein S23 mRNA (*Fragaria_x*), human homologue of yeast ribosomal protein S28 (Human_homo), *R. norvegicus* ribosomal protein S23 mRNA (*R.norvegic*), and *Saccharomyces cerevisiae* ribosomal protein S28 gene (*Saccharomy*). Each blue letter represents a nucleotide of the clone or amino acid residue of its deduced peptide. The green letters are the nucleotides or amino acids that are identical to those in all the sequences. The red letters represent the nucleotide or amino acid residues that are identical to those in the *psUVRib* clone or its deduced peptide, respectively. The black letters represent the nucleotide or amino acid residues that are divergent from those in the *psUVRib* clone or its deduced peptide. The first and stop codons of the deduced peptide are indicated as bold italic and bold letters, respectively. -, gap introduced for maximum alignment; identities (Id) of a sequence compared to those in *psUVRib* are shown at the end of each sequence.

a

<i>PsUVRib</i>	TCAACTC CATC	AAGAATGGGA	AGAAGATTGC	TCCTTTTGTG	CCTAATGATG	GTGCTTGAA	CTACAICGAA	GAAAAATGATG	80
<i>Fragaria_x</i>	TCAGCTTATC	AAGAACGGGA	AGAAGATTGT	TCCTTTTGTG	CCCAACGATG	TTTCTTAA	CTACATTAA	AAAAATGATG	
<i>Human_homo</i>	CCAGCTGATC	AAGAATGGCA	AGAAAATCAI	AGTCTTTTA	TCCAATGAC	TTTCTTAA	CTTATTGAG	AAAAATGATG	
<i>R.norvegic</i>	GCAGCTCATT	AAGAACGGCA	AGAAGATCAI	AGTCTTTGTG	CCCAATGAC	TTTCTTAA	CTTATTGAG	AAAAATGATG	
<i>Saccharomy</i>	TCAATTAATC	AAGAACGGTA	AGAAGGTCAC	TCCTTTCTTT	CCAAACGATG	TTTCTTAA	CTTTGTGAC	AAAAATGATG	
<i>PsUVRib</i>	AAGTTTTGAT	TCCTGGATT	GGAGAAAAG	GTGATGCTGT	GGGAGATATT	CCCGGAGTTA	GGTTC AAGGT	TGTGAAGGTT	160
<i>Fragaria_x</i>	AGTGTGAT	TCCTGGTTT	GGACTAAGG	GTGATGCTGT	GGTGTATATT	CCCGGAGTTA	GGTTC AAGGT	TGTGAAGGTC	
<i>Human_homo</i>	AAGTCTGGT	TCCTGGATT	GGTCCAAAG	GTGATGCTGT	GGTGTATATT	CCCGGAGTTA	GGTTC AAGGT	TGTGAAGGTC	
<i>R.norvegic</i>	AAGTCTGGT	TCCTGGATT	GGTCCAAAG	GTGATGCTGT	GGTGTATATT	CCCGGAGTTA	GGTTC AAGGT	TGTGAAGGTC	
<i>Saccharomy</i>	AAGTCTGGT	TCCTGGATT	GGTCCAAAG	GTGATGCTGT	GGTGTATATT	CCCGGAGTTA	GGTTC AAGGT	TGTGAAGGTC	
<i>PsUVRib</i>	TCCTGGTAT	CTCTCTTGC	CTTTTCAAG	GAAAAGAAGG	AAAACCAAG	ATCTEAGGT	TGTTGAATGT	CCTTTC-ATT	240
<i>Fragaria_x</i>	TCAGGTGTGT	CTCTCTTGC	CTCTTCAAG	GAAAAGAAGG	AGAAGGATAG	ATCTEAGGT	TATGGTTCAT	GAGTTGAGCT	
<i>Human_homo</i>	GCCAAATGTT	CTCTTTTGG	CTTATACAA	GGCAAAGAG	AAAGATTAAG	ATCTEAGGT	ATTATGAGT	AAAACACTGT	
<i>R.norvegic</i>	GCCAAATGTT	CTCTTTTGG	CTCTTACAA	GGCAAAGAG	AAAGATTAAG	ATCTEAGGT	ATTATGAGT	AAAACACTGT	
<i>Saccharomy</i>	TCCTGGTGTCT	CTCTGTGGG	TTTGTGGAA	GAAAAGAAGG	AAAAGGTAAG	ATCTEAGGT	CGAAAGTTAT	CCTCTCGAGG	
<i>PsUVRib</i>	TCCTTAGTTT	ATTGAAGTA	TGAAGAATTT	TCCTTCCGTT	TGAATGAGTT	--TTATAGAC	AGTTAT---G	TATTTCTGAT	320
<i>Fragaria_x</i>	ACTGAAATGT	TTTGATTGAG	ATACAATTTT	GTCTCTCTCT	TTAAGACCAT	GCTTGGCAAT	CAGATGCTTA	TGCTTTGTTT	
<i>Human_homo</i>	AGTAATAAAT	TTTCATATGC	-----	-----	-----	-----	-----	-----	
<i>R.norvegic</i>	AATAATAAAT	TTT-ATATCC	AGAAAAA---	-----	-----	-----	-----	-----	
<i>Saccharomy</i>	ACTACATTGT	TTTTGTACCT	TAATGTATCT	CTTTATTCTT	CCAGAAAGTT	CCTTCTAATT	TCTTATTAGG	AATGGGAGG	
<i>PsUVRib</i>	TATTTTGGG	AACCTATTT	TGACTTAGGA	ATGTTTAAAA	AAAAAAAAAA	AAAAAAA	Id (%)		
<i>Fragaria_x</i>	TTTGAATTT	CAGTGGTGT	TTCGGTCTTT	-----	-----	-----	62		
<i>Human_homo</i>	-----	-----	-----	-----	-----	-----	49		
<i>R.norvegic</i>	-----	-----	-----	-----	-----	-----	49		
<i>Saccharomy</i>	AATCACATCA	ATATTTTCTC	TTTAATAG-A	ATGTATAATT	CAATAGAAAA	AAACAAC	66		

b

<i>PsUVRib</i>	QLIKNGKKIAAEVNDGCLNYIEENDEVL IAGFGRKQHAVGDI PGVRFKVVKVS GVSLLALFKKKEKPPS	Id (%)
<i>Fragaria_x</i>	QLIKNGKKIAAEVNDGCLNYIEENDEVL IAGFGRKQHAVGDI PGVRFKVVKVS GVSLLALFKKKEKPPS	100
<i>Human_homo</i>	QLIKNGKKITAEVNDGCLNFEIENDEVL VAGFGRKQHAVGDI PGVRFKVVKVANV SLLALYKGFERPPS-	87
<i>R.norvegic</i>	QLIKNGKKITAEVNDGCLNFEIENDEVL VAGFGRKQHAVGDI PGVRFKVVKVANV SLLALYKGFERPPS	89
<i>Saccharomy</i>	QLIKNGKKVTAEVNDGCLNFEVDENDEVL IAGFGRKQKAGGDI PGVRFKVVKVS GVSLLALFKKKEKPPS	87

Figure 17

different organisms were almost identical, but their 3' ends diverged remarkably from each other. In addition, *psUVRib* was related to the ribosomal protein S23 of *Brugia malayi* and mouse, S12 of *Tetrahymena thermophila*, and chloroplast ribosomal protein S12 of many species (data not shown). A peptide of 71 amino acid residues deduced from *psUVRib* was nearly identical to the peptides encoded by the genes under comparison (Fig. 17b). The transcript, 1.0 kb in size, corresponding to this clone was increased 271% by UV-B irradiation (Fig. 15.1; Table 7).

The clone, *psUVGluc*, 374 bp in size, was obtained by using the zinc primer. It shared 66, 57, 51 and 50% nucleotide identity with *Cicer arietinum*, tobacco, pea and soybean β -1,3-glucanase genes, respectively (Fig. 18a). The most conserved regions of these genes were in the 5' portions, and several segments of different genes were nearly identical. The 3' portions of the genes, although highly related, showed much lower similarities. A peptide of 95 amino acid residues deduced from *psUVGluc* exhibited 43, 42, 59 and 42% identity with the peptides encoded by the pea, soybean, *Cicer arietinum* and tobacco β -1,3-glucanase genes, respectively (Fig. 18b). A FAMFDEN motif is identical in all the peptides of these genes. The *psGluc* mRNA, 1.2 kb in size, absent in non-irradiated leaves, was strongly induced by UV-B irradiation (Fig. 15.2; Table 7).

The clone, *psUVaux*, 472 bp in length, obtained from the bzip primer-amplified PCR products, shared 49% identity with an auxin-repressed protein gene found in *Prunus armeniaca* (Fig. 19a). One of its deduced peptides, containing 53 amino acid residues, showed 62% identity to the auxin-repressed

Table 7. Characteristics of UV-B-inducible clones

Clone	Size of the clone (bp)	Related protein	Putative function	Length of peptide (amino acids)	Size of mRNA (kb)	Increase in mRNA induced by UV-B (%)
<i>psUVRib</i>	371	Ribosomal protein	Protein synthesis	71	1.0	271.1
<i>psUVGlu</i>	374	β -1,3-glucanase	Pathogen resistance	95	1.2	NA*
<i>psUVaux</i>	472	Auxin-repressed protein	Growth inhibition	53	1.0	13.1

*NA: The mRNA was induced by UV-B but it was undetectable in non-irradiated plants.

Figure 18. Sequence analysis of *psUVGlu* and its deduced peptide

Nucleotide (a) and amino acid (b) sequence comparisons of *psUVGlu* to those for the glucan endo- β -1,3-glucosidase from *Cicer arietinum* (C. arr., AJ012751), *Hevea brasiliensis* (H. bra., U22147), tobacco (tobacco, X54430), pea (pea, L02212), and soybean (soybean, M37753). Each blue letter represents a nucleotide of the clone or amino acid residue of its deduced peptide. The green letters are the nucleotides or amino acids that are identical to those in all the sequences under comparison. The red letters represent the nucleotide or amino acid residues that are identical to those in the *psUVGlu* clone or its deduced peptide. The black letters represent the nucleotide or amino acid residues that are divergent from those in the *psUVGlu* clone or its deduced peptide. The first and stop codons of the deduced peptide are indicated as bold italic and bold letters, respectively. The putative poly(A) signal is indicated by a wave underline. -, gap introduced for maximum alignment; identities (Id) of a sequence compared to those in *psUVGlu* are shown at the end of each sequence.

a

<i>psUVGlu</i>	G AGGTGGAA	CTGGAGCTAG	TGCTGAAAT	GCTGCAACAT	ATTATGGGAA	TTTGATTAGG	CATGCTAAGG	70
Pea_beta-1	GATGGAGGCT	CTGCAACTTC	TTATGATAAT	TCGGGAATTT	ATCTCGATAA	CTTATTCGGT	CACHTTGGGA	
Soybean_be	TCTGGTGGAA	CTGCAACTTC	ACTTGATAAT	TAAGAAGCTT	ACAACACAAA	CTTATTCGGG	AAHTTGAAGC	
Cicerariet	CAAGTGGGAA	CTGGAGCTAG	TGTTGGAAAC	TCACAAACTT	ATTATGGAAA	CTTATTAAGG	CATGCTAAGG	
Tobacco_sp	GAAGGAAATT	CTGCAGCAAC	TATAGAAAAT	TCACAAACTT	ATTATAGAAA	TTTGTGTAAT	CATHTAAAG	
<i>PsUVGlu</i>	GTGGG----	-ACTCCTAAG	AGGCCTAATG	GACCTATTGA	GACTTATCTT	TTTGCATGT	TTGATGAAAA	140
Pea_beta-1	AAAGT----	-ACTCTTAGA	AGGCCTTGGG	CTAC---TAA	GGCTTATCTT	TTTCTATCTT	TTTATGAGAA	
Soybean_be	AAAGA----	-ACCCCTAAA	AGGCCTGGTG	CACCCCTTGA	AAHTTATGTG	TTTGCATCTT	TTTATGAAAA	
Cicerariet	GTGGG----	-ACACCTAAG	AGGCCTAATG	GACCTATTGA	AAHTTATCTT	TTTCTATCTT	TTTATGAAAA	
Tobacco_sp	GAGGGGCAGG	AAHTCTAAG	AAAGTGGAA	GAATTGTAGA	GACTTATCTG	TTTCTATCTT	TTTATGAAAA	
<i>PsUVGlu</i>	TCAGAAGCCA	GGTCCGTAAA	TTGAGAGACA	CTTTGGACTC	TTTAGCCCTG	A---TAAATC	ACCAAAGTAT	210
Pea_beta-1	TCAAAAEA--	-TCCCTGAAT	TGAGAGAAACA	TTTTTGTGTG	TTTTATCTTA	A---TAAACA	GAAGAAGTAT	
Soybean_be	TCAGAAAC--	-AGCCAGAGT	TTTAAAAATT	TTGGTGGCTC	TTTTCTCTTA	TAACTAAGCA	GCCCAATATC	
Cicerariet	TCAGAAAGCA	GATCCGTAAA	CCCAACGATA	TTTTTGTCTC	TTTTATCTTG	A---CAAATC	ACCTAATAT	
Tobacco_sp	TGAAAAAAT	GGAGAAATCA	CAGAGAAACA	TTTTTACTTC	TTTTATCTTA	A---TCGGAC	CGCAAAATAT	
<i>PsUVGlu</i>	CAACTAA--T	TCAATTG---	-AGATATA-C	TAGTGATTGG	TGTTTATGCA	TAATAAATAA	AC---- TAAA	280
Pea_beta-1	CCATTTGGAT	TTGGTGGAGA	AAGAAGGGAT	GGGGAAATTG	TTGAGGGGGA	TTTCAATGGA	ACTGTTTCTC	
Soybean_be	TCGATTAATT	TCAATTAATT	TTTAGCAGAA	GCATAATTTG	GTGTGAARTA	----AGGGCC	ACGAGTCCAA	
Cicerariet	CAAGTTAATT	TCAATTA---	-AGAAATA-T	CGAGAGGTG-	-GATTATGTA	ACTTGCATAA	GCCCAATACT	
Tobacco_sp	CAACTCAACT	TATGTAT--	-AGCGATACT	TAATAAGGTG	AAATTATGCT	TATTTCTCTG	GTTTTCAATA	
<i>PsUVGlu</i>	TGTAACTTGT	AAATAA ----	----AAGGGT	ACCTAGTGTCT	CATATGGTTA	TTTTTAGATA	TTTACTACTAA	350
Pea_beta-1	TTAAGAGT--	GATATGTAAG	ATGGAATCTA	GGTTTTCAAA	GTTTGAGGTG	TTTTATGGAT	AAATAAGAG	
Soybean_be	ATGAACTTC	AATAATGTTT	TTAGAAGAGG	CAGGTATAGA	ACTTTAATTT	ATCTGCATTC	TTGAGTCTG	
Cicerariet	AAGGGTACAT	TGTGCCCTTT	TGTTTACTTT	TAGATGTTTG	CATCAATGAA	TAATGATAAT	AA-TAAAC--	
Tobacco_sp	ATGTATTTGT	CAATGTCACT	TTGGAACATG	TTATTTCTCA	TTCTTAGAAT	TATTAATTTT	CAATCATT--	
<i>PsUVGlu</i>	TGAATAATGA	TAACAAATTC	TGTTGTGTAC	TCAAAAAATA	AAAAAATAAA	AA Id (%)		
Pea_beta-1	AAATCTTGT	GTTTGTGTTT	ATTTATCCTT	CCTTTTGTAA	TGTAATAAAGG	GA	51	
Soybean_be	CATGTACCAT	TTACGAGCTA	AGTTAATTAG	CTTTA-----	----ATAAAA	GT	50	
Cicerariet	--TTGATGTA	TA-----TG	TATTGTATAC	TCACAAAAAA	AAAAAATAAA	AA	66	
Tobacco_sp	--TCTACAAA	TCATATAAAG	TGTTATGGTC	GATGGACCGA	GTAATAAATAA	AA	57	

