On the Expression of Several Lhc Genes in Garden Cress (*Lepidium sativum* L.)

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The polymerase chain reaction was used to prepare gene-specific probes for several Lhc genes coding for chlorophyll *a/b*-binding proteins of cress (*Lepidium sativum* L.). Due to the presence of about 150 basepairs of the coding region, the isolated clones could be attributed to Lhc a 3 (1 clone), Lhc b 1 (5 clones), Lhc b 2 (1 clone) and Lhc b 3 (1 clone) genes. Probes prepared from the 3′non-coding regions of the clones did not cross-hybridize; they were specific for 3 different Lhc b 1 transcripts and one each of Lhc b 2, Lhc b 3 and Lhc a 3 transcripts. The transcript levels were higher in leaves than in cotyledons of light-grown seedlings; they decreased significantly in cotyledons from week 1 to week 4. The levels of 2 Lhc b 1 transcripts (detected with probes cd 1 and cd 2) changed from 1 week old cotyledons (30% cd 1, 28% cd 2) to 3 months old leaves (14% cd 1), 44% cd 2), stems (11% cd 1, 56% cd 2) and fruits (15% cd 1, 62% cd 2, all values percent of total transcripts), whereas transcript levels of another Lhc b 1 gene (detected with probe cd 3) and of a Lhc a 3 gene remained nearly constant. The level of Lhc b 2 and Lhc b 3 transcripts were 1–2 orders of magnitude smaller than those of the other Lhc transcripts. The data obtained with cress plants are compared with published data from other plants.

Introduction

Chlorophyll *a/b*-binding proteins are constituents of light-harvesting complexes (LHCs) that are associated with photosystem I and II of higher plants and green algae. The best investigated complex is LHC II that is – together with some minor complexes – connected to photosystem II; it contains about 50% of chlorophylls and total protein of the thylakoid membrane (Thornber, 1986; Peter and Thornber, 1991). Up to 6 different proteins have been detected in LHC II by isoelectric focusing (Bassi and Dainesse, 1990). LHC I – connected to photosystem I – seems to be less heterogeneous (Bassi *et al.*, 1990; Ikeuchi *et al.*, 1991).

The chlorophyll *a/b*-binding proteins are related to each other as deduced from the sequences of their genes: the Lhc genes form a large multigene family. Different degrees of homology allow to distinguish 3 types of Lhc b genes (coding for LHC II apoproteins) and up to 4 types of Lhc a genes (coding for LHC I apoproteins) (Morishige and Thornber, 1991; Schwartz *et al.*, 1991 a, b; Brandt *et al.*, 1992). Most numerous are Lhc b 1 (formerly cab II type I) genes: up to 16 copies encoding nearly identical polypeptides have been detected within one species (Dunsmuir, 1985).

Expression of Lhc genes depends on several factors. They are classical examples of light-regulated genes (Apel and Kloppstech, 1978) and exhibit a circadian rhythm of expression (Kloppstech, 1985; Paulsen and Bogorad, 1988; Nagy *et al.*, 1988; Miller and Kay, 1991). Expression of Lhc genes requires intact plastids (Taylor, 1989; Oelmüller, 1989); expression is restricted to green organs (leaves, stems *etc.*) and does not occur in roots (Piechulla *et al.*, 1986; Simpson *et al.*, 1986). Most studies on expression did not distinguish between single members of the Lhc gene family. In those few studies that compared single Lhc genes with each other, a differential pattern of expression was observed (Sheen and Bogorad, 1986; Karlin-Neumann *et al.*, 1988; Sun and Tobin, 1990; White *et al.*, 1992; Kellmann *et al.*, 1993).

We describe here the preparation of gene-specific probes for single Lhc genes and a study on differential expression in several organs and several developmental stages of cress plants.

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Materials and Methods

Growth of plant material

Etiolated cress seedlings (*Lepidium sativum* L.) were grown in Petri dishes (Ø 5 cm, 15 seeds per dish) on wet filter-paper in absolute darkness for 3 or 4 days at a temperature of 25 °C.

