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Full Length Research Paper

cDNA cloning and expression of anthocyanin biosynthetic genes in wild potato (*Solanum pinnatisectum*)

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The purple anthocyanins in potato tuber skin and flesh are primarily derived from petunidin. cDNA clones encoding the enzymes involved in anthocyanin biosynthesis, chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DRF), and UDP-glucose: flavonoid 3-0-glucosyltransferase (3GT), were isolated from *Solanum pinnatisectum* by RT-PCR with degenerated primers. Sequence comparison showed that they share 76-96% identities with each corresponding solanaceous gene reported previously. Each gene is a member of a multigene family. The spatial expression analysis indicated that these genes were preferentially expressed in flowers, stolons and terminal buds, and their transcripts could not detected in roots except 3GT. All the genes were induced expression in tuber skins by white light, and followed by anthocyanin accumulation. This is the first report cloning and expression of anthocyanin biosynthetic genes in wild potato (*Solanum pinnatisectum*).

Key words: Anthocyanin, expression, gene cloning, wild potato species.

INTRODUCTION

Anthocyanins are the largest subclass of flavonoids, which are the major color pigments in plants. Anthocyanins are responsible for red, blue and purple colors; these secondary products in plant have gained a great deal of attention over the years (Winkel-Shirley, 2001). For plants, Anthocyanins play important roles in phytoprotection, in attracting insects and animals for pollination and seed dispersal, in signaling between plants and microbes, in male fertility of some species, in defense as antimicrobial agents and feeding deterrents (Gould et al., 1995; Holton and Cornish, 1995). Anthocyanins are used as natural colourants for foods and beverages, and they have been shown to inhibit the growth of cultured human malignant cells (Kamei et al., 1993), and they have demonstrated excellent anti-inflammatory and antioxidant properties (Wang et al., 1999; Liu et al., 2002).

Anthocyanin biosynthesis and its regulation have been well characterized in several model plants like petunia (*Petunia hybreda*), maize (*Zea mays*) and arabidopsis (*Arabidopsis thaliana*) (Holton and Cornish, 1995; Bartel and Matsuda, 2003). In most crops, the identity of the genes underpinning natural variation in the types, levels, and tissue-specific expression patterns of anthocyanins are still not known (De Jong et al., 2004). Many genes that influence anthocyanin pigments have been isolated from Solanaceae. A few genes of anthocyanin synthesis have been isolated and characterizated from potato (*Solanum tuberosum* L.) such as chalcone synthase (CHS) (Jeon et al., 1996), dihydroflavonol 4-reductase (DFR) (De Jong et al., 2003) and flavonoid-3',5'-hydroxylase (F3'5'H) (Jung et al., 2005). Anthocyanins may produce anywhere in potato plant, e.g., in tuber, flowers, sprouts or stem (Jung et al., 2005). The tubers of wild potato species are only purple or white (Dodds and Long, 1955), and the purple anthocyanin pigments are primarily derived from the related compound petunidin, while the red anthocyanin pigments are derived from pelargonidin (Lewis et al., 1998). A schematic diagram of anthocyanin biosynthetic pathway is shown in Figure 1.