b

<i>PsUVGlu</i>	EGGTGASAGNAATYGNLIRHAK--GGTPKRPNGPIETYLAFMEDENQKPGPEIERHFQ	
Pea_beta-1	DGGSATSYDNARIYLDNLIHVG--KGTERRR-WATEAVLAFMEDENQKS-PELEKHFFQ	
Soybean_be	SGGTATSLDNARTYNTNLVRNVK--QITPKRPGAPLETYVAFMEDENQKQ-PEFEKFWG	
Cicer_arie	QGGTASVGNATYGNLIKHKAK--GGTPKRPNGPIETYLAFMEDENLTDPETERYFQ	
Tobacco_sp	EGNSAATIENAQTYRNLVNHVKGGATFKKPKRIVETYLAFMEDENEFNGEVTERHFQ	
<i>PsUVGlu</i>	LFRPDK-SPKYQLIQLRYTSDWWLCLINKLMVTCNKKGT	Id (%)
Pea_beta-1	VFYENK-QKNYPFGGGERRDGEIVEGDENGTVSLKSDM	43
Soybean_be	LFSPITKQPKYSINF-----	42
Cicer_arie	LFNFDK-SPKYQLNFN-----	59
Tobacco_sp	LFYENR-TAKYQLNEMYSDT-----	42

Figure 18

Figure 19. Sequence analysis of *psUVaux* and its deduced peptide

Nucleotide (a) and amino acid (b) sequence comparisons of *psUVaux* to those for putative auxin-repressed proteins from *Prunus armeniaca* (*Prunus_arm*, U93273), *Fragaria ananassa* (*Fragaria_a*, L44142), *Elaeagnus umbellata* (*Elaeagnus*, AF091513), the dormancy-associated protein from *Arabidopsis thaliana* (*Arabidopsi*, AF053747), *Pisum sativum* (*Pisum_sati*, AF029242), and apple fruit expressed protein (*Golden_del*, L15194). Each blue letter represents a nucleotide of the clone or amino acid residue of its deduced peptide. The green letters are the nucleotides or amino acids that are identical to those in all the sequences under comparison. The red letters represent the nucleotide or amino acid residues that are identical to those in the *psUVaux* clone or its deduced peptide. The black letters represent the nucleotide or amino acid residues that are divergent from those in the *psUVaux* clone or its deduced peptide. The first and stop codons of the deduced peptide are indicated as bold italic and bold letters, respectively. -, gap introduced for maximum alignment; identities (Id) of a sequence compared to those in *psUVaux* are shown at the end of each sequence.

a

<i>PsUVaux</i>	ACCAA-AGTG	GATCGCCTCC	GGCTTCTCCC	GCCGGTTCAG	TTACTCCGGT	GTCTCCGTTT	TCCGGAAC	TAGAACTACT	80
Prunus_arm	GGTAACAGCG	GATCGCCTCC	GATTTCCGCA	GCTGGGTCTA	CGCTCCGGT	GTCCCGGTTT	TCTGTGGCA	GCAGCATGGG	
Arabidopsi	AAGGATAACG	TGTGGAGG--	AGCGTCTTTA	ATCCGGGAAG	CAACCTCGCC	A-CTAGAGCC	ATCGGCTCCA	ACATCTTTGA	
F.ananassa	AAGGACAACG	TATGGAGG--	AGTGTGTTC	ACCCAGGTAG	CAACTGTCT	A-GCAGAGC	ATGSAACC	AGGTGTTTGA	
Elaeagnus	AAGGAAAACG	TATGGAGG--	AGTGTGTTC	ACCCAGGTAG	CAACTGTCC	A-CTCGTGGC	CTTGGGACTG	AGATGTTTGA	
Pisum_sati	GTTGACAACG	TTTGGAGG--	AGCGTGTTC	ATCCTGGGAG	TAACTCTGCT	A-CAAAGTCA	ATTGTGTCTC	ATGTTTTCGA	
Golden_del	AAAGACAACG	TTTGGAGG--	AGTGTGTTC	ACCCAGGTAG	CAACTGTCC	A-CCAAATCC	ATGSAACC	AGGTGTTTGA	
<i>PsUVaux</i>	TCCGTTTCGA	AGAAGTCCG	CATCGGATGC	GTTCGAGAAG	AAAAACCA--	-----AGA	CAGATCAAG-	-----CTCTT	160
Prunus_arm	AAGATTCCAG	AGGAGTCCG	CGTCCGATGC	ATACGAGAAG	GCAAGCCAGG	TTGGTGGAG	AGGAGCAAG	AGCAGCCCC	
Arabidopsi	TAARCCACCC	CATCCAAAT	CTCTCTCCGT	CTACGACTGG	TTGTACAGCG	--GTGACTCA	AGGAGTCAGC	ACC-GTTAGG	
F.ananassa	TAGCCACACG	C---CAAAC	CTCCACTGT	CTACGACTGG	ATGTACAGTG	--GAGAGACG	AGGAGCAAGC	ATC-ATCGTT	
Elaeagnus	CAAACCATCA	CAA-CCCAAC	TTCCACTGT	TTATGACTGG	CTCTACAGTG	--GAGAGACG	AGAAGCAAGC	ACC-GCTAAG	
Pisum_sati	CAARCCACTG	C---CCAACA	CTCCACTGT	CTATGACTGG	ATGTACAGTG	--GGGATAGC	AGAAGCAAGC	ACC-GTTGAT	
Golden_del	CAAGCCACAG	C---CCAAC	CTCCACTGT	CTACGACTGG	CTCTACAGTG	--GGGATAGC	AGGAGCATCC	ATC-ATCGTT	
<i>PsUVaux</i>	CTTCTCCTTT	CGATGTGTGA	GAATGAG-G	CACAGCATTT	T---CTAGT	CTCCAGAAGC	AGAGGATGTT	ACCTTTTGGC	240
Prunus_arm	GGTCTCCTTT	CGACGTGTGA	CCAAAGG---	-ACAACATGT	A---GTGAT	ATAATTAAGC	CCTTCTTTTG	CTAATTTGGG	
Arabidopsi	GATGGTCCGA	TGGAGTGTGA	CATCGGGTG	ATGTAATAT	GGTGCCTGA	TCTTCCGCCA	CCTTACTTTG	TTTTTTTTAT	
F.ananassa	AAGG---TGA	CAAGCTA-GC	GAC--AAGTG	ATGAGGATCT	A---TCGAG	GGTTCATGT	AAATAGTGT-	CGTTTTTCAT	
Elaeagnus	GAGA---ATC	TGTCCG-AC	TAC-----	-CCACGATC	GATGACCGAG	AA-TGATGT-	ATAAATAGTT	ATGTTTTCAT	
Pisum_sati	CAG---TGA	TCAAGT-GA	TAC-----	-CAACTCCC	T---TGG	TTTTGCATGT	AAATAGTGT-	TCTGTTTTGT	
Golden_del	GAGG---TGA	CGAAGAA-AC	AACTGAGCTG	ACCAGCAGCC	AGCCACCAGG	GGTTCATGT	AAATAGTGT-	ACCTTTTCAT	
<i>PsUVaux</i>	CTTAGATGTA	ACAAATTTTG	TTTGGTTTT	TTGTGTGTAT	GGGTGAGTT	GAATTGAAGT	TTTGTGCTGC	AGTTCAGGTT	320
Prunus_arm	TGTTGCTGGG	TGGTGTGGTG	TTTGTTTTGA	AGCTATATAT	ATAT-GTATT	GTATGTGATA	TATATA-TGT	AG---AGAGA	
Arabidopsi	A-TGTTTTT	GTGTACTTTA	TCAGAGTGA	TAAAGTGACG	GGGA---AT	TTTTTCTTT	TGTACA-AAA	AACTGTCTTC	
F.ananassa	T-TCAGTGT	TGCCAGTTG	TTGTTTTGG	TG-GAGTTTT	GTGA---AT	AGGGTTCG-T	CGTTTT-TCA	TTTCCAACTC	
Elaeagnus	T-TCTTTTG	TTTCAAGT-	TTTGTCTCAAG	TCCTATGGTA	GTGC---GT	TGATCTGTGA	TCATTA-CGT	ATTTGTGTCC	
Pisum_sati	--TCATCTC	TGCCACGT-S	TCTACATCAC	CGTTACGTGT	TGAG---AT	CTGATTTGTT	GTTCGA-GAG	TGTTTTTTTT	
Golden_del	C-TCAGTGT	TGCTACGT--	---GCCCGA	TGAGGGTTTT	CTGA---TG	CAATTCGAT	TAGTA-TCC	TTTTGGATTT	
<i>PsUVaux</i>	TGTTTATAAC	ATGTTTAAGT	TTGAAAGGAA	GTCCAAGGG	TAGGGTAATA	TGTATCTATG	TGTAGTGTAT	ATAGAGAGTC	400
Prunus_arm	GAGAGAGAGA	GAGAGAGAGG	GAGGGAGGGA	G---A-GAG	TAGTTTTGTA	TGTAAG-ATG	TAGAAATGT	GGATGGAGTT	
Arabidopsi	CC---ACCTA	AG---CAAT	GTGTGTAGT	TTCTGTTTTG	GGCTGTGTT	TGCAAGTTTT	TATTTTCAG	AGTTGTAGTG	
F.ananassa	TATCTAAACA	GGCTTTGGTT	TTGTGTAAAG	GCACTTAAGC	TCAGTGCCTA	ATG-AAGAGT	ATG-TGTTGT	AGTTGAGCTA	
Elaeagnus	TTAATGTTTT	GG-GCATGGA	CTTTATGAGA	ACTTATTTGA	ATTAATGGTT	TTT-TCITTA	TGTTCATTTT	GGATGAATTA	
Pisum_sati	TT--TATTTA	AGCATCTAAT	CTGAATCTGA	ATGTAATAATA	CTTAATAAAT	TGAATTAGTA	TCTTTTAAAA	AAAAAAAAAA	
Golden_del	TG--TACTTG	GG---GCTT	TCTTCCAAGA	GAGTCACTAT	TCTTGAATT	ATGATATGTG	AGATTATCTG	ATGAACACTT	
<i>PsUVaux</i>	TTTAAITTC	TGTGTTTTT	TTTTCTATGT	ATGTAAGTTT	TATCTTCTG	AATAATAATA	ATAACCTATA	TTCATTTCT	480
Prunus_arm	GCTGATTTAA	TCTGCTATAT	GTATC-ATGT	ATGTGTACAT	AGTGT--TG	TTCTTTGCA	AGTCGTCTC	TTTGGCATCT	
Arabidopsi	TAGTTGCAAG	TGGCGTTGG	ATCTTCACT	ATCT-TGTTG	GATTTTGAAG	ATGGTTGATG	CTTTTCTTT	AATGTTAAT	
F.ananassa	-GCTACTACA	CTACTTTTAC	CTTGCCATAT	ATGA-TATAT	GGTTGATTGG	TCTTGTGTTA	AAGCCAGATT	TACCGTGGTT	
Elaeagnus	-GTAAGTAAT	GTTTTTTTTA	TTTGTGTTAA	AAAA-AAAAA	AAAAAAA	-----	-----	-----	
Pisum_sati	-AAA-----	-----	-----	-----	-----	-----	-----	-----	
Golden_del	-GTGCGTTGA	TGATTGTTGG	GTGAGATCTA	CCGT-TGGAT	GGTTGTGAGG	AGTTGCTGTC	GTGTT---TT	TGCTATAATC	
<i>PsUVaux</i>	AAAAAAGAA	AAA	Id(%)						
Prunus_arm	GAATGAATAA	AAA	49						
Arabidopsi	AAATAAGCAT	ACT	31						
F.ananassa	TAGGGTGATA	ATC	34						
Elaeagnus	-----	---	26						
Pisum_sati	-----	---	27						
Golden_del	AAATAAATAA	ATA	33						

Figure 19

b

<i>psUVaux</i>	QSG---SPPASPAG----SVTPVSPFS--GTRESLRFRRRSASDAFEKKNQD-----RSSSSSPFDV	Id(%)
<i>Prunus_arm</i>	GSGNSGSPPISPAG----STPPVSPFS--GGSSMGRFRRRSASDAYEKASQVGGGGARSSPRSPFDV	62
<i>Fragaria_a</i>	PVTP--TTPISAR----KDNVWRSVFHPGSNLSSKTMGNQVFESF-QPNSPTVYDWMYSGETRSKH	15
<i>Elaeagnus</i>	SVTP--ATPSTQQ---LLDQRVRKTYGGVCSTQVATLPLVALGLRCLTNHHN-----PTSDCL---	8
<i>Arabidopsi</i>	PTTP--TTPTPR----KDNVWRSVENPGSNLATRAIGSNI FDKPTHPNSPSVYDWLYSGDSRSQH	17
<i>Pisum_sati</i>	PVTP--TTPSSAR---KVDNVWRSVENPGSNSATKSIGAHVFDPK-LPNTPTVYDWMYSGDTRSKH	15
<i>Golden_del</i>	PGTP--GTPGTPASARAKDNVWRSVFHPGSNLATKSMGNQVFDPK-QPNSPTVYDWLYSGETRSIH	16

Figure 19 (continued)

protein (Fig. 19b). However, the sequence similarities of this clone with other auxin-repressed proteins were rather poor; 26-34% at the nucleotide level (Fig. 19a) and 8-17% at the amino acid level (Fig. 19b). The *psUVaux* gene encoded a 1 kb mRNA, and UV-B irradiation increased the amount of *psUVaux* mRNA by 13% (Fig. 15.3; Table 7).

