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Suppression subtractive hybridization identifies differentially expressed genes in *Brassica napus* chlorophyll-reduced mutant

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Abstract

Suppressive subtraction hybridization (SSH) was used to identify differentially expressed genes caused by a chlorophyll-reduced mutation in *B. napus*. The cDNA fragments, derived from SSH positive subtractive library (tester: normal wild type, driver: mutant) were cloned into pMD18-T vector. Two hundred SSH cDNA clones were screened by dot blot array, and 151 clones were identified as differentially expressed cDNA fragments in Cr3529 line. Thirty-six positive clones which showed marked expression differences were selected and sequenced. After redundant cDNAs were removed, 33 differentially expressed unique cDNA section clones were obtained. Among the 33 clones, two clones possess different parts of the cDNA sequence of the same gene coding geranylgeranyl reductase, four clones belong to unknown proteins, and the rest share homology to genes of diverse class. Sequence analysis showed that at least 12 genes were discovered to be related to the photosynthesis, seven of them coded the proteins which belong to the subunit of photosystem 2. RNA gel blot analysis showed that compared with 3529, the gene expression of the chlorophyll *a*/*b*-binding protein *Lhc*b2 in photosystem 2 declined markedly in the cotyledons and seedling leaves of Cr3529, indicating that the reduced light-harvesting complex 2 accumulation in thylakoid membrane of Cr3529 was due to the decrease of the related gene mRNA level for translation.

Additional key words: oilseed rape, RNA gel blot analysis, sequence analysis, subtractive library.

Introduction

The *Brassica napus* line Cr3529 was a chlorophyll-reduced (CR) seedling mutant obtained from the doubled diploid inbred line 3529 induced by fast neutron and diethyl sulfate (Zhao *et al*. 2000). The chlorophyll content in the leaves of young CR seedlings was about one half of that of wild type (WT) seedlings, resulting in yellow-green cotyledons and leaves of the CR seedlings. After the five-leaf stage, the chlorophyll content of CR seedlings increased gradually as plants grew older, and the oldest leaves of mature plants had an appearance closer to that of the WT. The CR seedling trait was controlled by a pair of nuclear recessive genes and when the *Cr* gene was in the heterozygous condition, it had no deleterious effects on yield characteristics and disease resistance. Thus the CR trait can be used as a seedling marker to produce F_1 hybrids (Zhao *et al*. 2000, Wang *et al*. 2003).

Compared with the wild type, the plastids in Cr3529 had fewer and smaller grana and the average number of lamellae per grana was 5.45, about one half of that of the WT (Zhao *et al*. 2003). The mild electrophoresis of the pigment-protein complexes of thylakoid membrane revealed that the pigment-protein composition of Cr3529 changed and the protein gel blot analysis showed that the polypeptide of the major light-harvesting complex 2 (LHC 2) of photosystem 2 (PS 2) in *Cr*3529 thylakoid membrane decreased markedly (Zhao *et al*. 2001).

In this study, we used suppression subtractive hybridization (SSH) (Diatchenko *et al*. 1996) to identify differentially expressed genes in young seedlings between lines Cr3529 and its WT line 3529 in order to reveal the molecular mechanism of the mutation characters.

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Abbreviations: CR - chlorophyll-reduced; LHC 2 - light-harvesting complex 2; PCR - polymerase chain reaction; PS 2 - photosystem 2; SSH - suppressive subtraction hybridization; WT - wild type.

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Materials and methods

Plants: The line 3529 of *Brassica napus* L. is a homozygous diploid inbred from gynogenetic haploid development and the line Cr3529 was a CR seedling mutant obtained from the line 3529 induced by fast neutron and diethyl sulfate (Zhao *et al*. 2000). The line Cr3529 and its near-isogenic line wild type 3529 were cultivated in the test field or in flowerpot in Sichuan University, Chengdu, China. The sowing was during the first ten days of September. The temperature was about 25 °C, and seed germinated and seedling grew fast during this period. Forty days after sowing, the young leaves were selected for SSH from seedlings at five-leaf stage.