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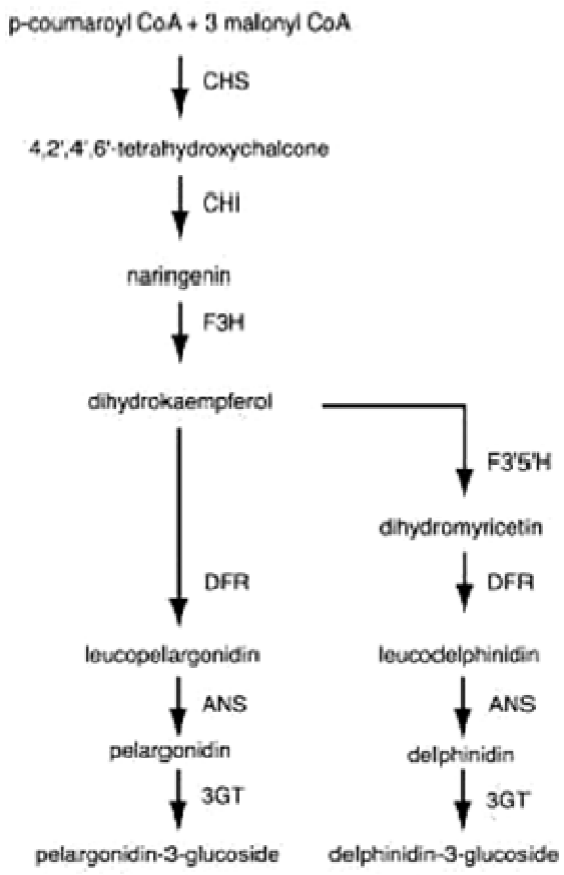

Figure 1. Schematic diagram illustrating the possible initial stages of the anthocyanin biosynthetic pathway, which could be involved in Solanaceae. Enzyme abbreviations are as follows: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol 4-reductase; F3'5'H, flavonoid 3', 5'-hydroxylase; ANS, anthocyanidin synthase; 3GT, UDP-glucose anthocyanidin 3-O-glucosyltransferase.

The stimulation of anthocyanin biosynthesis in plants can be influenced by many factors such as light (Lancaster, 1992; Sato et al., 1996), temperature (Mori et al., 2005), phytohormone (Mori et al., 1994; Pasqua et al., 2005), sugar (Pasqua et al., 2005; Do and Cormier, 1991), and the presence of ions (Naumann and Horst, 2003). Although these factors have been determined in other plants, they have rarely been investigated in potato.

In this study, we first isolated the four genes of anthocyanin biosynthetic enzymes, chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DRF), and UDP-glucose:flavonoid 3-0-glucosyltransferase (3GT), from *Solanum pinnatisectum* by reverse transcriptase polymerase chain reaction (RT-PCR) with degenerated primers. We also studied the spatial expression of anthocyanin biosynthetic genes, and molecular mechanism of inducing anthocyanin accumulation by light.

MATERIALS AND METHODS

Plant materials

Wild potatoes (Solanum pinnatisectum) were grown in the greenhouse at Nanjing Agricultural University. The roots, stems, leave, flowers and tubers were harvested in adult stage, respectively. Samples were frozen in liquid nitrogen and stored at -80^{O}C until analyzed. Two to three tubers per plants were wrapped in foil immediately after harvest, and maintained in darkness at 8^{O}C and 90-95% relative humidity (RH) for 20 days. The remaining tubers were exposed to the light treatments, and maintained at 8^{O}C and 90-95% RH under a 16 h (day)/8 h (night) photoperiod with light provided by fluorescent lamps at an irradiation intensity of 90μmol m-2s-1 for 20 days. Five randomly selected discs (5 mm diameter) were removed from the light treatments or dark treatments of tubers for anthocyanin content or total RNA isolation.