In addition to the *Prunus armeniaca* auxin-repressed peptide, blastx search revealed that a peptide deduced from the complementary strand of the *psUVaux* nucleotide sequence shown in Fig. 19 shared 25-28% identity with fragments containing 60-108 amino acid residues of telomerase protein-1 of *Rattus norvegicus*, *Mus musculus*, and *Homo sapiens* (data not shown). Furthermore, the poly-A signal, AATAAA, was present in this complementary strand but not the one listed in Fig. 19. Therefore, the actual sense strand of this clone was uncertain. To determine the sense strands of *psUVaux* and another clone, *psUVzinc*, that will be described later and whose sense strand was also uncertain, asymmetrical 3' RACE PCR was used to generate single-stranded probes. The single-stranded probes were poorly stained with ethidium bromide and they migrated slower than double-stranded probes in 1% agarose gel (Fig. 20). Only one of the two single-stranded probes derived from each clone detected transcripts on northern blots (Fig. 21). Probes from the clones, *psUVGlu* and *psUVRub*, served as controls because of the certainty of their sense strands. The probes complementary but not the ones identical to their mRNAs detected the messengers (Fig. 21a, b), which confirmed the reliability of the single-stranded probes. Similarly, probes generated from *psUVaux* and *psUVzinc* by PCR with excessive amounts of the adapter primer

Figure 20. Verification of single-stranded probes generated by asymmetrical PCR

Asymmetrical PCR was performed as described in Materials and Methods. Clones containing the cDNA inserts of *psUVaux* (lanes 1, 2), *psUVRub* (lanes 3, 4), *psUVGlu* (lanes 5, 6), *psUVdeh* (lanes 7, 8), *psUVRib* (lanes 10, 11) and *psUVzinc* (lanes 9, 12 and 13) were used as templates. The primer ratios used were either the same as defined in Materials and Methods (lane 9), 1:10 bZIP:adapter (lanes 1, 3, 10), 1:10 adapter:bZIP (lanes 2, 4, 11), 1:10 zinc finger:adapter (lanes 5, 7, 12) or 1:10 adapter:zinc finger (lanes 6, 8, 13). Biotin-14-dATP was included in the PCR reaction mixture. The products were either directly viewed by ethidium bromide staining of the gel (a), or blotted onto an N⁺ nylon membrane and detected by chemiluminescence (b) as described in Materials and Methods.

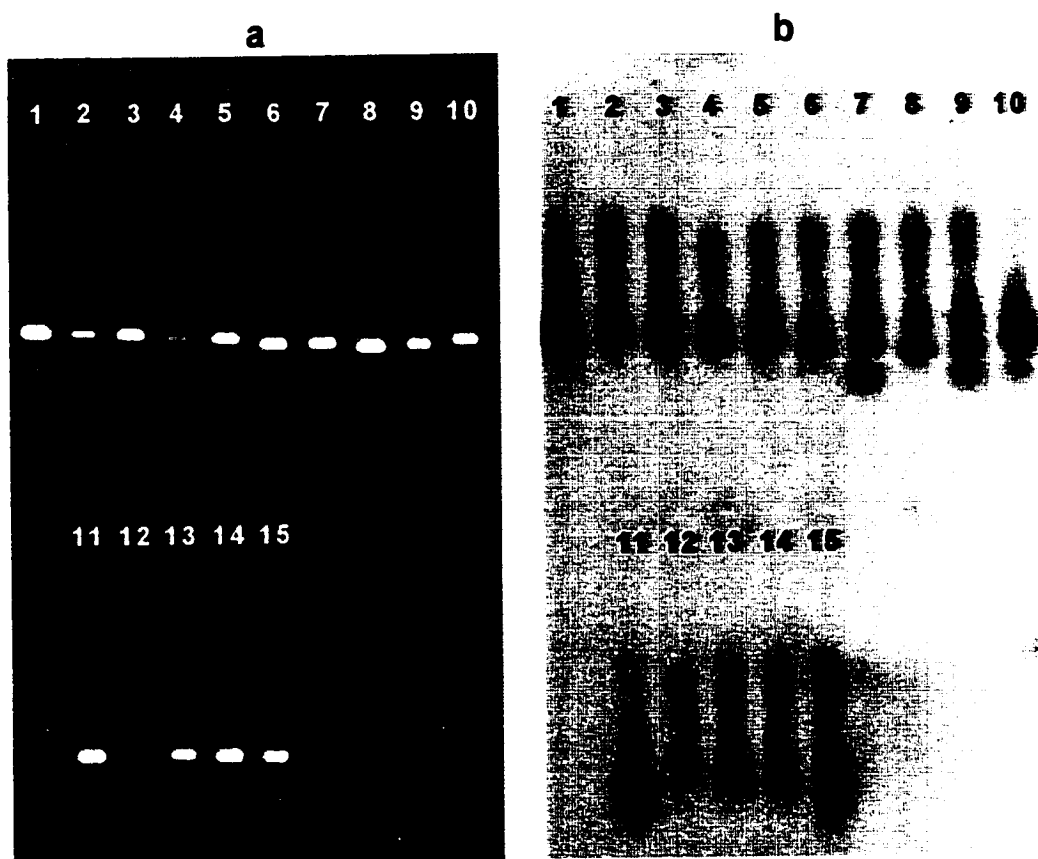


Figure 20

Figure 21. Determination of sense and anti-sense strands

Northern blots identical to those used in Figures 15 and 16 were hybridized to the probes prepared by asymmetrical PCR. The probes were not denatured by boiling before adding into hybridization buffer. a, *psUVGlu*; b, *psUVRub*; c, *psUVaux*; d, *psUVzinc*; 1, probe prepared with an excessive amount of adapter primer; 2, probe prepared with an excessive amount of degenerate primer (refer to the legend to Figure 20 for details). Other symbols are the same as defined in the legend of Figure 15.

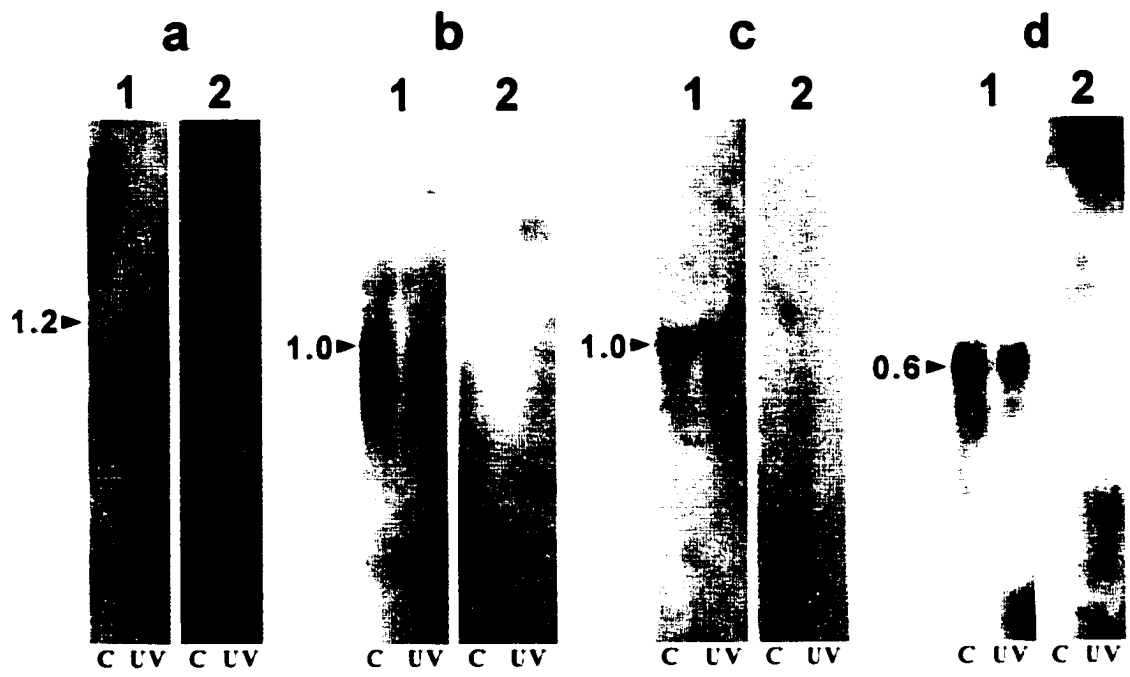


Figure 21

detected the mRNAs on northern blots (Fig. 21c, d), confirming that the sequences presented in blue are the sense strands of *psUVaux* (Fig. 19a) and *psUVzinc* (Fig. 24).

3.5.3 UV-B-repressible genes:

Clone *psUVRub*, 447 bp in size, obtained from the bzip primer-amplified PCR products, shared 27-57% identity with the 3' portions of Rubisco activase genes from several plant species (Fig. 22a). A 44 amino acid residue peptide deduced from the sense strand of the clone exhibited 50-90% identity with peptides of the genes under comparison (Fig. 22b). The 160 nucleotides of the 5'-translated region of *psUVRub* were nearly identical to the homologues (Fig. 22a), but the similarity of its 3' non-translated region with these genes was lower. The *psUVRub* mRNA on northern blots was always a smear, ranging from 0.35-1.0 kb, which was reduced 58% by UV-B (Fig. 16.1; Table 8).

Clone *psUVDeh*, obtained from the zinc primer-amplified PCR products, was 398 bp in length and highly related to the pea dehydrin cognate gene B6 in both nucleotide (Fig. 23a) and peptide sequence (Fig. 23b), sharing 86% and 62% identity, respectively. Except for the first 60 nucleotides at the 5' end and several nucleotides on the 3' end, the clone was identical to the pea dehydrin cognate gene, but it showed only 29-47% identity with other dehydrin genes (Fig. 23a). The peptide deduced from this clone consisted of 105 amino acid residues with 65 internal residues identical to those in pea dehydrin cognate. Except for a conserved hydrophilic amino acid-enriched, lysine-rich, cysteine- and tryptophan-free motif **KIKEKLPG** and its flanking regions near the C-terminus, the peptide differed

Figure 22. Sequence analysis of *psUVRub* and its deduced peptide

Nucleotide (a) and amino acid sequence (b) comparisons of *psUVRub* to Rubisco activase from *Phaseolus vulgaris* (Phaseolus, AF041068), *M. domestica* (*M. domestica*, Z21794), *Lycopersicon pennellii* (*Lycopersic*, AF037361), *C. sativus* (X67674), *Nicotiana tabacum* (*Nicotiana*, U35111), and Spinach (Spinach_ru, J03610). Each blue letter represents a nucleotide of the clone or amino acid residue of its deduced peptide. The green letters are the nucleotides or amino acids that are identical to those in all the sequences under comparison. The red letters represent the nucleotide or amino acid residues that are identical to those in the *psUVRub* clone or its deduced peptide. The black letters represent the nucleotide or amino acid residues that are divergent from those in the *psUVRub* clone or its deduced peptide. The first and stop codons of the deduced peptide are indicated as bold italic and bold letters, respectively. -, gap introduced for maximum alignment; identities (Id) of a sequence compared to those in *psUVRub* are shown at the end of each sequence.