Total RNA extraction and mRNA isolation: Fresh tissue (4 g) was put in liquid nitrogen in a mortar and grinded quickly to a fine powder. Total RNA was extracted according to the method described by Clark (1997). The total RNA was dissolved in 0.05 cm^3 ddH_2O that had been treated with diethyl pyrocarbonate. *Smartsec*^{TM}*plus s*pectrophotometer (*Bio-Rad Laboratories,* Hercules, CA, USA) was used to determinate the RNA concentration and purity. The Poly(A)-RNA was purified from the total RNA by using polyATtract mRNA isolation systems III kit (*Promega*, Madison, Wisconsin, USA).

Suppression subtractive hybridization: SSH was performed with the PCR-select cDNA subtraction kit (*Clontech*, Palo Alto, CA, USA) as directed by the manufacturer's instructions. Double-stranded cDNA was prepared from 2 μg of $Poly(A)^+$ wild type RNA (tester population) and mutant RNA (driver population). The cDNA was then digested with *Rsa*I. In two separate ligations, the tester cDNA was ligated to adapters 1 and 2. In the first hybridization, an excess of driver cDNA was hybridized at 68 °C for 8 h with the tester cDNA ligated to adapter 2 in reaction 2. In the second hybridization, reactions 1 and 2 were hybridized together in the presence of fresh driver cDNA at 68 °C overnight. The substractive product was amplified by PCR using oligonucleotides that were complementary to adapters 1 (5'-CTAATACGACTCACTATAGGGCTCGAGCGGCC GCCCGGGCAGGT-3' 3'-GGCCCGTCCA-5' and 2 (5'-CTAATACGACTCACTATAGGGCAGCGTGGTCG CGGCCGAGGT-3' 3'-GCCGGCTCCA-5').

Polymerase chain reaction (PCR) was performed according to the following parameters: 75 °C for 5 min and 27 cycles at 94 °C for 30 s, 66 °C for 30 s, and 72 °C for 1.5 min. Then, a nested PCR was performed as follows: 12 cycles at 94 °C for 30, 66 °C for 30 s, and 72 °C for 1.5 min. The final PCR product was identified as dominantly expressed cDNA and corresponded to the gene population over expressed in the WT seedlings.

In addition, the *B. napus* chloroplast triazine-resistance protein (*psb*A) gene was amplified with subtracted and unsubtracted cDNA populations as templates, and the amplified products were compared to evaluate the efficiency of cDNA subtraction by electrophoresis.

Construction of the subtracted cDNA library (T/A cloning): Products of the secondary PCR from the forward subtraction were purified using *E.Z.N.A* cycle-pure kit (*Omega Bio-tek*, Doraville, GA, USA). The purified products were then ligated into a *pMD-18T* vector (*TaKaRa Biotechnology*, Dalian, China) to transform *Escherichia coli* JM109 cells. Colonies were grown on Luria-Bertani (LB) agar plates containing 50 μg cm^{-3} ampicillin (*Amresco*, Solon, OH, USA), 1 mM isopropyl-D-thiogalactopyranoside (*Gibco BRL*, Grand Island, NY, USA) and 80 mg 5-bromo-4-chloro-3-indolyl bd-galacto-pyranoside (*Gibco BRL*). Transformation efficiency was approximately 10^7 colonies per 1 μg of starting DNA. Individual recombinant white colonies were picked up and grown in LB medium containing ampicillin (50 μg) on 96-well microtiter plates.

Differential screening of subtracted library: Bacterial culture of white colonies was amplified directly using PCR in a volume of 0.025 cm^3 with nested primer 1 (5'-TCGAGCGGCCGCCCGGGCAGGT-3') and nested primer 2R (5'-AGCGTG GTCGCGGCCGAGGT-3'). One mm^3 of PCR products was then dot blotted onto nylon membranes (*Hybond* N^+). Four identical membranes with cDNA arrays in duplicate were prepared.

DIG-labelled screening probes were prepared according to DIG DNA labelling and detection kit (*Roche Molecular Biochemicals*, Penzberg, Germany). Four DIG-labelled differential screening probes were prepared: two subtracted cDNA probes and two unsubtracted cDNA probes. Four different DIG-labelled probes were hybridized to four identical membranes. Dot blot hybridization and washing were carried out according to the manufacturer's instructions. Result analysis and classification of differentially screened clones were performed according to the protocol recommended.

cDNA sequencing and sequence analysis: The PCR products were cloned into the *p*MD18-T vector (*TaKaRa Biotechnology*, Dalian, China) and sequenced by the chain termination reaction using an automated sequencer (*SEQLAB*, Gottingen, Germany). The homology searches for the nucleic acid and protein were performed using the *BLAST* program at the National Center of Biotechnology Information.