Cloning of *S. pinnatisectum* CHS, F3H, DFR and 3GT cDNA

Total RNA from purple spouts was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. The first strand cDNA was synthesized with 1 μg purified total RNA using the reverse transcription (RT) system (Promega) according to the manufacturer's protocol. The oligo (dT)15 was used as a primer and the reverse transcription reaction was incubated at 42^{O}C for 60 min in a total volume of 20 μl. Degenerated primers for the amplification of CHS, F3H, DFR and 3GT were designed from consensus amino acid and similarity nucleotide sequence of these genes conserved among other Solanaceae species. Oligodeoxyribnucleotide primers were synthesized by TaKaRa (Dalian). The degenerate primers used in this study were as follows: for CHS, 5' A(T/A)G(G/C)T(C/G)ACCGT(G/C)(G/A)AG(G/A)A(G/A/T)(T/G)(A/T) C 3' and 5'CTAAG(A/C/T)(T/A)G(C/A)(A/G/C)ACACT(G/A)TG(A/G)AG(A/C) AC3'. The reaction conditions were an initial denaturation at 94oCfor 4 min, 30 cycles at 94oC for 1 min, 58oC for 60 s, 72^{O}C for 1 min10 s and a final 72oC for 6 min. For F3H, the primers were 5' (A/T)TG(G/A)(C/T)(T/A)T(C/A)(A/T)A(C/A)A(C/T)TAACAG(C/A)T(C/ T)T(A/G)GC 3' and 5' (C/T)TTA(A/G)GCAA(G/A)(A/G)AT(T/C)TC(C/T)TCAAT(G/A)GGC 3'. The reaction conditions were an initial denaturation at 94^{O}C for 4min, 30 cycles at 94^{O}C for 1 min, 56^{O}C for 60 s, 72^{O}C for 1 min 10 s and a final 72^{O}C for 6 min. For DFR, the primers were 5'C(T/A)GA(A/T)AA(T/A)G(G/C)CA(A/T)G(T/T)GA(A/T)G 3' and 5'G(G/T)T(T/A)CT(A/T)GAT(T/A)T(C/G)ACC(A/T)T(T/A)(G/C)G 3'. the reaction conditions were an initial denaturation at 94^{O}C for 4 min, 30 cycles at 94^{O}C for 1 min, 50.5^{O}C for 60 s, 72^{O}C for 1 min 10 s and a final 72^{O}C for 6 min; for 3GT, the primers were 5'A(T/A)GAC(T/A)A(C/G)T(T/A)CT(C/G)A(A/T)CTTCA(T/C)AT(T/A) GCA(C/T)3' and 5'T(C/T)AAGT(A/T)(A/G)(G/A)CTTG(T/A)GA(C/G)A(T/A)T(T/A)AAC (T/A)AGC3', the reaction conditions were an initial denaturation at 94^{O}C for 4 min, 30 cycles at 94^{O}C for 1 min, 56.5^{O}C for 60 s, 72^{O}C for 1 min 10 s and a final 72^{O}C for 6 min. The PCR systems are as follow: 1 μl RT product was amplified in a 50 μl volume containing 5 μl of 10X PCR buffer with 3.8 μl of 25 mM MgCl2, 1 μl of 10 mM dNTP mixture, 1.5 U of Taq polymerase (TaKaRa, Dalian) and 1 μl of 25 pmol each primer. PCR was performed on a thermal cycler PTC-200 (MJ Research, USA). The PCR products were run on 1% agarose gel and purified with agarose gel DNA purification kit (TaKaRa, Dalian) according to the manufacturer's protocol. The purified product was then cloned into the pMD 18-T vector (TaKaRa, Dalian) and sequenced (invitrogen, Shanghai).

Sequence analysis

The sequences were compared with those in the database using BLAST Program at Genbank (http://www.ncbi.nlm.nih.gov/blast). The molecular weight was calculated by compute PI/Mw (http://us.expasy.org/tool/pi_tool.html). Nucleotide sequences were translated to amino acid sequences by Expasy (http://us.expasy.org/tool/pi_tool.html). DNAssist program (version 2.2) was used for sequence analysis. Protein sequence similarity comparisons were performed using DNAmam (version 5.22).