For poly A^+-RNA isolation 3 days old etiolated seedlings were harvested under dim green safe light. "Red light-induced" seedlings were grown 4 days in the dark then illuminated with a 2 min red light pulse (λ_{max} = 665 nm, 10 W/m^2) and harvested after a subsequent dark period of 4 h. Green seedlings were grown in garden soil at 20 °C with a daily light phase of 15.5 h and a white light fluence (Osram L 58 W/11 lamps) of 22 W/m^2. Adult cress plants were cultivated in a greenhouse at the Botanical Institute, University of Munich from beginning of March to end of May 1992. During this time plants were exposed to the naturally occurring day and night cycle. After harvest all plant material was immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

Isolation of RNA

Total cellular RNA was prepared with the usual precautions (Ausubel *et al.*, 1987) from different cress organs as described by Kittsteiner *et al.* (1991). For preparation of poly A^+-RNA total RNA was precipitated with LiCl added to a final concentration of 2 M (instead of isopropanol precipitation). After incubation at 4 °C for 14 h, the precipitate was collected by centrifugation and washed with 70% ethanol. After drying *in vacuo* total RNA was dissolved in 400 µl 0.5 M LiCl per mg. Poly A^+-RNA isolation on a oligo dT-cellulose column was done according to Ausubel *et al.* (1987).

PCR amplification of cDNA ends

The PCR-based amplification of specific 3′cDNA ends was performed following the method of Frohman *et al.* (1988). Oligo-dT adapter primer for first strand cDNA synthesis (5′GACTCGAGTCGACATCGAT_{17}3′) and adapter primer for subsequent amplification of 3′cDNA ends (the same without AT_{17} at 3′) were taken directly from Frohman *et al.* (1988). The Lhc gene-specific primer necessary to amplify Lhc-related cDNAs in combination with the unspecific adapter primer has the following sequence which was degenerated at one position:

5′AGATTGGCTATGTTCTCTATGTT$\begin{matrix}C\\T\end{matrix}$GG3′.

cDNA first strand was synthesized with 1 µg poly A^+-RNA using 200 U M-MLVH$^-$ reverse transcriptase (Superscript, Gibco BRL) and 0.2 µg oligo-dT adapter primer according to the manufacturer's instructions. The 20 µl reactions were stopped by adding 80 µl H_2O and freezing the sample at −80 °C until further use.

To amplify Lhc-cDNA ends, 1.5 µl of a cDNA first strand reaction were combined with 1.2 U Taq polymerase and 0.25 µg each of gene-specific and adapter primer in 50 µl reactions containing 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatine and 0.2 mM dATP, dCTP, dGTP and dTTP. After 30 cycles of 1 min denaturation (94 °C), 2 min annealing (50 °C) and 3 min polymerization (72 °C) 5 U of Klenow polymerase were added and incubated for 15 min at 37 °C.

For further sequence analysis the resulting PCR products were cloned into a SmaI linearized pBluescript vector. After purification recombinant plasmid DNAs were sequenced using the T7-Sequencing kit (Pharmacia).

Subcloning of 3′non-coding regions

The 3′non-coding regions of Lhc cDNAs were subcloned using either suitable restriction sites and partial digestion with exonuclease III in combination with mung bean nuclease as follows: cd1: restriction enzymes MspI (5′site) and Ksp632I (3′site), cd2: MspI (5′site) and exonuclease III (3′site), cd3: HindIII (5′site) and TaqI (3′site), cd4: MspI (5′site) and exonuclease III (3′site), cd5: RsaI (5′site) and exonuclease III (3′site), cd6: Sau3AI (5′site) and HaeIII (3′site). After blunting ends with Klenow polymerase the subclones were ligated into the SmaI site of pBluescript plasmid.

Labeling of hybridization probes

The inserts of the linearized plasmid DNAs containing cd1–cd6 were then amplified in a PCR reaction using only one primer (either T3 or T7) to obtain single-stranded antisense DNA probes.

Final concentrations of nucleotides were 5 μM dATP, dGTP, dTTP, 3.5 μM dCTP and 50 μCi α[^{32}P]dCTP in a 10 μl reaction volume.

Labeling was achieved by partially replacing unlabeled dCTP with α[^{32}P]dCTP (3000 Ci/mmol). Linear amplification was performed through 30 cycles of denaturation (30 sec, 94 °C), annealing (30 sec, 40 °C) and polymerization (30 sec, 72 °C). Non-incorporated nucleotides were then separated with a Sephadex G 50 spun column (Maniatis *et al.*, 1982).