a

<i>PsUVRub</i>	AAGATGACAC	TAGAAAAGCT	GTTGGAATAT	GGTAACATGC	TTGTCCAAGA	ACAAGAGAAT	GTGAAGAGAG	TCCAATTGGC	80
Phaseolus	AAGATGACTC	TGGACAAGCT	CTTGGCTGTAT	GCTAGCATGC	TTGTCCAAGA	ACAAGAGAAT	GTGAAGAGAG	TCCAATTGGC	
M.domestic	AAGATGACTA	TGGA AAAAGCT	CCTCGAGTAC	GGAAACATGC	TTGTCCAAGA	GCAAGAGAAT	GTGAAGAGAG	TCCAATTGGC	
Lycopersic	AAAAATGACCC	TTGAGAAGCT	CCTTGAGTAT	GGTAACATGC	TTGTCCAAGA	GCAAGAGAAT	GTCAAGAGAG	TCCAATTGGC	
C.sativus	GAAACTTCTC	ATGAAATCCC	ACTTGGAAAT	GCAAAATGAGG	ATGCTGTTC	GTCAAAAAC	TCTTATGAA	TTGCTGTCA	
Nicotiana	AAGATGACCA	TTGAGAAGCT	CCTCGAGTAC	GGTAATATGC	TTGTCCAAGA	GCAAGAAAAT	GTGAAGAGAG	TTCAAGTGGC	
Spinach_ru	GAAATGACCT	TACAAAAGTT	GATGGAGTAC	GGAAACATGC	TTGTCCAAGA	GCAAGAGAAT	GTCAAGAGAG	TCCAACTTGC	
<i>PsUVRub</i>	AGACAAGTAT	TTGAGTGAGG	CTGCTCTTGG	TGATGCTAAT	GAAGATGCTA	TCAAAAAGGGG	AACCTTCTCA	A---ATGCAG	160
Phaseolus	TGACCAGTAC	TTGAATGAGG	CTGCTCTTGG	AAATGCTCAAC	GAGGATGCTA	TCAAGAGTGG	ATCTTCTTTC	---A ATAG	
M.domestic	TGATAAGTAC	TTGAGCGAGG	CTGCTCTTGG	TGATGCTAAC	TCAGATGCTA	TGAATACAGG	AACCTTCTAT	G---GT-TAG	
Lycopersic	TGAAAACCTAT	CTTAAGAGG	CAGCTCTCGG	AGATGCTAAC	GCTGATGCTA	TCAACACTGG	AAATTTCTAG	AACCTCACCA	
C.sativus	AGATGCAACT	GAGGATGTTG	TTCACTCGGA	AATTTCTGAA	GAACCGTTC	TTATGGGTTC	AAATGGGAT	G---TCGTTCA	
Nicotiana	TGACAAATAC	CTCAAGAGG	CTGCACTTGG	TGATGCTCAAT	GCTGATGCTA	TAAACAATGG	ATCTTCTTTT	GCTAGTTAGT	
Spinach_ru	TGACCAGTAC	ATGAGCTCGG	CTGCACTTGG	TGATGCTCAAC	AAAGATGCTA	TTGACAGAGG	AACCTTCTTC	GGCAA GCAG	
<i>PsUVRub</i>	ACTC-TTAAA	---CAAGAGG	CCTTTGTTGG	AGATATTGCT	-----	----ATGTTT	GTGTTCTTGA	----TTCAAAA	240
Phaseolus	ATC--ATTGA	AGGCAAGCA	ACCCGACRAG	TAAATATTC	TGTCCTGTAT	GGCTGTGCTG	GTCCAAGTGC	TAG-CAACAG	
M.domestic	ATCC--TTGAT	ATTTAGAGAG	CTCCCTGTGG	AGGCCAAGCA	GCCGACGCA	GTGAATGTTT	CAGTTCTCTGA	AGG-TTGAC	
Lycopersic	ATC--TCAAA	AGTGGTCTAA	ACAATGAAGA	GGCGAAAATA	GCCGACAT-	GTCAACTTCC	AGG--AGTGA	TGA-TGTATT	
C.sativus	GTCAGGAAGT	TCTGAAGCTG	CTACTCTCGG	TGCAAAATGAG	GATGTTGTTG	TACAGTCCGG	AACCTTCTAT	GAATCAATGG	
Nicotiana	ATC---TTGCT	CGACTTAAAG	CTTAACAAG	AGGAAAAGAA	GCACAACAT-	TTTGTATCTC	TTGTTGCTGA	AGG-CTCTAC	
Spinach_ru	CTCAGCAAGT	AAGTTTGCCA	GTTGCTCRAG	GTTGTACAGA	CCCTGAGGCC	AAAAACTATG	ATCCAACCTGC	AAG-GAGTGA	
<i>PsUVRub</i>	AGCCTCA-A-	-----ACT	TTGATCCAAC	TACTTGAAGT	TGTGGTGGCA	CGTA-----	-ACATTCTCA	ATGTATCAAT	320
Phaseolus	TGTATTACAT	TCTTAGCATT	CTCAATGTGT	TGTGTGCAAA	GAGAAGATGC	TAAGTTTGAA	ATAATTTTAC	CATGTTCTT	
M.domestic	TGATCCA-AC	TGAGAAAAC	TTGATCCAAC	GGCTAGAAGT	GATGATGGA	GTTGCTTTTA	CACATTTTAA	GTCATTTTCT	
Lycopersic	TTATTT--CT	TCAACTTTTT	TTGAGGCAAT	TTGTGAGGGT	AGCTATAGAA	TGTGACT---	CAACTGTTTA	ATATTGTTAT	
C.sativus	GGGAGAGGA	ACGCAACAAG	TTAATATCAT	TGTTCTGTAA	GGCTGTGCG	ATTCATCTAC	TAAAACCTAT	GTCCCAACAG	
Nicotiana	TGATCAATT	CGAACRAAAC	TTGATCCAAC	TTCTATGGCT	GAAGATGGA	GTTATCTTTT	CAAAATTTAA	GGGCACCTTT	
Spinach_ru	TGATGGGAGC	TGCACGTACA	ATTTGTAGGT	CTTACTCAAT	TTGTGCAAC	TGATATGTAG	AAAAAGGGGA	ACAAGTTTTA	
<i>PsUVRub</i>	TT-----	-----	--GGTGAAG	GAAAAGATTC	NTNGTTEGAG	ATG-----A	TTTTATAATA	TTTTCTTTT	400
Phaseolus	ATTGGAC---	----TGTCTT	CTATAATTTT	GGTTACAGTT	TGCACTTAAT	G-A-----A	TTGGTGCCTT	TTTCTTTT-	
M.domestic	TTTAGGTTAT	AATTGAGGGG	AGGGAGGAAG	GAGAGAAGCA	CTTTTAAAGG	ATGTTAAGGG	TAAAAAATA	ATAATGTTG	
Lycopersic	GTAATC---	----AGTTGT	GTTCCAAGT	TAAGGGAATA	CRAACTCCAA	ATA-----A	TCTTTGTTT	TCAGTTTGT	
C.sativus	CCAGATTGGA	CGACAAAAGC	TCCAGCATT	TACTCTTAGG	AAATTTCTAC	A--CTTTTGC	TCCAGTAATG	TAAATTGTTA	
Nicotiana	GTGGG-----	----AGTAGC	TATTTGATAT	GACCAAGATT	GTTATTTAT	GAA-----A	TCAGTCTCTG	TTTGTAAAT-	
Spinach_ru	GTTAA-----	----TTTGG	CTCTCTTAAT	TAGAGGGATT	ATTATTCAC	TTC-----G	TACTCTTTC	CTATTTTT-	
<i>PsUVRub</i>	TGGAGTGTCT	CCCTTTGTAA	TTTTGTTATA	TG---TACA	ATGTTAAAT	TGATTTTGA	TCTTTCTC	TTTCTGCCA	480
Phaseolus	TTTCTATGT	CGGATTGGTG	TCATGTAATC	TTGTATTGTA	ATGTACACTA	TGCAGTAGGT	TCTCTCTTTC	TTTCTGCCG	
M.domestic	TCCATTATAT	TCTTCTGTAA	TCAACTTGT	GATT-TGGAA	CTTCAATATA	TTTGCTTTGT	-----	-----	
Lycopersic	---CTGTAAT	AAAACCTTCA	TTAAAAGGTA	TGATATCATT	AAACGATAAA	CAAATTTCT	AGTCATTAAC	AAT-----	
C.sativus	---ACAAGAT	TTGGTTG-AG	CTTTTGTATC	AGTT-TTGTA	ATGCTATGTA	ATCAATTTCT	ATTTTTG---	-----	
Nicotiana	---TTCTTTT	TTAATTTTGT	TCTGGTGGT	GAGT-TGTTT	GTCTGTAAAT	TTGTGTT--	-----	-----	
Spinach_ru	TTACACCCCTT	GATTTCTTTA	AAAAAAAAAA	AAAAAAAA	Id (%)				
Phaseolus	-----A	AAAAAAAAAA	AAAAAA--		42				
M.domestic	TCAGTTGTTT	TAGTTAGTTC	AGTTAGTCAT	GTTGTGCT	57				
Lycopersic	-----A	AAAAAAAAAA	AAAAAA--		48				
C.sativus	-----	-----	-----		27				
Nicotiana	-----A	AAAAAA--	-----		50				
Spinach_ru	-----	-----	-----		40				

b

<i>PsUVRub</i>	KLLEYGNMLVQEQENVKRVQLADKYLSEAALGDANEDA	IKRGT F	Id (%)
Phaseolus	KLLLYASMLVQEQENVKRVQLADQYLNEAALGNANEDA	IKSGS F	84
M.domestic	KLLEYGNMLVQEQENVKRVQLADKYLSEAALGDANSAMNITG	F	90
Lycopersic	KLLEYGNMLVQEQENVKRVQLAETYLKEAALGDANADA	INTGIS	84
C.sativus	KLLEYGNMLVMEQENVKRV	LVTS-----I-----	50
Nicotiana	KLLEYGNMLVQEQENVKRVQLADKYLKEAALGDANADA	INNGS F	88
Spinach_ru	KLMEYGNMLVQEQENVKRVQLADQYMSSAALGDANKDA	IDRGT F	84

Figure 22

Table 8. Characteristics of UV-B-repressible clones

Clone	Size of the clone (bp)	Related protein	Putative function	Length of peptide (amino acids)	Size of mRNA (kb)	Increase in mRNA reduced by UV-B (%)
<i>psUVRub</i>	447	Rubisco activase	Molecular chaperon	44	0.35 - 1.0	57.9
<i>psUVDeh</i>	398	Dehydrin	Dehydration resistance	114	0.8	46.2
<i>psUVzinc</i>	420	PsbX	Unknown	80	0.6	42.5

Figure 23. Sequence analysis of *psUVDeh* and its deduced peptide

Nucleotide (a) and amino acid (b) sequence comparisons of *psUVDeh* to those of the genes encoding pea dehydrin-cognate (*Pea_dhn-co*), *Phaseolus vulgaris* dehydrin (*Phaseolus*), pea dehydrin DHN3 (*Pea_mRNA_f*), pea dehydrin Dhn2 (*Pea_Dhn2_m*), *Lavatera thuringiaca* LTCOR18 (*Lavatera_t*), pea dehydrin DHN1 (*Pea_DHN1*), and *A. thaliana* cor47 (*A.thaliana*). Each blue letter represents a nucleotide of the clone or amino acid residue of its deduced peptide. The green letters are the nucleotides or amino acids that are identical to those in all the sequences under comparison. The red letters represent the nucleotide or amino acid residues that are identical to those in the *psUVDeh* clone or its deduced peptide. The black letters represent the nucleotide or amino acid residues that are divergent from those in the *psUVDeh* clone or its deduced peptide. The first and stop codons of the deduced peptide are indicated as bold italic and bold letters, respectively. -, gap introduced for maximum alignment; identities (Id) of a sequence compared to those in *psUVDeh* are shown at the end of each sequence.