RNA gel blot analysis: Ten μg total RNA was electrophoresed on 1 % agarose-formaldehydegels, then transferred and cross-linked to nylon membrane (*Boehringer* Mannheim, Germany) using the standard methods (Sambrook *et al*. 1989), and hybridized in a solution containing 0.5 M sodium phosphate (pH 7.0), 1 mM EDTA, 1 % BSA, and 7 % SDS for 20 h at 65 °C. After hybridization, the blot was washed twice with a

solution containing 0.1× SSC and 0.1 % SDS for 5 min at room temperature, followed by two washes of the same solution at 55 °C for 20 min. Hybridization was performed with the α-^{32}P-dCTP labeled probe of selected cDNA clone by the random priming method (Sambrook *et al*. 1989). The blot was exposed to *Kodak* X-ray film between two intensifying screens at -80 °C. The autoradiogram was developed after 100 h of exposure.

Results

Qualitative analysis of RNA: Plenty of total RNA with high quality is the base for suppression subtractive hybridization. The use of the protocol described here resulted in RNA with high quality. RNA examined by electrophoresis on 1 % agarose/TAE gels showed a 28s rRNA band which was more abundant than the 18s rRNA band, indicating that little or no RNA degradation occurred during the extraction (Fig. 1). As a chloroplast itself possesses rRNAs, more than two rRNA bands were observed (Fig. 1).

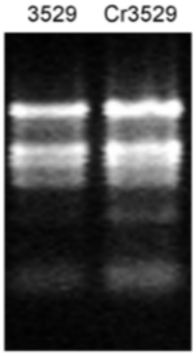

Fig. 1. The total RNAs isolated from young seedling leaves of 3529 and Cr3529. Total RNA was extracted according to the method described by Clark (1997).

The typical A_{260}/A_{280} absorbance ratios of the RNA ranged from 1.8 - 2.0, indicating that little or no protein contamination has occurred (Schultz *et al*. 1994). The A_{260}/A_{230} ratios were greater than 1, indicating that little or no polysaccharide or polyphenol contamination existed (Schultz *et al*. 1994). The A_{260}/A_{280} absorbance ratios of the purified poly(A)$^+$ RNA of both leaves were greater than 1.9. A clear smeared band with a M_r of greater than 0.5 kb was present on the 1 % agarose gel (Fig. 2), indicating that the quality of the obtained poly (A)$^+$ RNA was sufficient for further use.

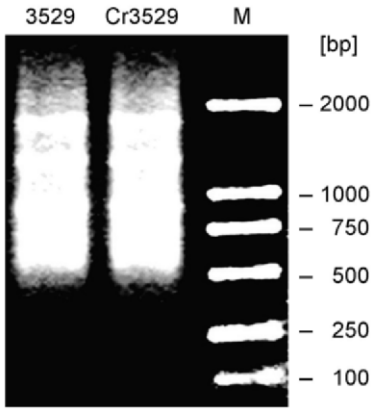

Fig. 2. The mRNAs isolated from young seedling leaves of 3529 and Cr3529. Poly(A)-RNA was purified from total RNA by using polyATtract mRNA isolation systems III kit (*Promega*).

Evaluation of subtraction efficiency: The key to obtaining successful SSH result was to effectively eliminate uniform cDNA appearing in both testers and drivers (Diatchenko *et al*. 1996). The *B. napus psb*A gene sequence (GenBank Accession No. M36720) was analyzed for restriction sites of *Rsa*I. Two primers, psbA5 and psbA3, were designed to amplify a segment (466 bp) between the two *Rsa*I restriction sites. PCR amplification of this segment showed that it appeared after 18 cycles when using the unsubtracted tester cDNA as a template but did not appear until after 23 cycles when using the subtracted cDNA as a template (Fig. 3). This indicated that cDNA homologous to both tester and driver has been eliminated to a certain extent. The difference of the amplification patterns between subtracted and unsubtracted cDNAs indicated a successful subtraction (Fig. 4).