DNA slot blot

Plasmid DNA inserts were denatured as described by Ernst *et al.* (1987) and blotted to nitrocellulose membranes with the help of a slot blot apparatus (Minifold II, Schleicher & Schuell). Each slot contained about 100 ng DNA. DNAs were fixed to the membrane by heating to 80 °C *in vacuo* for 90 min.

RNA gel electrophoresis, Northern blots

RNA samples were denatured as described by Paulsen and Bogorad (1988) and loaded onto 1.2% agarose gels containing 2.2 M formaldehyde. After electrophoretic separation gels were used for Northern blotting of RNA to nylon membranes (Biodyne A, PALL) with 10×SSC (1×SSC: 0.15 M NaCl, 15 mM sodium citrate, pH 7.0). After blotting RNAs were fixed to the membranes with UV light.

Hybridization and RNA quantitation

Prehybridization and hybridization were performed as described by Paulsen and Bogorad (1988) at 42 °C. After hybridization filters were washed as recommended by the manufacturer; the most stringent wash was in 0.1×SSC, 0.1% SDS at 50 °C for 20 min. The wet filters were exposed to Hyperfilm MP films at −80 °C using intensifying screens. Autoradiograms were scanned with a densitometer (TLD 100, Vitatron Scientific Instruments, The Netherlands).

For comparison of the hybridization activity of a RNA sample with the different gene-specific probes, the labeling grade of the single probes given by the number of dGTPs in the amplified sense strand (see Fig. 1) was taken into account.

Results and Discussion

Starting material for preparation of gene-specific probes was poly A$^+$-RNA from cress seedlings. We had already seen that the amount of total Lhc mRNA in dark-grown cress seedlings varies in 3 days old and in 4 days old seedlings; light induction of Lhc mRNA is higher in 4 days old than in 3 days old seedlings (Kittsteiner *et al.*, 1991). In order to imply as many different Lhc genes as possible, we used 3 RNA preparations from: 1. three days old etiolated seedlings, 2. five days old seedlings grown in day/night cycles (10 h white light per day), harvested 4 h after begin of the last irradiation, and 3. four days old etiolated seedlings that received a 2 min pulse of red light, harvested 4 h after the light pulse. According to the RACE method (rapid amplification of cDNA ends) of Frohmann *et al.* (1988), the first cDNA strand was synthesized with an oligo-dT adapter primer (see Materials and Methods). For the subsequent polymerase chain reaction (PCR), the adapter primer (without oligo-dT tail) and a gene-specific primer coding for the N-terminal part of the third membrane-spanning helix (see Materials and Methods) were used. Comparison of the known Lhc genes had revealed that sequences of the first and third membrane spanning helices are highly conserved (Green *et al.*, 1991). Choosing the sequence of the third helix should yield PCR products containing 250–400 bp. Fragments of this size were obtained as main products from all 3 RNA preparations; hybridization with a Lhc-specific probe (not shown) confirmed amplification of the desired Lhc gene-specific cDNA.

After amplification PCR products were ligated blunt end into a linearized pBluescript vector. Subsequent transformation of *E. coli* DH5α yielded a high number of recombinant clones. Analysis of 52 of these clones resulted in 8 different Lhc-specific clones; the sequences of these are given in Fig. 1. Due to the position of the gene-specific primer, the cloned sequences contained about 150 bp of the coding region of the genes. The deduced amino acid sequences (Fig. 2) allowed the identification as Lhc genes and also the attribution to different types of cab genes (Table I). Clones CC1, CC1A, CC1B, CC2 and CC3 are 100% identical with each other and with the corresponding region of several Lhc b1 genes.