a

<i>psUVDeh</i>	-CGAATGCTT AAGCTCTGGG TGGGCACCTA ATGCTTGAAG CTTTGGG-AG GGCACAAGAA AA-----CA GA-----GGAAG 80
<i>Pea_dhn-co</i>	-AGGAAGAGA AGGGTTTCCT TGACAAAATT AAGGAGAAGC TTCCTGG-AG GACACAAGAA AA-----CA GA-----GGAAG
<i>Phaseolus</i>	GAGAAAGTTG AGGTTTGGG AAGTGAGGAA AAGAAGGGGT TCCTGGG-AG AGATTAAGGA GAAGCTACCA GG-----GCACA
<i>Pea_mRNA_f</i>	GGAGATAAGA AAGGGTTTAT GGACAAGATT AAGGAAAAGA TTCCTGGTAC GGAACAATCA AGAACCAATA CTGATGGAAC
<i>Pea_Dhn2_m</i>	GGAGATAAGA AAGGGTTTAT GGACAAGATT AAGGAAAAGA TTCCTGGTAC GGAACAATCA AGAACCAATA CTGATGGAAC
<i>Lavatera_t</i>	TGTGAAAAGA AGGGTGTAT GGAGAAGATC AAGAGAAAC TGCTGG-AC RACATAAGAA AG-----AT GA-----AGAGG
<i>Pea_DHN1</i>	GGAGATAAGA AAGGGTTTAT GGACAAGATT AAGGAAAAGA TTCCTGGTAC GGAACAATCA AGAACCAATA CCGATGGAGC
<i>A.thaliana</i>	GAAGAGAGA AAGGATTATG TGAGAAGATC AAGGAGAAGC TTCCTGG-TC ACCACGACGA GAAAGCAGAG GATTCCACCAG
<i>psUVDeh</i>	TAACAACCTCC TCCACCAGTT GTCGCTGC-T CATGTTCCAA CTG-AGACAA CAGCTACAAC AACAACTATT CATGAGGGAG 160
<i>Pea_dhn-co</i>	TAACAACCTCC TCCACCAGTT GTCGCTGC-T CATGTTCCAA CTG-AGACAA CAGCTACAAC A-----ACTATT CATGAGGGAG
<i>Phaseolus</i>	AGAAGAGTGA GAGGGCGGCA GCTCCTCC-T CCGCACCCAC CTG-CTGCCA CCTCAATCGGA ACATGAAGGA GAGGGAAGG
<i>Pea_mRNA_f</i>	AGGGTATGGA TCAACTGGTC AAGGGTATGT GAGAGAGCAG CAGCATGGAA CAGGGTATGG ATCAACTGGT CAAGGGTATG
<i>Pea_Dhn2_m</i>	AGGGTATGGA TCAACTGGTC ATGG----- -AGCTAGTGG TGG--TGGAA T-----TGG GAACACTGGT CAAGAATATG
<i>Lavatera_t</i>	TTGTCAACGC ACCGCCACCA CCTGCTCC-T GTGTCAAGTG AGA-GCATTG TGGTTAGCA T-----TGG ACATGAAGGA GAGTCCGAGG
<i>Pea_DHN1</i>	AGGGTATGGA TCAACTGGTC ACCG----- -AGCTAGTGG TGG--TGGAA T-----TGG AACACCGGT CAAGAATATG
<i>A.thaliana</i>	CTGTCACTC CACGCGTGT GTTGTAC-G GAGCATCCCG TGG-AGCCTA CCGCAGGACT TCCAGTGGAA CATCCGAGG
<i>psUVDeh</i>	AGA-AGAAAG GTATTTTGA AAGATCAAA GAGAAGCTTC CTGGTTATCA TGCTAAGACT GCTACTGATG GTGAAGACAA 240
<i>Pea_dhn-co</i>	AGA-AGAAAG GTATTTTGA AAGATCAAA GAGAAGCTTC CTGGTTATCA TGCTAAGACT GCTACTGATG GTGAAGACAA
<i>Phaseolus</i>	AGA-AGAAAG GTATTTTGA AAGATCAAA GAGAAGCTTC CTGGTTATCA TGCTAAGACT GCTACTGATG GTGAAGACAA
<i>Pea_mRNA_f</i>	TGAGAGAGCA GCAGGATGTT CATCATGGAG ATGAGCAACA TGGA-GAGAA GAAAGGATT ATGGAGAAGA TTAAGGAGAA
<i>Pea_Dhn2_m</i>	TGAGAGAGGA GCGTCACTTT CATCCTGGAG ATAGAAAACA TGGAAGCGCT GGTCAAGAGT ATGTGAGAGA GAGCATCAT
<i>Lavatera_t</i>	AGA-AGAAAG GTATTTTGA AAGATCAAA GAGAAGCTTC CTGGTTATCA TGCTAAGACT GCTACTGATG GTGAAGACAA
<i>Pea_DHN1</i>	TGAGAGAGGA GCATCGTGT GATCATGGA- ----- -GAGAA GAAAGGATT ATGGATAAGA TTAAGGAGAA
<i>A.thaliana</i>	AGA-AGAAAG GTATTTTGA AAGATCAAA GAGAAGCTTC CTGGTTATCA TGCTAAGACT GCTACTGATG GTGAAGACAA
<i>psUVDeh</i>	A----- GATCATCACA AGGATGCAGA CT-ACCTCTC ATTGAATATG ATCGATTGGT TGTGTTGGTT TGTGGCTTTG 320
<i>Pea_dhn-co</i>	A----- GATCATCACA AGGATGCAGA CT-ACCTCTC ATTGAATATG ATCGATTGGT TGTGTTGGTT TGTGGCTTTG
<i>Phaseolus</i>	GAG----- TGTGGTCACT GAGATTGAGA GA-AGGGTTG TGTGTTGGGG TTTGGTTGGA GATGATTGTG GTGTGCTTTG
<i>Pea_mRNA_f</i>	GCT---TCCT GGTACTGGAG CATGTACTGG ----ACACTA GATCATCATC ATGATTATAA TCTATAGATA TATGCATATG
<i>Pea_Dhn2_m</i>	GTTCA-TCCT GGAGATAAGA AACATGGAAG CGCTGGTCRA GAGTATGTGA AAGAGGAGCG TCGTGGCATT GGGAACACTG
<i>Lavatera_t</i>	GACCAGTACT GCTGCTAAC ATTAAAAAAT CA-ATATCAT GTTTTTGAAG TAAAATAAAA TAAATGCTTG TGGGATTGTG
<i>Pea_DHN1</i>	GCT---TCCT GGTACTGGAG GATGTACTGG ----ACACTA GATCATCATC ATGATTATAA TCTATAGATA TATGCATATG
<i>A.thaliana</i>	AGAAAAGAG TCTGATGATT AAGCAA-AAT GG-TTAAAGA ATGAATAATG ATGTGGGAGT GGGACATTCG CTGTGTTTTG
<i>psUVDeh</i>	-GGGGTGTTT TGTTTAAT- --AATTACTA TCATTAAAGC CTTGTGTGGT GATTTCTTTC TTGAATTAAT GGTATATGTA 400
<i>Pea_dhn-co</i>	-GGGGTGTTT TGTTTAAT- --AATTACTA TCATTAAAGC CTTGTGTGGT GATTTCTTTC TTGAATTAAT GGTATATGTA
<i>Phaseolus</i>	-TTTTCAATTT CATCATATC- --AAACACTT GTGGGCTTTC TTATCTTTTGT TTACTCTTTC TCATCATATC TCTATTTTTT
<i>Pea_mRNA_f</i>	ATCAATAATA AATAAACTTA GAGTAATTTG TTTGGTGTGG TGTATTTTAT AATCTCATGT GAGGGATATA ATAAATGATA
<i>Pea_Dhn2_m</i>	GTCAGAGTA TGTGAGAGAG GAGCATCGTG TTGATCATGG AGAGAGAGAA GGGATTATGG ACAAGATTAA GAGAGAGCTT
<i>Lavatera_t</i>	-TGGTTTTATT TATTTGCTGG G-AGTGTCTG TAACTTTTGT TAACTTTTTC TTACTATATC ATAGTATAG TTTGTGTTTT
<i>Pea_DHN1</i>	ATCAATAATA AATAAACT- ----- TTTG TTTGAAAAA AAAAAAATAA AAAAAAATAA AAAAA-----
<i>A.thaliana</i>	-TGATCATT TCTTTCTCT --TTAAGTTG TTATTGTGGC TTTGTTGAT TGCAAT-TTGA TCCTTTATT TGTAAATTTCA
<i>psUVDeh</i>	TTTGGTTCTT AAAAAAAAA AAAAAA Id (%)
<i>Pea_dhn-co</i>	TTTGGTTCTT AACTTGTCTT TGTGGG 86
<i>Phaseolus</i>	TATTATTATT ATTGTGATG TATCAT 47
<i>Pea_mRNA_f</i>	TAGATAAAA TAATGCTGT TTTAAA 35
<i>Pea_Dhn2_m</i>	CCTGGTACTG GAGGATGTAC TGGACA 32
<i>Lavatera_t</i>	TCCCTTGCTT CGTAAGTA-A TAAAAC 45
<i>Pea_DHN1</i>	----- 29
<i>A.thaliana</i>	TTCTATCTT TATATAAGT TTGCAT 45

b

<i>psUVDeh</i>	MLEALGGHKKTEEVTPPPV-----VAHVPTETATTT--IHEGKKGILEKIKELPGYH-AKTATDGEDKDHKKDADYF
<i>Pea_dhn-co</i>	KEKLPFGHKKTEEVTPPPV-----VAHVPTETATTT--IHEGKKGILEKIKELPGYH-AKTATDGEDKDHKKDETTS
<i>Phaseolus</i>	EKELEGYHKED-----TSVPVEKVVES-----EKKKFLKIKELPGYH-KSEEAAPP P P P P P P AATSSSE
<i>Pea_mRNA_f</i>	GTGYGSGTNPDYGSTNTSGYGTGYGSGTTEYVREE--HHGDKRGMVKIKELIPGTEQSRINTDGTGYGSTGQGYVR
<i>Pea_Dhn2_m</i>	GTGYGSS-----GTTEYVREE--HHGDKRGMVKIKELIPGTEQSRINTDGTGYGSTGQGYVR
<i>Lavatera_t</i>	KEKLPQGHKKDEEVVTPPP-----PPAAVSSSESVVEHHE-EESKEKGMEXIKELPGYH-SKTEDEKKEKSTAAANH--
<i>Pea_DHN1</i>	GTGYGSS-----GTNEVRED--HHGDKRGMVKIKELIPGTEQSRINTDGTGYGSTGQGYVR
<i>A.thaliana</i>	KEKLPGHDKTAEDDVP-----VSTTTPVVSSESVVEHDHPEEKKGLVEMIKELPGYH-DEKAEDSPAVTSTPLVVTE
<i>psUVDeh</i>	SLNMIW-----LCWFVALGVFLINYHH Id (%)
<i>Pea_dhn-co</i>	H----- 62
<i>Phaseolus</i>	HEGEAK-----E---KKGILEKI 20
<i>Pea_mRNA_f</i>	E-----QQHG-----TGYGSTGQGYVREQDQVHH 22
<i>Pea_Dhn2_m</i>	GGIGNTGQGYVREERHVPDGGKHHGSAQGYVREHHVHPGDKKHGSAGQGYVKEERRIGNTGQGYVREERVDH 21
<i>Lavatera_t</i>	----- 31
<i>Pea_DHN1</i>	G-----IGNTGQGYVREERVDH 20
<i>A.thaliana</i>	HPVEPTT-----ELPVEHPEEKKGILEKI 26

Figure 23

markedly from other dehydrins found in pea and other species, showing 20-31% identity. In addition, both of the *psUVDeh* and pea dehydrin cognate peptides contained a stretch of residues at the N-terminal side to the KIKEKLPG motif that is missing from other pea dehydrins (Fig. 23b). A 0.8 kb mRNA was detected by the probes of this clone, and the UV-B exposure reduced the amount of this messenger by 46.2% (Fig. 16.2; Table 8).

A clone designated *psUVzinc* and containing a 420 bp cDNA fragment was obtained by using the zinc finger degenerate primer (Fig. 24). No homologue of this clone was found in the GenBank database. No poly(A) signal was found in either strand of this clone. Northern blots hybridized to the single-stranded probes demonstrated that the sequence with a poly(A) tail was the sense strand (Fig. 21d). Thirty-eight out of the 80 amino acid residues of the longest peptide deduced from its sense strand showed 45% identity to *Odontella sinensis* photosystem II reaction center X protein (Fig. 24). The *psUVzinc* transcript was 0.6 kb in size, and was reduced by 43% upon 3 h of UV-B irradiation (Fig. 16.3; Table 8).

Figure 24. Sequence analysis of *psUVzinc* and its deduced peptides

The letters in red and black represent amino acid residues of the *Odontella sinensis* photosystem II reaction center X protein retrieved from GenBank. Blue letter, nucleotide of the clone; pink, turquoise or green letter, amino acid residue of the peptide deduced starting from either the first, the second or the third nucleotide; red letter, amino acid residue identical to that in the peptide deduced from *psUVzinc*; black letter, amino acid residue different from that in the peptide deduced from *psUVzinc*. The stop codons of the deduced peptide are indicated as bold letters. -, gap introduced for maximum alignment.

TCAAAGTCTA **ACGGAAGGTT** **TCAAGTGAAA** **GCGTCGCTGA** **AGGAGAAAGT** **TGTGACAGGG** 60
 S K S N G R F Q V K A S L K E K V V T G
 Q S L T E G F K
 K V

CTGACTGCAG **CTGCACTGAC** **AGCTCAAATG** **GTGATTCCTG** **ATGTGGCTGA** **AGCTGCTACC** 120
 L T A A A L T A Q M V I P D V A E A A T

GTTTCACCTT **CTTTGAAGAA** **TTTCTTGCTC** **AGTATTGTTT** **CTGGTGGAGT** **TGTGGTTACG** 180
 V S P S L K N F L L S I V S G G V V V T
 M T P S L A N F I S S L T A G G L V V L

GCCATTCTTG **GTGCTGTTAT** **CGGTGTTTCC** **AATTTGACC** **CTGTCAAGCG** **AGGCTCCCAA** 240
 A I L G A V I G V S N F D P V K R G S Q
 T I A V A L I V I S R T D R V T R F

TGAATCTGAT **TCTCAGGCC** **AACCGAGGGT** **ATTTTAGTAT** **TTCTCTCATT** **GTTATATTTA** 300

TCTGTATAAT **GTATTCAGAA** **ATGAACTCTG** **TGTTGTGTGT** **AATATCATAA** **TATGTGACTC** 360
TTGATCTCCT **CTTGAGCATT** **AGTTTTCTTA** **TTCTTAAAAA** **AAAAAAAAAA** **AAAAAAAAAA** 420

Figure 24

IV. Discussion

4.1 Pea *lhcb* genes have similar UV-B response mechanisms

The amount of *lhcb* mRNA in pea, all of which is produced in the nucleus, is dramatically reduced upon UV-B irradiation, responding much more rapidly than the chloroplast-encoded *psbA* transcript (Jordan et al., 1991). UV-B modulates the amount of *lhcb* mRNA by affecting transcription of the genes, rather than through post-transcriptional mechanisms (Mackerness, personal communication). This response is developmentally regulated (Jordan et al., 1994), with the amount of *lhcb* mRNA decreasing in green leaves but increasing in etiolated buds following UV-B exposure. Due to the sequence similarity of the seven *lhcb* genes, northern blotting used in previous work (Jordan et al., 1991; 1994) did not distinguish the transcripts from each gene. To reveal the contribution of single genes in this developmentally regulated response, the amount of mRNA for the seven *lhcb* genes was individually determined in this study by use of RT-PCR. UV-B irradiation decreased the amount of mRNA for each of the seven pea *lhcb* genes in green leaves, but increased these transcripts in etiolated buds. It appears, therefore, that UV-B modulates transcription of all the seven genes via stimulative mechanisms in etiolated buds, but through repressive pathways in green leaves.

4.2 UV-B- and red light-responsive pathways of pea *lhcb* genes may be linked

Based on sensitivity to a red light pulse, the pea *lhcb* genes fall into two groups. The mRNAs for *lhcb1*2*, *lhcb1*3* and *lhcb1*5* are barely detectable after a red light pulse, while the transcripts for *lhcb1*1*, *lhcb1*4*, *lhcb2*1* and *lhcb3*1*

accumulate (White et al., 1992). These two groups of genes also differed in response to UV-B. In etiolated buds, the *lhcb* genes insensitive to red light were slower to respond to UV-B than those that were sensitive, but they reacted more quickly in green leaves. Thus, the two subfamilies of genes may respond to common pathways differentially, or each subfamily is regulated separately through different pathways that may have similarities. Additionally, the similarity between UV-B and red light in classification of pea *lhcb* genes suggests that the UV-B- and red light-responsive pathways regulating the genes are linked, possibly via phytochromes.

4.3 Does UV-B repress *lhcb* mRNA accumulation under red light by damaging a chloroplast component of the red light pathways?

As a specific inhibitor of phytoene desaturase, the rate-limiting enzyme of carotenoid biosynthesis, norflurazon has been used widely to obtain carotenoid-deficient plants for studying the relationship between nuclear and chloroplast genomes. Therefore, it was employed in this study to increase the sensitivity of chloroplasts to photo-damage in order to determine the role of chloroplast photo-damage in UV-B-induced regulation of *lhcb* genes. In the first trials, pea seedlings were grown in potting soil with several concentrations of norflurazon, none of which always inhibited carotenoid biosynthesis to the same extent (data not shown). However, when potting soil was substituted with vermiculite, carotenoid synthesis was consistently suppressed by 100 μM norflurazon without significantly influencing the levels of chlorophylls a and b. Under continuous dim red light, these carotenoid-free plants resembled controls that

were grown in the absence of norflurazon until the 7th day, except for the absence of carotenoids and increase in UV-B-absorbing pigments. The increase in UV-B absorption by pea leaves is probably due to phytoene accumulation (Rabourn et al., 1953). As demonstrated previously (Taylor, 1989; Demming-Adams and Adams, 1992), the carotenoid-free plants were extremely sensitive to photo-oxidative stress, which was evident in the reduction of transcripts from the nuclear encoded *lhcb* and chloroplast-encoded *psbA* genes, as well as loss of chlorophyll following a 3 h exposure to white light.