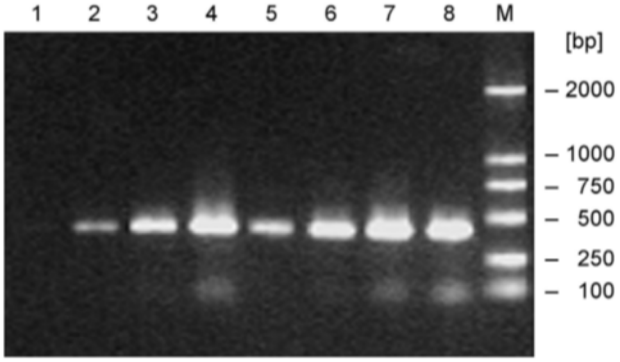

Fig. 3. Estimation of the subtraction efficiency by means of amplifying the *Psb*A gene. The *B. napus psb*A gene sequence (GenBank Accession No. M36720) was analyzed for restriction sites of *Rsa* Ⅰ. Two primers, psbA5 and psbA3, were designed to amplify a segment (466 bp) between the two *Rsa* Ⅰ restriction sites with subtracted and unsubtracted cDNA populations as templates, respectively. *Lanes* 1 to 4: the subtracted cDNA as a template; *Lanes* 5 to 8: the unsubtracted tester cDNA as a template; *Lane* 1, 5: 18 cycles; *Lane* 2, 6: 23 cycles; *Lane* 3, 7: 28 cycles; *Lane* 4, 8: 33 cycles.

Differential screening of SSH clones: The forward secondary PCR products were cloned into pMD-18T vectors after purification and transformed into the competent cells of *E. coli* JM109. A total of 1000 white clones were obtained. There were 900 fully separated

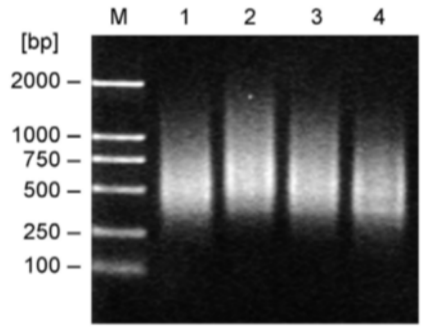

Fig. 4. The second PCR products of subtractive hybridization. SSH was performed with the PCR-Select cDNA subtraction kit (*Clontech*) as directed by the manufacturer's instructions. The substractive product was amplified by PCR using oligonucleotides that were complementary to adapters 1 and 2. *Lane* 1: PCR products of forward subtracted hybridization; *Lane* 2: PCR products of forward unsubtracted hybridization; *Lane* 3: PCR products of reverse subtracted hybridization; *Lane* 4: PCR products of reverse unsubtracted hybridization.

positive clones that were selected for further PCR amplification. The results revealed that the clones had different sizes of cDNA inserts (ranged from 200 - 1000 bp) (Fig. 5).

Two hundred positive clones confirmed by PCR amplification were randomly selected and screened by dot-blotting analysis. This experiment was repeated once to avoid obtaining pseudo positive result. Partial hybridization results with forward and reverse subtracted probes were shown in Fig. 6. Finally, 151 clones potentially overexpressed in wild type were screened out.

Sequence data analysis: Among the 151 positive clones, 36 clones which showed the marked expression differences were selected to have their inserts sequenced and Blast searches for the 36 clones were performed. After removing redundant cDNAs, 33 differentially expressed unique cDNA section clones were obtained. Among the 33 clones, D5 and D22 possess different parts of the cDNA sequence of the same gene coding

Table 1. Features of forward subtraction clones and results of *BLAST* search.