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                  10          20          30          40          50
CC1: AGA TTG GCT ATG TTC TCT ATG TTT GGA TTC TTT GTT CAA GCC ATT GTC ACT GGT AAG
CC1A *** *** *** *** *** *** *** *** *** *** **C *** *** *** *** *** *** **C ***
CC1B *** *** *** *** *** *** *** *** *** *** **C *** *** *** *** *** *** **C ***
CC2: *** *** *** *** *** *** *** *** *** *** **C *** *** *** *** *** *** **C ***
CC3: *** *** *** *** *** *** *** **C *** *** **C *** *** *** **C *** **C **A ***
CC4: *** **A *** *** *** *** *** *** *** *** **C *** *** *** *** **T *** *** **A
CC5: *** *** *** *** *** *** *** **C **C *** *** *** *** *** *** **T **C **A **A
CC6: *** *** *** *** *** *** *** **C **T *A* *** A*C *GG *GA C*A **G **C *** GTT

       60          70          80          90          100         110
CC1: GGA CCT TTG GAG AAC CTT GCT GAC CAT TTG GCC GAT CCA GTC AAC AAC AAC GCA TGG
CC1A *** **A *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
CC1B *** **A *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***
CC2: *** **A *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** **T ***
CC3: *** **A *** *** *** *** **C *** *** *** **T *** *** *** *** *** *** *** ***
CC4: **T **C A*T *** *** T*G TT* **T *** G** **T *** **T **G GCT *** **T **T ***
CC5: **T *** *** **A *** **C TTG *** *** C*C *AT A*C **G *** GCT *** **T **C ***
CC6: *** *** *AC C** *** *** CT* **T *** *** *** *** **C *** *** *** *** ATC *T*

          120         130         140         150         160         170
CC1: GCC TTC GCC ACC AAC TTT GTT CCC GGA AAG TGA GCG ACT TTT ATC TGT ATT TCG CTT
CC1A *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *T* ***
CC1B *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *T* ***
CC2: *** *** *** *** *** *** *** *** GGA AAG TGA GCA AAG TTC TAT CTG TTT GTA ATT
CC3: *** *** *** **T *** *** **C *** *** *** TGA *** *** **A GCT TAT TAT GTG AGT
CC4: T*T *A* *** **T *** *** *** *** *GA AAG TAA TTA CCA TCT GTA TTT GCT TCT TAA
CC5: **T *** **A **A **G *** **A CCT GGA GCT TAG ATT TTA AAT CTT AAT GTG ATT CTT
CC6: A** AG* CT* *AG TT* CAC TAA GAT *AT CAT CAT CCC ATG TGT CCT TTG CTC TTG TAT

              180         190         200         210         220
CC1: CAG TCT TTG TGT CGT GTG AAT GTG AAA ACT GAA AGA GAG GTT GCT CAG ATG TAA AAT
CC1A *** *** *** *T* *** **T *** *** *** *** *** *** *** *** *** *** *** *** ***
CC1B *** *** *** *T* *** **T *** *** *** *** *** *** *** *** *** **A
CC2: TGT TTT TTT AAG TTT TTT TAA TTC GAG TAA GAG TGA GAG ACG GGA GAA(n) GAA AGA CCA
CC3: GAG AGC AGA GAA AGA GAG TTT GTT TGT GGT CTT TTC TAT GTA CAT TTA TGA AAT TTC
CC4: TCT CTT TAC CTT GTG TAA CTC GTG ACT GTA TTA ATG GTT TTA TGT GTC AAT TTG GAA
CC5: GTG ATG ATA TGA ACA TAA TCC TTG TAG TTC CTT TCA AGT TAC AAA TCT CTA TTC GCT
CC6: CTT TTC TAT CTT TCG TTA CCA TCG GCT ACT TTC TTA GTA TTG TAA CAC CCT GTG AAA

     230                         250         260         270         280
CC1: TTG CAA GAC TCT ATG ATT TTG TTT CAG GAA TTA AGA ACT CTT CTT TTC TCT TAA A(16)
CC1A *** **G A*A *G* *** *** *** *A* *T* A** *C* *T* *** *** *CC *C* AAA A(n)
CC2: GGT TTG TAT TTG GCG TGG ATG GTT TGG GAC TTT CAG ATG TAA CTT TGC AAG ACT
CC3: CTT TGT GCA ATC TTT TTA TGA TTT TAT CAG AAA TCG AAC CTC TTT TTC CCA A(16)
CC4: CTT AAT AAC GTT TCT TTT TGC GAA GTA AAA AAA AAA AAA A(30-50)
CC5: ATG CAC ATA AGA AGT CTC AAA AAA ACT CTT TCT CTA AAA AAA AAA AAA AAA
CC6: ACA TAT TCG TGT TTC GTA AGA ATG ACA ATG TCA ATG GCC AA(17)