Of the pea *lhcb* multiple genes, *lhcb1*2* and *lhcb1*4* typify two extremes, with *lhcb1*4* transcribed at a high level and *lhcb1*2* at a low level upon either red or blue light exposure (White et al., 1992). Transcription of *lhcb1*4* occurs in both etiolated buds and green leaves (White et al., 1992), but accumulation of *lhcb1*2* transcript does not begin until de-etiolation takes place to some extent (Argüello et al., 1992; White et al., 1992). In addition, transcription of *lhcb1*4* is much more sensitive to red and blue light induction than *lhcb1*2* (White et al., 1995). In this study, it was found that removal of carotenoids differentially affected *lhcb* mRNA accumulation under dim red light, with *lhcb1*4* mRNA slightly reduced while *lhcb1*2* transcript was increased. Therefore, the signal transduction pathways regulating *lhcb1*2* and *lhcb1*4* are likely to be different from one another. However, after the carotenoid-free chloroplasts were partially photobleached by white light, mRNA accumulation under dim red light for both *lhcb1*2* and *lhcb1*4* was reduced to the same extent, indicating that both genes share a chloroplast component that is essential for red light induction of *lhcb* genes (Blackbourn, 1997; Bolle et al., 1994;

Taylor, 1989). In support of this idea, individual members of the tobacco *lhca/b* multigene family have similar sensitivities to chloroplast photodamage (Bolle et al., 1994), although *lhca/b* gene expression is more sensitive than the nuclear genes encoding other chloroplast proteins (Bolle et al., 1994; Sagar et al., 1988). In contrast, UV-B reduced mRNA accumulation of *lhcb1*4* and *lhcb1*2* differentially under dim red light in carotenoid-free pea plants. These data, thus, suggest that UV-B represses *lhcb* mRNA accumulation under red light through a factor other than the photobleaching-sensitive chloroplast component of the red light pathway shared by *lhcb1*4* and *lhcb1*2*. In support of this notion, Greenberg et al. (1989) found spectral evidence demonstrating that visible light and UV-B induce degradation of the D1 photosystem II reaction center protein via different photosensitizers located in chloroplasts, with photosynthetic pigments mediating visible light and plastoquinone mediating UV-B.

4.4 UV-B protects the chloroplast component mediating red light induction of *lhcb* genes from photodamage

Synergistic inhibition of photosystem II activity by UV-B and photo-oxidative white light has been documented, and the basis for this synergism is that the same molecule, such as D1, one of the reaction center proteins, is damaged by both wavebands (Friso et al., 1994). Therefore, it is likely that the repressive effects of UV-B and white light on *lhcb* genes in carotenoid-free plants are synergistic, or at least additive, if they down-regulate the genes by damaging the same chloroplast component. However, UV-B exposure prevented reduction of *lhcb* mRNA induced by subsequent white light exposure in carotenoid-free plants. Although an increase

in transmittance of blue light into plant tissues resulting from carotenoid-deficiency may promote photoreactivation, the photorepair of UV-B-induced DNA damage, by itself, cannot explain why the amount of mRNA in UV-B-irradiated leaves was higher than in non-UV-B-irradiated leaves. In addition, by increasing photosynthesis rate with low pressure sodium light and CO₂, Mackerness et al. (1996) provide evidence that photoreactivation repair is not important in pea for protection of the nuclear genes encoding chloroplast proteins against UV-B. One explanation could be that prior UV-B exposure protects the red light-linked chloroplast component against white light-induced chloroplast photodamage. Therefore, this study argues against the possibility that UV-B down-regulates the *lhcb* genes by damaging the photobleaching-sensitive chloroplast component of the red light pathways.

4.5 Chloroplast integrity and maturation modulate UV-B effects on pea *lhcb* mRNA accumulation

UV-B induces photo-oxidative damage to chloroplasts (Malanga and Puntarulo, 1995; Takeuchi et al., 1995; Strid et al., 1996), while carotenoids function as antioxidants in photosystem II to prevent chloroplast damage by dissipating excessive light energy (Demming-Adams and Adams, 1992). Chloroplasts and *lhcb* transcription are, thus, thought to be prone to UV-B damage in carotenoid-free plants. Moreover, carotenoid-free plants lack light-harvesting complex II because carotenoids are components essential to the assembly of *lhcb* proteins into thylakoid membranes. Due to the absence of light-harvesting complex II, photosynthesis-dependent protection of *lhcb* gene expression against UV-B inhibition (Mackerness et al., 1996) may also be defective in carotenoid-free plants.

Therefore, damage to chloroplasts, by removing carotenoids and exposing the carotenoid-free plants to white light, is believed to make *lhcb* transcription more sensitive to UV-B repression. Surprisingly, as detected 5 h after UV-B exposure (4.2 MED h⁻¹), UV-B irradiation caused an increase rather than a reduction in *lhcb* transcripts when chloroplasts were damaged by norflurazon and photo-oxidative treatments. Therefore, the integrity of chloroplasts seems to influence whether UV-B stimulates or inhibits pea *lhcb* mRNA accumulation. In addition, hypocotyls of all the UV-B irradiated plants, both norflurazon-treated and non-treated, wilted 21 h after UV-B exposure (data not shown), suggesting that the mechanisms by which UV-B regulate *lhcb* mRNA accumulation and hypocotyl wilting are separated.

Jordan et al. (1994) first reported that UV-B exposure increases the amount of *lhcb* transcript in etiolated pea buds but reduces the same transcripts in green pea leaves. In a more detailed study, Mackerness et al. (1998) found that only if chlorophylls accumulated to some extent during de-etiolation did UV-B reduce the amount of *lhcb* mRNA; otherwise, exposure to UV-B increased *lhcb* transcripts. These coincident observations, and that UV-B stimulates *lhcb* transcript in the artificially generated chloroplast-deficient plants, suggest that maturation of chloroplasts is an important determinant for the effect of UV-B on *lhcb* mRNA accumulation. From results obtained by treating the *Arabidopsis* genomes uncoupled mutant, believed to uncouple nuclear and chloroplast communications, with norflurazon and UV-B, Jordan et al. (1998) proposed that chloroplasts are not involved in UV-B-induced regulation of *lhcb* genes. This result, unlike those obtained in this study, may reflect inter-species difference between *Arabidopsis* and

pea. Furthermore, in their experiments, carotenoid-deficient plants were generated by spraying seedlings with norflurazon, and although the inner leaves photobleached, the outer leaves remained green (Jordan et al., 1998), indicating uneven and incomplete inhibition of carotenoid biosynthesis. Therefore, these results cannot rule out the presence of chloroplast components synthesizing intercellular trafficking signals that mediate UV-B-induced regulation of *Arabidopsis lhcb* genes. It has also been established that once norflurazon supply ceases, carotenoid biosynthesis resumes rapidly (Markgraf and Oelmüller, 1991), suggesting that spraying norflurazon is not an appropriate approach for obtaining carotenoid-free plants. Finally, as discussed above, UV-B-induced repression of pea *lhcb* genes is not due to damage to the photobleaching-sensitive chloroplast component of the red light pathways, raising the uncertainty of whether or not the genes that are altered in the *Arabidopsis* genomes uncoupled mutant connect communications between chloroplast and nuclear genomes in response to UV-B.

4.6 UV-B-induced repression of both *lhcb1*4* and *lhcb1*2* is dependent on carotenoids

UV-B loses its capacity to reduce *lhcb1*2* and *lhcb1*4* mRNA when carotenoid biosynthesis was inhibited, indicating that carotenoids are necessary for UV-B-induced down-regulation of the responsible genes in green leaves. Neither the idea that carotenoids protect chloroplasts against UV-B, nor the notion that UV-B-induced *lhcb* transcript reduction results from chloroplast damage, is favoured by these observations. Additionally, the difference in UV-B response between *lhcb1*2* and *lhcb1*4* was not substantially influenced by

removing carotenoids. A carotenoid-related component, therefore, seems to equally mediate the UV-B responses of these two genes. Coincidentally, carotenoids content in soybean leaves is negatively correlated with photosynthesis efficiency in response to UV-B irradiation, which was, however, used to argue that carotenoids protect soybean photosynthesis against UV-B (Middleton and Teramura, 1993). In addition, it is unclear why the effects of carotenoids on UV-B responses of pea *lhcb* genes are contradictory to the observation that carotenoids increase the resistance of the fungus *Fusarium aquaeductuum* to UV-B (Rau et al., 1991).

In higher plants, carotenoids are synthesized by nuclear-encoded multienzyme complexes located in plastid envelopes, and they accumulate exclusively in plastid membranes (Norris et al., 1995). Carotenoid-free chloroplasts develop an ultrastructure typical of chloroplasts in plants grown under low intensity light and accumulate a normal complement of both nucleus- and plastid-encoded chloroplast proteins under nonphotobleaching light conditions such as dim red light (Taylor, 1989). All components in the chloroplasts of norflurazon-treated plants appear to be relatively unaffected, except for the absence of functional photosystem II because carotenoids are essential for integration of Lhcb proteins into thylakoid membranes (Taylor, 1989; Karapetyan et al., 1991; Markgraf and Oelmüller, 1991). Therefore, it is possible that the carotenoid-dependent UV-B repression of *lhcb1*2* and *lhcb1*4* was due either to carotenoids per se, or photosystem II. In addition, norflurazon reduces intracellular abscisic acid because carotenoids are its precursors (Taylor, 1996), and abscisic acid down-regulates

transcription of *Arabidopsis* and *Lemna gibba lhcb* genes (Weatherwax et al., 1996). Moreover, although norflurazon is believed to be a specific inhibitor of phytoene desaturase (Chamovitz et al., 1993), it may affect other intracellular enzymes such as fatty acid desaturase. Thus, it remains to be determined if the alteration in UV-B responses of *lhcb1*2* and *lhcb1*4* mRNA caused by carotenoid deficiency are due to changes in ABA biosynthesis, fatty acid saturation or other intracellular events.

4.7 A photobleaching-sensitive component mediates UV-B effects on *lhcb1*2* but not *lhcb1*4*

In the plants where chloroplasts were partially photobleached, the difference in UV-B response between *lhcb1*2* and *lhcb1*4* disappeared. This resulted mainly from the loss of UV-B's ability to reduce *lhcb1*2* mRNA, while the UV-B response of *lhcb1*4* was not substantially affected. Thus, an intracellular component that is sensitive to chloroplast photobleaching may mediate the effect of UV-B on *lhcb1*2* but not *lhcb1*4*. It is established that when carotenoid-deficient plants are partially photobleached, intracellular compartments and events remain fairly unaffected, with exception for the degradation of chlorophylls, chloroplast thylakoid and inner envelop membranes (Bolle et al., 1994; Taylor, 1989). Thus, the component that specifically mediates the UV-B effects on *lhcb1*2* is likely to be associated with either chlorophylls or components such as plastoquinone located in chloroplast membranes (Greenberg et al., 1989). Another phenomenon worthy of mention is that reduced UV-B (2.8 MED h⁻¹) did not cause an increase in mRNA in partially photobleached plants as seen in the experiments with the high intensity UV-B (4.2

MED h^{-1}). As an explanation, both UV-B-repressive and stimulative mechanisms may function in the partially photobleached chloroplasts, and under higher intensity UV-B the stimulative mechanism overwhelmed the repressive one. Alternatively, the UV-B-repressive mechanism responded to high but not low UV.

4.8 Application of 3' RACE PCR to identify UV-B-responsive genes

Identification of the putative chloroplast components mediating the UV-B response of *lhcb* genes is a challenge. Although a red light-responsive chloroplast component was proposed over ten years ago and indirect evidence for its existence has been obtained, it has not yet been identified. It is believed that any signal transduction pathways affecting transcription eventually involve interactions between transcription factors and cis-acting elements of target genes. Therefore, searching backwards along the UV-B-responsive pathways by identification of transcription factor genes may increase the possibility of identifying the putative chloroplast components. To initiate such studies, 3' RACE PCR was used as a general approach to obtain UV-B-responsive transcription factor genes. Degenerate primers were designed on the basis of conserved peptide residues of basic leucine zipper and C₂H₂ zinc finger transcription factors, two of the largest transcription factor families with light-responsive members yet identified in plants. Six clones obtained in these experiments hybridized to UV-B-responsive transcripts on northern blots. Of them, *psUVzinc* shows no homology with any sequence registered in GenBank. Therefore, it represents a novel UV-B-responsive gene. The other five UV-B-responsive clones had homologues in the GenBank, but none of them were transcription factor genes. Partial sequence complementation

between the cDNA and degenerate primers led to the cloning of these genes, suggesting that annealing temperature used in the PCR reaction should be higher, and that nested degenerate primers might be considered to increase the specificity of PCR amplification. In addition, identifying UV-B- and chloroplast signal-responsive *lhcb* cis-elements and then using them as probes to screen expression libraries would be a useful approach to clone transcription factor genes mediating the UV-B responses of *lhcb* genes.

4.9 Identification of UV-B-responsive genes reveals new features of UV-B-dependent regulation of gene expression

In addition to rRNAs, a ribosome is composed of many different proteins. The *psUVRib* cDNA clone identified in this study is closely related to the ribosomal protein S23 of strawberry, human, *Brugia malayi* and mouse, S28 of yeast and S12 of *Tetrahymena thermophila*, and the chloroplast ribosomal protein S12 of many species. Northern blotting illustrated that the transcript corresponding to this clone in pea was increased by UV-B irradiation, and all UV-inducible genes identified to-date are plant defense- or pathogen-related (references in Table 1). *PsUVRib* is the first UV-B-responsive translation machinery protein gene identified in plants; however, the biological significance of the UV-B-induced transcript increase in this gene remains elusive.

β -1,3-glucanases consist of basic and acidic isozymes, with the basic isozymes containing a C-terminal extension that is cleaved off during targeting to vacuoles. The acidic isozymes lack the C-terminal extension, and they accumulate extracellularly (Linthorst et al., 1990). Both the basic and acidic

isozymes are encoded by multigene families in plants (De-Loose et al., 1988; Linthorst et al., 1990). The *psUVGlu* clone is closely related to the pea β -1,3-glucanase gene and it represents the second gene identified from the pea β -1,3-glucanase family. This clone also shares sequence similarity with the genes for both basic and acidic β -1,3-endoglucanases from other species.