Code	Clone	Size [bp]	DNA homology	Accession number	Identities (aa/aa)
1	A26	173	carnitine/acylcarnitine translocase-like protein (*A. thaliana*)	AAM97040	35/48 (72 %)
2	A44	737	chlorophyll a/b-binding protein CP26 in PS 2 (*B. juncea*)	CAA65042	160/177 (90 %)
3	A47	224	putative alanine aminotransferase (*A. thaliana*)	AAK25905	74/75 (98 %)
4	B5	206	PS 2 protein W homolog T5F17.110 (*A. thaliana*)	T10660	37/39 (94 %)
5	B29	236	neoxanthin cleavage enzyme-like protein (*A. thaliana*)	CAA16706	51/54 (94 %)
6	B44	222	unknown protein (*A. thaliana*)	AAL36158	46/51 (90 %)
7	B50/C22	291	signal recognition particle receptor beta subunit-like protein (*A. thaliana*)	BAB09661	75/86 (87 %)
8	B51	170	plastidic glutamate--ammonia ligase precursor (*B. napus*)	CAA51280	56/56 (100 %)
9	B52	335	elongation factor 1 alpha subunit [*Malusx domestica*)	CAA11705	103/106 (97 %)
10	B54/C11	258	calcium-binding protein, putative (*A. thaliana*)	AAK76479	37/38 (97 %)
11	C2	181	unknown protein (*A. thaliana*)	AAL91144	56/59 (94 %)
12	C3	144	unknown protein (*A. thaliana*)	AAB87114	25/28 (89 %)
13	C9/C47	198	LHC 2 Type III chlorophyll a /b binding protein (*B. napus*)	CAA43802	44/44 (100 %)
14	C16	601	PS 2 32 kDa protein (*Bryum coronatum*)	AAN85803	170/187 (91 %)
15	C17	196	hypothetical protein (*Guillardia theta*)	AAK39727	14/30 (47 %)
16	D5	162	geranylgeranyl reductase (*A. thaliana*)	CAA74372	52/53 (98 %)
17	D12	189	uridylyltransferase-related (*A. thaliana*)	NP_564010	58/62 (93 %)
18	D19	480	PS 2 reaction center W (PsbW) protein-related (*A. thaliana*)	NP_180615	79/120 (65 %)
19	D22	172	geranylgeranyl reductase (*A. thaliana*)	CAA74372	56/57 (98 %)
20	D23	257	stress enhanced protein 1 (SEP1) (*A. thaliana*)	NP_567958	36/59 (61 %)
21	D28	333	chitinase-like protein 1 (CTL1) (*A. thaliana*)	NP_172076	105/110 (95 %)
22	D30	122	dehydration-induced protein (ERD15) (*A. thaliana*)	NP_181674	17/19 (89 %)
23	D34	134	chlorophyll a/b-binding protein-like (*A. thaliana*)	CAB39787	16/19 (84 %)
24	D42	185	40S ribosomal protein S29 (RPS29A) (*A. thaliana*)	NP_189984	49/54 (90 %)
25	E7	315	serine hydroxymethyl transferase (*A. thaliana*)	CAB71289	96/101 (95 %)
26	E10	189	plasma membrane intrinsic protein 2 (*B. napus*)	AAD39374	60/62 (96 %)
27	E11	61	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, putative (*A. thaliana*)	NP_850971	16/19 (84 %)
28	E33	169	S-adenosyl L-homocystein hydrolase (*A. thaliana*)	AAL24370	31/52 (59 %)
29	E39	114	fructose-1,6-bisphosphatase precursor (*B. napus*)	AAD12243	37/37 (100 %)
30	E40	127	succinate dehydrogenase cytochrome b subunit family protein (*A. thaliana*)	NP_196522	35/42 (83 %)
31	E44	232	chlorophyll a/b-binding protein *Lhc* b2 (*A. thaliana*)	T52322	64/68 (94 %)
32	E46	303	vacuolar ATP synthase 16 kDa proteolipid subunit 5 (*A thaliana*)	NP_179244	63/76 (82 %)
33	E53	266	40S ribosomal protein S26 homolog (*A. thaliana*)	CAB87433	57/72 (79 %)