              290         300         310         320
CC2: TTG TAT CAT TTA TCA TTA GTC AAA TAA CTC AAT TTT CTC TCA A(23)
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Fig. 1. Nucleotide sequences of cDNA clones (CC1–CC6) containing the 3′ parts of Lhc genes, prepared from poly A$^+$-RNA of cress plants with the RACE method (see Materials and Methods). The sequence of the gene-specific primer at 5′ is given in italics, the stop codon in bold face. The sequences of gene-specific probes (cd1–cd6) derived from the 3′non-coding region are underlined.

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          5    10    5    20    5    30    5    40    5
CC1: RLAMFSMFGF FVQAIVTGKG PLENLADHLA DPVNNNAWAF ATNFVPGK
CC2: ********** ********** ********** ********** ********
CC3: ********** ********** ********** ********** ********
CC4: ********** ********** *I***F**V* ***A****SY ********
CC5: ********** ********** *****L***D N**A****** **K*****A
CC6: **********Y *IRGL***V* *YQ**L**** ******ILTS LKFH
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Fig. 2. Amino acid sequences of the coding regions of cDNA clones CC1–CC6 (see Fig. 1).

Table I. Comparison of amino acid sequences of the coding regions of Lhc cDNA clones (this paper) with published sequences. The values are percent sequences identity determined with the FASTA program.

Clone or type	CC4	CC5	CC6	Lhc b1[b]	Lhc b2[c]	Lhc b3[d]	Lhc a3[e]
CC1[a]	85	85	52	100	77	80	57
CC4	–	77	44	85	92	80	57
CC5	–	–	44	85	80	95	52
CC6	–	–	–	52	46	46	86
Lhc b1[b]	–	–	–	–	77	80	57
Lhc b2[c]	–	–	–	–	–	80	51
Lhc b3[d]	–	–	–	–	–	–	51

[a] Clones CC1A, CC1B, CC2 and CC3 are 100% identical with clone CC1 and therefore not listed separately.
[b] Lhc b1 (formerly cab II type I) sequences of cab 1A–C, 3C from tomato (Pichersky *et al.*, 1985) and cab 13, cab 25 from petunia (Dunsmuir, 1985).
[c] Lhc b2 (formerly cab II type II) sequence of cab 5 from tomato (Pichersky *et al.*, 1987).
[d] Lhc b3 (formerly cab II type III) sequences of cab 13 from tomato (Schwartz *et al.*, 1991b).
[e] Lhc a3 (formerly cab I type III) sequence of cab 8 from tomato (Pichersky *et al.*, 1989).

The highest homology is found between CC4 and Lhc b2, between CC5 and Lhc b3, between CC6 and Lhc a3 (see Table I). We attribute the clones correspondingly to these types of Lhc genes.

The coding regions of the different types of Lhc genes are so similar to each other that gene-specific probes can not be obtained from these regions. The non-coding regions are less similar; sequence identity is throughout below 50% (Table II). These regions should be suitable for preparation of gene-specific probes. Only the non-coding regions of CC1A and CC1B are highly homologous to that of CC1 (CC1A: 96% identity in 72 bp, CC1B: 88% identity in 132 bp). We have to consider the group CC1, CC1A and CC1B as a subfamily of Lhc b1 genes. Only one gene probe (from CC1) was prepared for this subfamily. The existence of subfamilies with a high degree of homology in the 3′non-coding regions was also found for *Petunia* (Dunsmuir, 1985) and *Arabidopsis* (Leutwiler *et al.*, 1986).

Table II. Comparison of 3′non-coding DNA sequences of Lhc cDNA clones. The values are percent sequence identity, determined by pairwise computer-aided comparison.