UV radiation increases the resistance of plants to pathogenic attack, however the mechanism is only partially resolved. Cloning of the pea UV-B-inducible β -1,3-glucanase gene, *psUVGlu*, increases our understanding of the relationship between UV-B irradiation and pathogen resistance, because β -1,3-glucanases hydrolyze yeast and fungal cell walls (de-la-Cruz et al., 1995). Moreover, release of elicitor-inducible carbohydrates from fungal cell walls by β -1,3-endoglucanase of host tissues, leading to host defense responses such as phytoalexin production, is one of the earliest processes following fungal invasion (Okinaka et al., 1995). This may be why tobacco class I β -1,3-glucanase acts synergistically with class V chitinase against *Fusarium solani* germlings (Melchers et al., 1994). The amount of mRNA for β -1,3-glucanases is repressed by glucose (de-la-Cruz et al., 1995), and induced by either virus infection, salicylate treatment (Linthorst et al., 1990), fungal cell wall polymers, or autoclaved yeast cells and mycelia (de-la-Cruz et al., 1995). Thus, the observation that the *psUVGlu* mRNA is induced by UV-B irradiation suggests that UV-B and pathogens regulate β -1,3-glucanase genes in a common manner.

Auxin is a plant hormone that promotes growth and inhibits dormancy of plants. Accumulation of auxin-repressed protein gene mRNAs is correlated with

strawberry growth inhibition (Reddy and Poovaiah, 1990) and bud dormancy (Stafstrom et al., 1998). Therefore, these proteins function as growth repressors in plants. *PsUVaux* cloned in this study is related to a putative auxin-repressed protein gene of apricot, being the first putative UV-B-inducible plant growth repressor identified in plants. However, the sequence similarity of this clone with other auxin-repressed protein genes is rather poor.

To my knowledge, *psUVRub* is the first clone of the pea Rubisco activase gene. This cDNA clone, and a peptide deduced from its sense strand, share high sequence similarity, respectively, with Rubisco activase genes of other species and their encoded proteins. Rubisco activase, functioning as a molecular chaperone in an ATP hydrolysis-dependent manner, is essential for the activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1. 39). It binds preferentially to non-native Rubisco and dissociates after addition of ATP (Sanchez et al., 1995). The stoichiometry of Rubisco to Rubisco activase is about 20 Rubisco active sites per activase tetramer in *Arabidopsis* (Eckardt et al., 1997). A small decline in the amount of Rubisco activase results in great decrease in Rubisco activity, therein inhibiting photosynthesis (Eckardt et al., 1997; He et al., 1997) and plant growth (He et al., 1997). In addition, Rubisco activase mRNA was always a smear on my northern blots, while other mRNA bands were sharp, suggesting that Rubisco activase mRNA is prone to degradation. Therefore, continuous transcription of the Rubisco activase gene may be critical to Rubisco activity. When characterizing the response of Rubisco to UV-B irradiation, Jordan et al. (1992) found that the activity of Rubisco

declined more rapidly than its abundance, but it was unclear how this happened. My finding that the amount of Rubisco activase mRNA remarkably decreases upon UV-B irradiation sheds light on the UV-B response of Rubisco.

Dehydrins are a large family of proteins enriched in hydrophilic amino acids and characterized by several conserved motifs that are arranged in a linear order. They are normally glycine-rich and cysteine- and tryptophan-free (Close et al., 1989; Momma et al., 1997). Of the various motifs, the most conserved is a lysine-rich KIKEKLPG sequence near the C-terminus, present once or as multiple copies (Artlip et al., 1997; Robertson and Chandler, 1994). Another motif, a DEYGNP segment, may be found, usually as repeats, at the N-terminus of some of these proteins (Artlip et al., 1997; Momma et al., 1997). Other than these regions, the remainder of the proteins often differ markedly from one another. The *psUVdhn* clone exhibited the highest sequence homology with *B61*, a pea dehydrin cognate gene. The KIKEKLPG motif and the flanking regions of a peptide deduced from *psUVdhn* are almost identical to the corresponding *B61* segments. In addition, *psUVdhn* and *B61* contain a stretch of common residues that are missing from other pea dehydrins. The expression of most dehydrin genes is induced by dehydration (Close et al., 1989; Cellier et al., 1998; Parra et al., 1996; Robertson and Chandler, 1992), abscisic acid (Cellier et al., 1998; Parra et al., 1996; Robertson and Chandler, 1992), cold (Kemp et al., 1997; Muthalif and Rowland, 1994; Wolfrain et al., 1993), mannitol (Parra et al., 1996) or NaCl (Parra et al., 1996). However, it is unknown why the pea *B61* mRNA is reduced upon dehydration and has no response to abscisic acid (Robertson and

Chandler, 1994). The mRNA of *psUVdhn* identified in this study is reduced by UV-B irradiation, and is thus another stress-responsive dehydrin gene showing atypical stress responses in pea.

The last clone, *psUVzinc*, identified in this study was repressible by UV-B. It has no homologue in the GenBank databases, indicating that it is a novel UV-B-repressible gene in pea. Thirty-eight out of the 86 amino acid residues of the longest peptide deduced from its sense strand showed 45% identity to *Odontella sinensis* photosystem II reaction center X protein, but obtaining a clone containing the remaining portion of the cDNA is essential for determining the open reading frame of this clone.

4.10 Conclusions

Based on the findings of this work, a model for the signal transduction pathways mediating the UV-B responses of *lhcb* genes is proposed in Fig. 25. Normally, UV-B represses *lhcb* genes via a UV-B-specific pathway, but weakly activates them through a red light pathway. The UV-B pathway requires a carotenoid-associated intermediate (CxU, Fig. 25) to down-regulate all these genes, and a chlorophyll-associated intermediate (ChU) to more strongly repress the *lhcb1*2*-represented subfamily. The red light pathway contains a chlorophyll-associated intermediate (ChR) that mediates induction of all the *lhcb* genes, and a carotenoid-associated intermediate (CxR) that attenuates the induction of the subfamily of genes represented by *lhcb1*2*. When the chloroplast intermediates of the UV-B-specific pathway are either damaged or not formed, UV-B loses its ability to repress *lhcb* genes, but it still induces them via the red light pathway. UV-B

Figure 25. A proposed model for UV-B-induced regulation of pea *lhcb* genes

ChR, a chlorophyll-associated intermediate that mediates red light induction of both *lhcb1*4* and *lhcb1*2*; CxR, a carotenoid-associated intermediate that attenuates the effect of red light on *lhcb1*2* accumulation; CxU, a carotenoid-associated intermediate that mediates UV-B-induced down-regulation of both *lhcb1*4* and *lhcb1*2*; ChU, a chlorophyll-associated intermediate that enhances the UV-B-induced down-regulation of *lhcb1*2*; P_r: red light-absorbing form of phytochrome. Doted lines represent repression pathways; solid lines represent induction pathways. Thickness of the lines indicates strength of the actions.

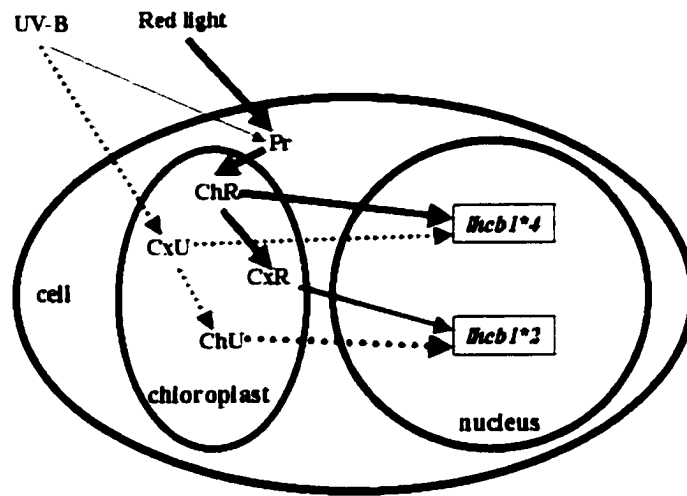


Figure 25

prevents photodamage to the chloroplast intermediates of the red light pathway.

It is unclear whether the chloroplast intermediates of the UV-B pathway are carotenoids and chlorophylls per se, or light-harvesting complex II, plastoquinone, abscisic acid and thylakoid membrane lipid. Moreover, the identification of UV-B-responsive genes in this study indicates that UV-B induces the expression of genes involved in translation, pathogen defense and auxin responses, and represses those encoding molecular chaperons, drought-responsive proteins and genes of unknown functions. This probably makes the mechanisms more complicated because the UV-B responses of *lhcb* genes may be affected by regulation of other genes. Thus, how to identify the chloroplast intermediates of the UV-B pathway regulating *lhcb* genes is a challenge. Evidently, identifying UV-B-responsive *lhcb* cis-elements and then using them as probes to screen expression libraries may provide direct evidence for the intermediates of these signal transduction pathways, and characterization of more UV-B-responsive genes will facilitate our understanding of UV-B-dependent regulation of plant growth and development.

Appendix I. Solutions and Recipes

Solution used for inhibition of carotenoid biosynthesis

100 μ M Norflurazon: 0.386 g Zoria Rapid80
10 l distilled water

Solutions for total RNA extraction

1M Tris pH 8.0: 12.1 g Tris
80 ml DEPC water
adjust pH to 8 with HCl
add DEPC water to 100 ml
autoclave

0.25 M EDTA pH 8.0: 9.31 g disodium EDTA-2H₂O
80 ml DEPC-treated dH₂O
adjust pH to 8.0 with NaOH
add dH₂O to 100 ml
autoclave

PAS-TNS solution: 4% PAS (p-aminosalicylic acid) sodium salt
1% TNS (Tris-iso-propylnaphthanlene sulfonic acid)
50 mM Tris pH 8.0
autoclave

Phenol reagent: 0.1 g 8-hydroxyquinoline
100 ml phenol/chloroform/isoamyl alcohol
(25:24:1,
pH 5.2)

100 mM ATA: 2.365 g ATA (aurin tricarboxylic acid)
100 ml 50 mM Tris pH 8.0
autoclave

Extraction buffer (quantities/sample):

4 ml PAS-TNS solution
40 μ l 100 mM ATA
80 μ l β -mercaptoethanol
206 μ l 20% SDS
prepared just prior to use

12 M LiCl: 25.43 g LiCl
dissolved and brought up to 50 ml with dH₂O
filtered through 0.22 μ Acrodisc

Resuspension buffer:	10 mM Tris pH 7.5 2.5 mM EDTA pH 8.0 10 μ M ATA autoclave
50 μM ATA:	50 μ l 100 mM ATA stock 100 ml 50 mM Tris pH8.0 autoclave
3 M sodium acetate:	12.3 g sodium acetate 40 ml DEPC-treated dH ₂ O adjust pH to 5.2 with glacial acetic acid add DEPC-treated dH ₂ O to 50 ml

Solutions for 1st strand cDNA synthesis:

TE:	10 mM Tris pH 8.0 0.1 mM EDTA pH 8.0
Stop mix:	0.1 M EDTA

Solutions for RT-PCR

0.3125 mM dNTPs containing biotin-14-dATP:	0.2734 mM dATP 0.039 mM biotin-14-dATP 0.3125 mM dTTP 0.3125 mM dCTP 0.3125 mM dGTP
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Solutions for Southern blotting

6x DNA loading buffer:	0.025 g bromophenol blue 0.025 g xylene cyanol FF 3 ml glycerol dH ₂ O to 10 ml autoclave
50x Tris-acetate EDTA:	24.2 g Tris 5.71 ml glacial acetic acid 10 ml 0.5 M EDTA pH 8.0 dH ₂ O to 100 ml
DNA denaturation buffer:	0.2 M NaOH 0.6 M NaCl

Sodium phosphate buffer (pH 6.5):
25 mM NaH₂PO₄, pH 6.5

Solutions for chemiluminescent detection

10x Phosphate Buffered Saline (PBS):
0.58 M Na₂HPO₄
0.17 M NaH₂PO₄·H₂O
0.68 M NaCl
autoclave

0.5% (w/v) I-block buffer: 0.5% (w/v) I-block powder
1x PBS
dissolved by microwave (do not boil)
0.5% SDS

0.2% (w/v) I-block buffer: 0.2% (w/v) I-block powder
1x PBS
dissolved by microwave (do not boil)
0.5% SDS

1:30000 streptavidin alkaline phosphatase (SA-AP) conjugate solution:
3.34 μl SA-AP
20 ml 0.5% I-block buffer
filter through 0.45 μ Acrodisc
add 0.5% I-block buffer to 100 ml

Wash buffer: 1x PBS
0.5% SDS

Assay buffer: 0.96% diethanolamine, pH 10
0.02% (w/v) MgCl₂

125 μM CDP™: 100 μl CDP™ (Tropix, Bedford, USA)
10 ml assay buffer

Solutions for cloning

0.1 M CaCl₂: 0.735 g CaCl₂·2H₂O
add dH₂O to 50 ml
filtered through 0.22 μm Acrodisc

SOC medium: 2% bacto tryptone
0.5 % yeast extract
10 mM NaCl

	2.5 mM KCl adjust pH to 7.0 with 5 N NaOH autoclave 10 mM MgCl ₂ 10 mM MgSO ₄ 20 mM glucose filter through 0.22 μm Acrodisc
X-gal:	20 mg ml ⁻¹ N,N-dimethylformamide
IPTG:	2 g isopropylthio-β-D-galactoside add dH ₂ O to 10 ml
LB broth:	25 g LB medium powder 1 l dH ₂ O autoclave
LB/ampicillin broth:	25 g LB medium 1 l dH ₂ O autoclave cool to ~50 °C, add 4 ml 5 mg ml ⁻¹ ampicillin
LB plate:	25 g LB medium powder 15 g bacto-agar 1 l dH ₂ O autoclave pour plates
LB/ampicillin plate:	25 g LB medium powder 15 g bacto-agar 1 l dH ₂ O autoclave cool to ~50°C, add 4 ml 5 mg ml ⁻¹ ampicillin pour plates