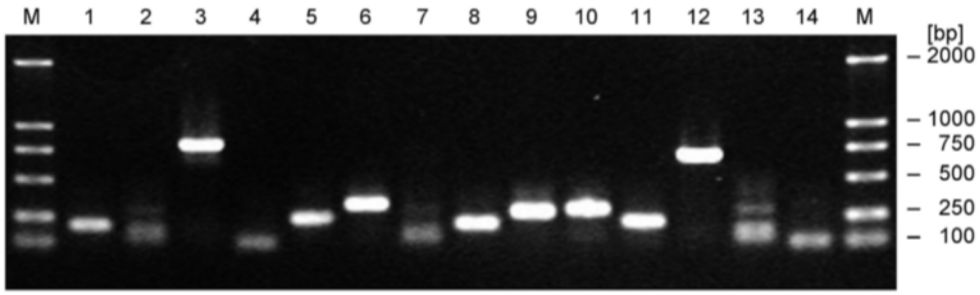

Fig. 5. The insets of 14 randomly selected clones in forward subtracted library. Products of the secondary PCR from the forward subtraction were purified and ligated into a pMD-18T vector to transform *E. coli* JM109 cells. Bacterial culture of white colonies was amplified directly using PCR in a volume of 0.025 cm^3 with nested primer 1 (5'-TCGAGCGGCCGCCCGGGCAGGT-3') and nested primer 2R (5'-AGCGTG GTCGCGGCCGAGGT-3').

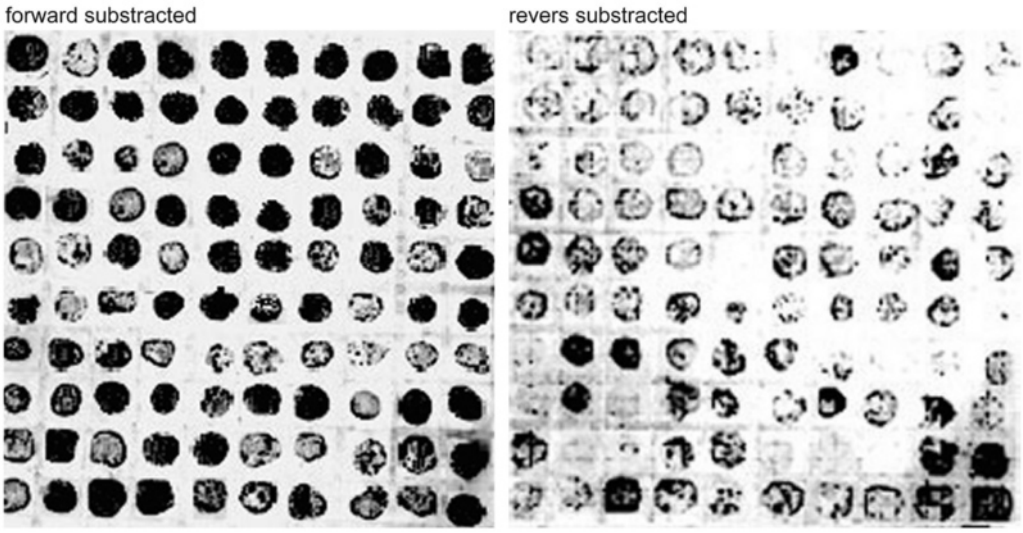

Fig. 6. The positive clones confirmed by PCR amplification in forward subtracted library were dot blot analyzed. DIG-labeled screening probes were prepared according to DIG DNA labeling and detection kit (*Roche Molecular Biochemicals*). 1 mm^3 of PCR products in forward subtracted library was dot blotted onto nylon membranes (*Hybond-N*$^+$) and hybridized with forward subtracted cDNA probe and reverse subtracted cDNA probe respectively.

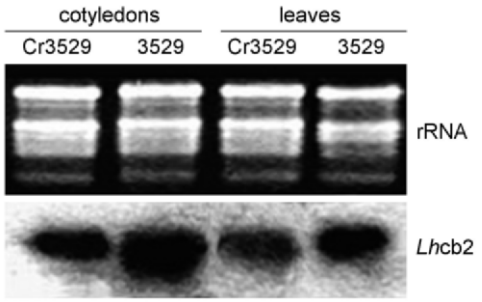

Fig. 7. RNA gel blot verification of *Lhc*b2 in cotyledons and seedling leaves of Cr3529 and 3529. 10 μg of total RNAs isolated from cotyledons and seedling leaves of Cr3529 and 3529 respectively were separated in a formaldehyde gel and blotted onto a *Hybond N*$^+$ membrane. RNA gel blotting was used to hybridize with the probe, the clone E44 fragment labeled with the α-^{32}P-dCTP by the random priming method. The cotyledons were germinated for six days and seedling leaves were at five-leaves stage.