Clone	CC2	CC3	CC4	CC5	CC6
CC1	45	30	51	30	30
CC2	–	49	39	28	27
CC3	–	–	42	28	39
CC4	–	–	–	43	29
CC5	–	–	–	–	33

Subcloning of the 3′non-coding regions was achieved by restriction and (in part) partial digestion with exonuclease III; this treatment removed also the poly A tails (although not completely in the case of CC4 and CC5). The fragments were – if necessary - treated with the Klenow enzyme in order to obtain blunt ends and ligated into the pBluescript vector (see above). The correct cloning of the fragments (named cd1–cd6, see Fig. 1) was checked by sequencing.

The specificity of the [^{32}P]dCTP-labeled probes cd1–cd6 was tested with inserts of all clones CC1–CC6. As shown in Fig. 3, each probe detected only the insert from which it was derived; no cross-hybridization was detectable.

Expression of the single Lhc genes was then investigated with 3 months old adult cress plants. The plants were grown under natural day/light cycles and were harvested 4 h after sunrise. Total RNA was isolated from several organs. The Northern blot (Fig. 4) shows a single-hybridizing signal (at about 1000 bases) with all probes; the signal is seen in all green organs, namely in leaves, upper part of the stems and fruits, but not in roots. A single signal of the same size (not shown) was also obtained with RNA isolated from cotyledons and primary leaves of 1 to 4 weeks old seedlings. The degree of expression is different in the different plant organs and is also different for the single Lhc genes within one organ (Table III). The level of all Lhc mRNAs decreases in the cotyledons from week 1 to week 4; this might be a sign of senescence. Much less decrease is seen in the primary leaves from week 2 (highest levels) to week 4. The highest level of transcription is found throughout in leaves; only for probe cd2 (detecting one member of Lhc b1 gene products), the signal in fruits is as high as that in leaves. Only with probe cd6 (detecting a Lhc a3 gene product), the signal in fruits is smaller than that in stems. The signals obtained with probes cd4 and cd5 are to small too be quantitated in the 3 months old plants.

The relative amounts of transcripts reacting with the single probes can be estimated under consideration of several factors (*e.g.* labeling degree of single probes, exposition time to the film). The total

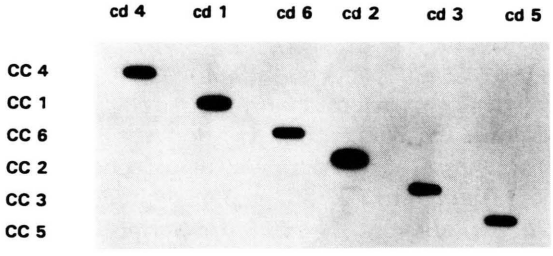

Fig. 3. Autoradiograph showing the hybridization of cDNA clones CC1–CC6 with gene-specific probes cd1–cd6 (see Fig. 1). Inserts of the clones were denatured and fixed to nitrocellulose by slot-blot. Each nitrocellulose sheet containing the inserts of all six clones CC1–CC6 was hybridized with a different [^{32}P]dCTP-labeled probe (cd1–cd6).

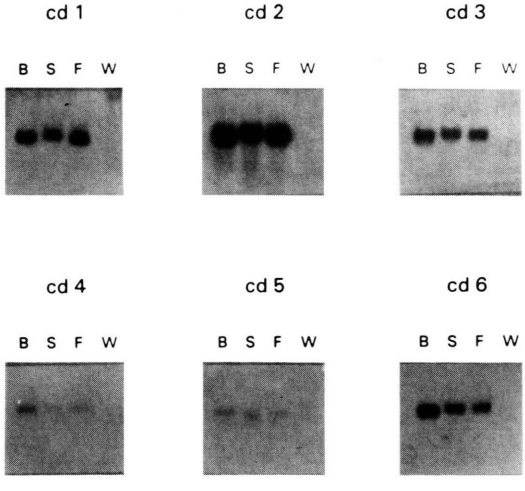

Fig. 4. Northern blots of total RNA prepared from organs of 3 months old cress plants with labeled probes (cd1–cd6) specific for divers Lhc genes (see Fig. 1). RNA was isolated from leaves (B), stems (S), fruit (F) and roots (W).