Solutions for plasmid mini-preparation

ZE lysis buffer:	10 mM Tris pH 8.0 1 mM EDTA pH 8.0 2 mg ml ⁻¹ lysozyme 0.2 mg ml ⁻¹ heat-treated RNase 0.1 mg ml ⁻¹ bovine serum albumin
Solution I:	50 mM glucose 25 mM Tris pH 8.0 10 mM EDTA pH 8.0

	autoclave
Solution II:	0.2 N NaOH 1% SDS
Solution III:	5 M potassium acetate 11.5% glacial acetic acid
TE containing RNase:	10 mM Tris pH 8.0 0.1 mM EDTA pH 8.0 20 $\mu\text{g ml}^{-1}$ heat-treated RNase

Solutions for northern blotting

RNA denaturation buffer:	40 mM MOPS 12% formaldehyde 5 mM DEPC-treated EDTA pH 8.0 50% de-ionized formamide stored at -20°C
1% formaldehyde agarose gel:	0.45 g agarose 28.35 ml DEPC-treated water dissolve and leave at 65°C for 10 min 9 ml 10 x MOPS 7.65 ml formaldehyde 29.93 μl 100 mM ATA
10x MOPS running buffer:	46.3 g MOPS 16.7 ml 3 M sodium acetate 800 ml DEPC-treated dH ₂ O adjust pH to 7.0 with DEPC-treated NaOH 20 ml 0.25 M DEPC-treated EDTA pH 8.0 add DEPC-treated dH ₂ O to 1 L filtered through 0.22 μ filter
20x SSC :	175.3 g NaCl 88.2 g Na-Citrate 800 ml dH ₂ O adjust pH to 7 add dH ₂ O to 1000 ml, autoclave
RNA loading buffer:	50% glycerol 5 x MOPS 2.5% bromophenol blue 2.5% xylene cyanol FF 0.1% DEPC, 37 °C overnight, autoclave

0.5 M disodium phosphate buffer:

0.5 M Na₂HPO₄
85% H₂PO₄
pH 7.2 as mixed

Hybridization buffer:

1 mM DEPC-treated EDTA pH 8.0
7% SDS
0.25 M disodium phosphate, pH 7.2

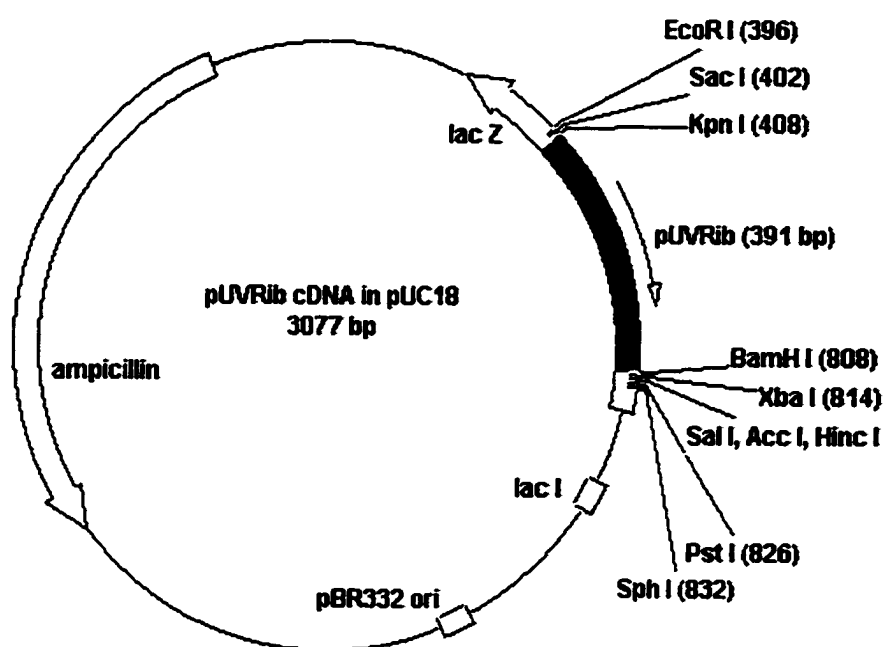
Probe stripping buffer:

0.05 x SSC
0.01 M EDTA pH 8.0
boiling
0.1% SDS

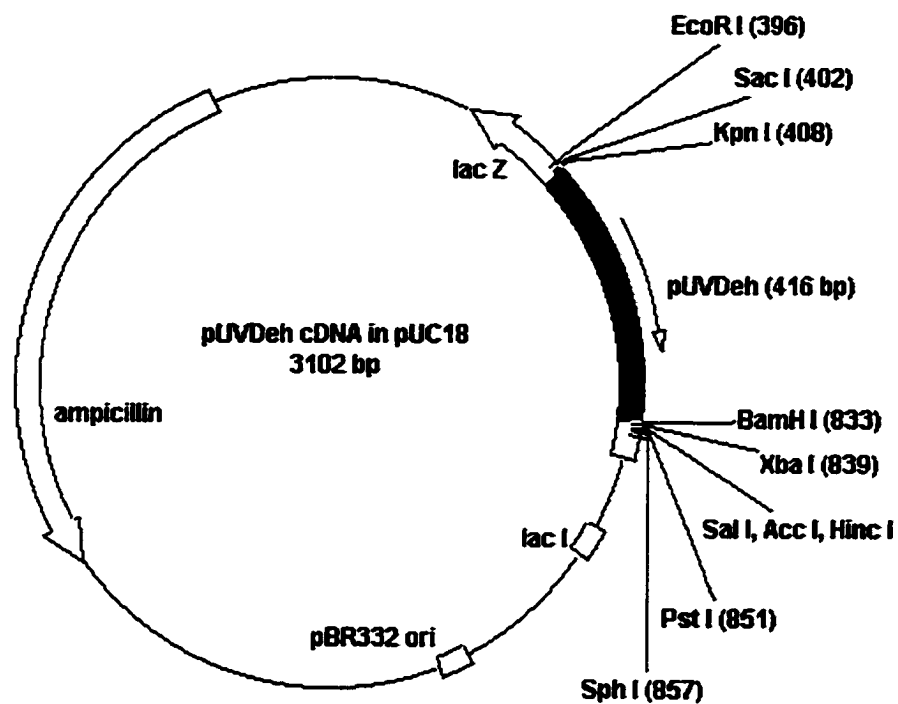
Solutions for staining RNA makers on northern blots**Methylene blue:**

0.5 M sodium acetate
0.04% methylene blue

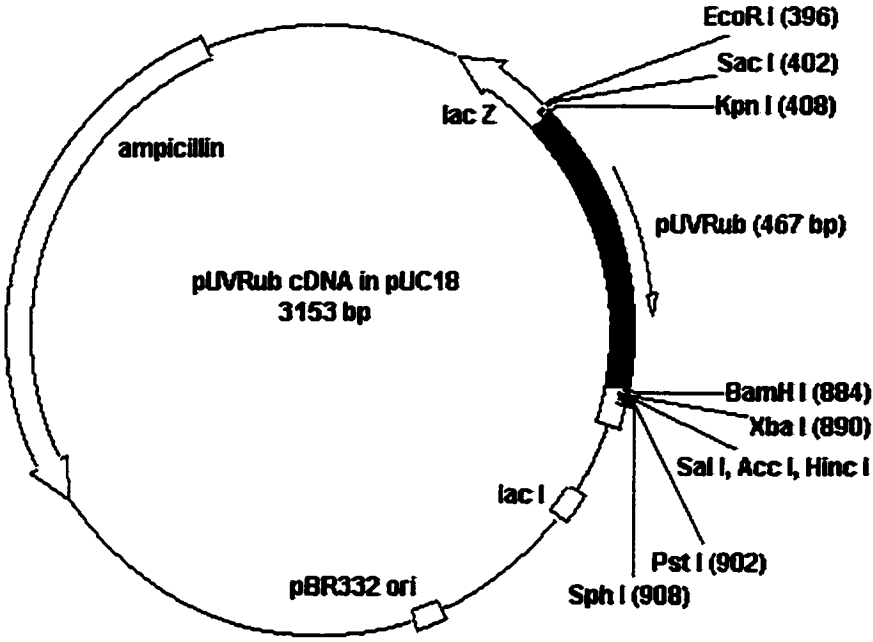
Appendix II. Schematic representation of *pUVRib* cDNA in *pUC18*



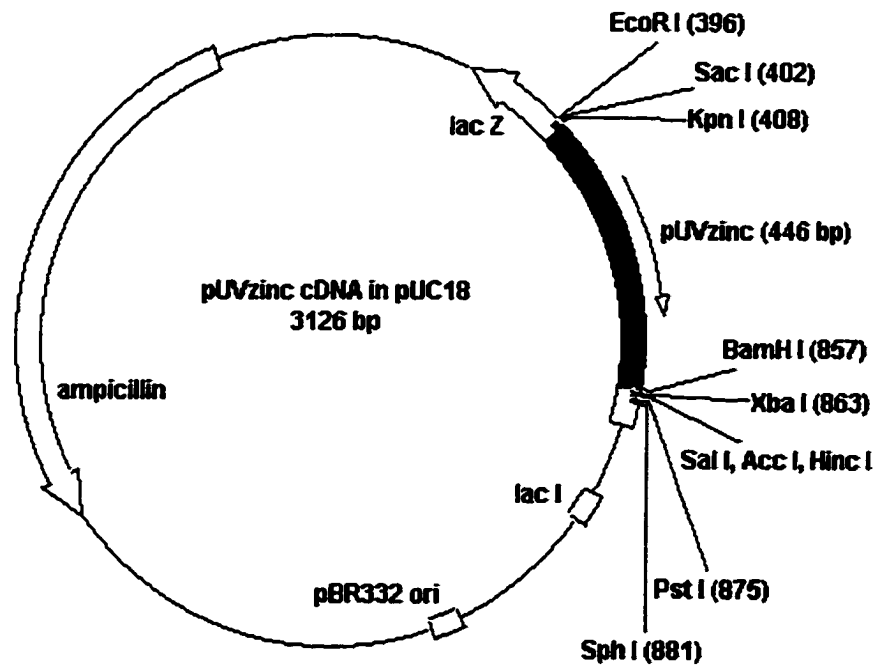
Appendix III. Schematic representation of *pUVDeh* cDNA in *pUC18*



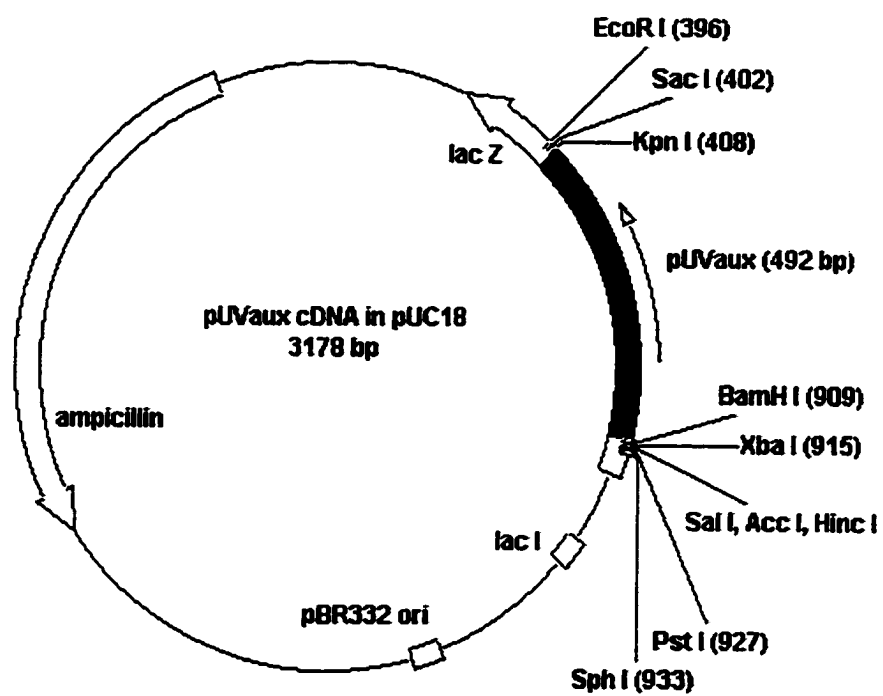
Appendix IV. Schematic representation of *pUVRub* cDNA in *pUC18*



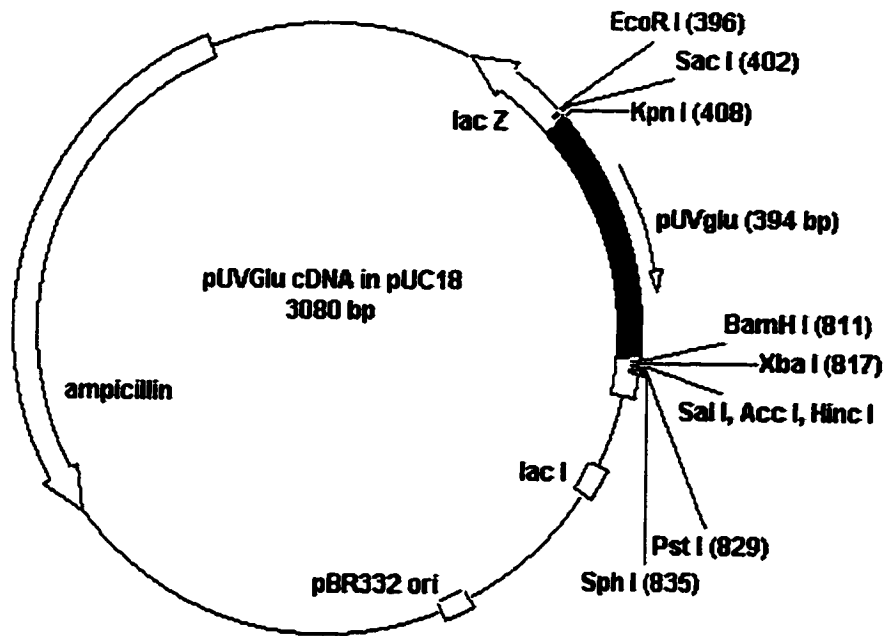
Appendix V. Schematic representation of pUVzinc cDNA in pUC18



Appendix VI. Schematic representation of *pUVaux* cDNA in *pUC18*



Appendix VII. Schematic representation of *pUVGlu* cDNA in pUC18



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