geranylgeranyl reductase, four clones belonged to unknown proteins (B44, C2, C3, C17), and the rest of the clones shared homology to diverse classes of genes with *B. napus, B. juncea*, *Arabidopsis thaliana*, *Malus* × *domestica*, *Bryum coronatum* and *Guillardia theta* in GenBank (Table 1). Sequence analysis showed that at least 12 genes were discovered to be related to the photosynthesis, seven of them coded the proteins which belonged to the subunit of PS 2, *i.e.* chlorophyll *a*/*b*-binding protein CP 26 (A44), PS 2 protein W homolog T5F17.110 (B5), LHC 2 Type III chlorophyll *a*/*b* binding protein (C9/47), PS 2 32 kDa protein (C16), PS 2 reaction center W protein-related (D19), chlorophyll *a*/*b*-binding protein-like (D34) and chlorophyll *a*/*b*-binding protein Lhcb2 (E44). D5 and D22 clones were homologous to a putative *chl P* gene (geranyl-geranyl reductase) which was supposed to be related to the synthesis of chlorophyll (Addlesee *et al*. 1996, Tanaka *et al*. 1999).

RNA gel blot analysis: The gene expression of the chlorophyll *a*/*b*-binding protein Lhcb2 which corresponds to the clone E44 codes was examined in cotyledons and seedling leaves of 3529 and Cr3529 by RNA gel blot analysis. The cDNA fragment of the clone E44 labeled with α-^{32}P-dCTP was used as a probe in hybridization of equal amounts of total RNA from cotyledons germinated for 6 d and seedling leaves at five-leaf stage, respectively. The RNA gel blot showed that the signal intensity in 3529 is much stronger than that in Cr3529 either in cotyledons or in seedling leaves and that the signal intensity in cotyledons is quite strong compared to that of leaves in both Cr3529 and 3529 (Fig. 7).

Discussion

As the result of a single gene mutation occurred in Cr3529, chloroplast structure and most growth characteristics changed (Zhao *et al*. 2000, 2001, 2003). In this study, SSH results showed that the expressions of many genes were reduced in Cr3529 and some down-regulated genes were related to the chloroplast development and the process of photosynthesis which might cause the change of the chloroplast structure and growth characteristics, which result in the low seed yield of the line Cr3529.

It was found that the composition of the pigment-protein of Cr3529 was changed and the content of LHC 2 in Cr3529 was about one-third of that in the wild type (Zhao *et al*. 2001). The result of SSH showed that 7 down-regulated genes in Cr3529 belonged to the subunit of PS 2. RNA gel blot result confirmed that the gene expression of the chlorophyll *a*/*b*-binding protein Lhcb2 coding the sub unit protein of LHC 2 was reduced markedly in Cr3529. These results indicated that the reduced LHC 2 accumulation in thylakoid membrane of Cr3529 was due to a decrease level of the mRNA of the related gene for translation.

The enzyme geranyl-geranyl reductase (Chl P), which the clone D5 and D22 encode, catalyzes the reduction of free geranyl-geranyl diphosphate to phytyl diphosphate, providing the side chain to chlorophylls, tocopherols and plastoquinones (Addlesee *et al*. 1996, Tanaka *et al*. 1999). As a chemical singlet oxygen quencher, α-tocopherol can protect thylakoid membranes against photodestruction through lipid peroxidation and maintain PS 2 structure and function (Trebst *et al*. 2002, Havaux *et al*. 2005). The *chl P* gene is up-regulated during etioplast to chloroplast and chloroplast to chromoplast development (Keller *et al*. 1998). In transgenic tobacco plants expressing antisense RNA for geranyl-geranyl reductase, the reduced activity of geranyl-geranyl reductase leads to loss of chlorophyll and tocopherol (Tanaka *et al*. 1999, Havaux *et al*. 2003). The phenotype of transgenic tobacco plants was similar to that of Cr3529. Therefore, the expression reduction of the *chl P* gene in Cr3529 may be one of the factors causing fewer and smaller grana in chloroplast and the reduced chlorophyll content in *Cr*3529 leaves.