Table III. Results of densitometry of Northern blots with total RNA from cotyledons (K1–K4, 1–4 week old seedlings) and from primary leaves (L2–L4, 2–4 weeks old seedlings) of cress plants, hybridized with Lhc gene-specific probes. All values are percentages based on the signal of leaves of 2 weeks old seedlings = 100%.

	cd 1	cd 2	cd 3	cd 4	cd 5	cd 6
K1	64	51	63	72	71	79
K2	61	38	57	77	75	76
K3	26	25	24	50	32	42
K4	25	22	32	45	23	29
L2	100	100	100	100	100	100
L3	82	75	55	74	56	76
L4	87	65	71	70	92	75

Table IV. Results of densitometry of Northern blots with total RNA from several organs of 3 months old cress plants, hybridized with Lhc gene-specific probes. All values are percentages based on the signal of leaves = 100%.

	cd 1	cd 2	cd 3	cd 4
Leaves	100	100	100	100
Stems	53	89	75	50
Fruits	79	103	75	32
Roots	–	–	–	–

amount of transcripts is highest in leaves (100%), less in green fruits (75%) and stems (70%). The relative amount of the transcript reacting with probe cd 2 is the highest in all organs except cotyledons of young plants; it makes up 55–60% in stems and fruits and 44% in leaves of adult plants (Table V). The other Lhc b 1 transcripts (reacting with the probes cd 1 and cd 3) make up about 25% in all organs of adult plants. The Lhc a 3 transcript (reacting with probe cd 6) varies between 31% (leaves) and 11% (fruits).

Comparison of the relative amounts of single transcripts during development of cress plants reveals a change mainly of 2 Lhc b 1 transcripts (Table V): In 1 week old cotyledons, the cd 1-specific transcript (30%) is about as abundant as the cd 2-specific transcript (28%). The amount of the cd 1 transcript decreases from 2 weeks old leaves (29%) to 3 months old leaves (14%) and also in stems (11%) and fruits (15%); the amount of the cd 2 transcript increases from 34% (2 weeks old leaves) to 44% (3 months old leaves), 56% (stems) and 62% (fruits). The cd 3-specific transcript does not change its percentage (11–13%) during development.

Table V. Relative amounts of transcripts reacting with the indicated 3′probes in various organs of cress plants during development. All values are percentages of total transcripts = 100%.

Percentage and organ	3′Probes cd 1	cd 2	cd 3	cd 4	cd 5	cd 6
Cotyledons, 1 week old	30	28	13	3	1	26
Leaves, 2 weeks old	29	34	13	3	1	20
Leaves, 3 months old	14	44	11	<1	<1	31
Stems, 3 months old	11	56	12	<1	<1	22
Fruits, 3 months old	15	62	11	<1	<1	11

Several Lhc b 1 genes are clustered in the genome of *Petunia* (Dunsmuir, 1985), tomato (Pichersky *et al.*, 1985), *Arabidopsis* (Leutwiler *et al.*, 1986) and *Nicotiana plumbaginifolia* (Castresana *et al.*, 1987). The genes reacting with the probes cd 1, cd 2 and cd 3 are also located close to each other on the genomic DNA of cress (Brunner, 1993). The biological significance of differential expression of such genes during development (here: genes hybridizing with cd 1 and cd 2 (see Table V) is not clear; it can not even be predicted whether the differential expression could be detected on the protein level since the amino acid sequences of these genes – as far as they were determined in CC1 and CC2 – are 100% identical.

Highly significant is the difference between Lhc b 1 genes (detected with probes cd 1, cd 2 and cd 3) and Lhc b 2 (probe cd 4) or Lhc b 3) (probe cd 5) genes. The transcript levels of Lhc b 2 and Lhc b 3 genes are 1–2 orders of magnitude smaller than the level of Lhc b 1 transcripts in cress throughout development (see Table V). A similar difference between Lhc b 2 and Lhc b 1 transcripts was found in leaves of tomato (Piechulla *et al.*, 1991) and buds and leaves of pea (White *et al.*, 1992). A higher amount (about 25% of total transcripts) was found for Lhc b 3 transcripts in pea, contrary to the levels found in cress. The usually low level of Lhc b 3 apoprotein (Jansson, 1992; Green *et al.*, 1992; Peter and Thornber, 1991) corresponds to the low manuscript level in cress rather than the relative high transcript level in pea. For detailed consideration of physiological significance, the apoprotein levels have to be determined as well as transcript levels in future investigations.

Acknowledgements

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