Besides the photosynthesis, the down-regulated genes in Cr3529 are related to many aspects of plant cells such as signal transduction, the metabolism of amino acids, saccharides and fatty acids, secondary metabolites, stress resistance, *etc*. Our data suggest that, although the mutation was on a single gene locus, the CR mutation causes changes of a series of genes at the transcription level and affect various metabolism processes in Cr3529.

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Jerusalem artichoke (*Helianthus tuberosus* L.) is one of rich plant sources for inulin and other fructooligosaccharides {as *e.g.* yacon [*Smallanthus sonchifolius* (Poepp. *et* Endl.) H. Robinson]} that may provide dietary health benefits for obesity, diabetes, and several other health issues and with its possible use for biofuels is drawing tremendous recent interest. With its ready cultivation and minimal pest and disease problems, Jerusalem artichoke is an underutilized resource that possesses the potential to meet major health and energy challenges. "Biology and Chemistry of Jerusalem Artichoke" is a comprehensive, up-to-date book, which presents the unique biological and chemical properties of this crop. Citing a diverse cross-section of references, it reviews the history, classification, morphology, and anatomy of the plant. It details inulin chemistry addressing its properties and structure, extraction, and modification using microbes, enzymes, and a wide range of chemical processes. The book examines the use of Jerusalem artichokes as a biofuel and the role of inulin derived from the crop in combating obesity and diabetes, as well as promoting bone, blood, bowel, and immune health. A comprehensive chapter addresses genetic resources, breeding, breeding methods, hybridization, and the heritability of important traits. The book details developmental biology in terms of maximizing yield and determining resource allocation as well as controlling pests and disease. It concludes with practical information on agronomic methods, storage, the economics of crop production, and future prospects for utilization. This book provides the most comprehensive resource to date on this extremely useful crop and could serve as a valuable single reference source. The book focuses on Jerusalem artichoke as a source of inulin production and presents an up-to-date review of research on inulin and other fructooligosaccharides (FOS), and their derivatives with healthy and beneficial effects against diabetes and obesity. The authors provide industrial applications of Jerusalem artichoke, as well as its use as a feedstock for the production of biofuel. They address genetic resources, breeding, and the heritability of important traits and explain developmental biology within the context of maximal yield and resource allocation as well as offer information on agronomic methods, storage, economics, and future prospects for utilization. The book is divided into fourteen chapters detail dealing with all aspects of this crop – beginning from its nomenclature, identification, origin and history, distribution, following morphological differences between cultivars and clones. Significant chapter is devoted to tuber chemical composition, inulin and FOS chemistry, methods of their isolation, extraction, purification, fractionation, analysis, drying and storage, sources of insulin, uses for native and fractionated insulin, microbial and enzymatic modification of insulin, chemical modification of inulin and value in human and animal diet. In the other part the use of biofuel biomass, direct combustion, and biological conversion is discussed. Other chapters inform a reader about genetic resources, breeding and cultivars, breeding programs, cytology, interspecific hybrids, controlled crosses, traditional breeding, breeding techniques, flowering time, manipulation, irradiation, selection criteria, selection sequence, transgenic plants, genetic resources, molecular genetics, cultivars and clones. There is also in detail described fructan metabolism, additional metabolic pathways, yield, growth analysis and modelling, environmental factors affecting yield, agronomic practices, planting date, planting, weed control, fertilization, irrigation, harvesting and handling, as well as different pests and diseases, insect pests, molluscs, nematodes and other pests, fungal, bacterial and viral diseases characteristic for the Jerusalem artichoke. On the basis of 25 years running of their own research work with this plant and an extensive bibliography, the authors discuss propagation, tubers, rhizomes, tissue culture, slips, cuttings, seed developmental biology, resource allocation, and yield, developmental stages, photo-synthesis, respiration, assimilate allocation strategy, carbon transport, sink strength in relation to allocation, assimilate allocation and redistribution, storage options, storage losses and alterations in composition during storage, controlled atmosphere storage, effect of irradiation and economics and future prospects for artichoke utilizing. Thus, this book could be a very useful source of up-to-date information for both, experimental botanists, biochemists and physiologists, as well as for specialists, who are interested in the breeding, cultivation and many-sided utilization of this crop.

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