Gene expression semi-quantitative RT-PCR analysis

Total RNA extraction from the roots, stems, leaves, flowers, tubers, stolon and terminal buds, and reverse transcription were performed using previously described methods. The RT product was used as template, with specific primers for semi-quantitative RT-PCR. These specific primers were as follows: for CHS, 5'ATGGTCACCGTGGAGGAGTATC3' and 5'CTAAGATGCAA-CACTGTGAAGAAC3'; for F3H, 5'ATGGCTTCAACACTAACA-GCTCTAGC3' and 5'CTTAAGCAAGAATTTCCTCAATGGGC3'; for DFR, 5'CTGAAAATGGCAAGTGAAG3' and 5'GGTTCTAGAT-TTCACCATTGG3'; for 3GT, 5'ATGACTACTTCTCAACTTCA-TATTGCAC3' and 5'TCAAGTAAGCTTGTGACATTTAACTAGC3'. The reaction conditions were the same as above, but reduced to 26 cycles. The RT-PCR reaction for the house-keeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) using specific primers GAPDH-S (5'-CAAGGACTGGAGAGGTGG-3') and GAPDH-A (5'-TTCACTCGTTGTCGTACC-3') was performed as mentioned above to determine the amount of RNA among samples used in the RT-PCR reaction (Knud, 2004). The PCR products (15 µl) were separated on 1% agarose gels stained with ethidium bromide (5 µg/ml). The homogeneity of each PCR product from each gene was confirmed by direct sequencing.

Anthocyanin extraction and quantification

Tubers periderm was broke carefully with a 5 mm diameter cork borer, and discs were peeled from the cored areas. The discs were used for anthocyanin extraction and total RNA extraction. Five randomly selected discs were used per assay and extracted with 1 mL of methanol-HCl (0.1% HCl, v/v) solution and held 4^{O}C for 24 h in the dark. A 50 µL aliquot was measured spectrophotometrically at 541 nm using Ultrospec 3000 UV/visible spectrophotometer (Amersham Pharmacia, England). Anthocyanin content is expressed as content per surface area (Chen et al., 1997; Aaron et al., 2002)

RESULTS

ISOLATION OF ANTHOCYANIN BIOSYNTHESIS GENES

Four anthocyanin biosynthesis-related genes encoding CHS, F3H, DFR, and 3GT were successfully isolated from the *S. pinnatisectum* purple spout cDNA by degenerated primers designed from the conserved regions of corresponding gene of other Solanaceae plants. The comparison of identities among these genes isolated in this study and those reported from other species is shown in Table 1.

The *S. pinnatisectum* CHS cDNA (designated as SpCHS) contains an open reading frame (ORF) of 1170 bp encoding an estimated 43 kDa polypeptide composed of 389 amino acid residues. The deduce CHS protein showed more than 80% identity to CHS of other plant species. The highest identities of the encoded polypeptide SpCHS was with the CHS from Solanaceae of S. tuberosum (93%), *Lycopersicon esculentum* (92%), *Nicotiana tabacum* and *Petunia hybrida* (89%) (Table 1). The phylogenic tree based on the deduced amino acid sequence demonstrates that SpCHS belong to the corresponding CHS-subfamily enzymes (data not shown). The *S. pinnatisectum* F3H cDNA (designated as SpF3H) is an ORF of 1078 bp with a 358 deduced amino acid sequence and a predicted molecular mass of 40 kDa. The SpF3H has a high degree of homology (67-96%) to other F3Hs. The deduced amino acid sequence of SpF3H showed 96% identity with that *S. tuberosum* F3H, 88% identity with that P. hybrida F3H, and 85% identity with that Nierembergia F3H. The *S. pinnatisectum* DFR cDNA (designated as SpDFR) was isolated using PCR, of which degenerated primers were designed from the conserved regions of previously characterized DFRs (De Jong et al., 2003; Bongue-Bartelaman et al., 1994; Beld et al., 1989). The amplified PCR fragment is 1159 bp long with an OFR of 1149 bp encoding 382 amino acid residues, and the calculated molecular mass was 43 Kda. The SpDFR has highly homology (58-95%) with other plant DFRs (Table 1), and presence of a NADP-binding domain, which is a substrate specificity region of DFR enzymes (data not shown), and shows 95% identity to S. tuberosum DFR, 92% identity to *L*. esculentum DFR, 86% identity to *P. hybrida*, and 85% identity to Nierembergia DFR. The *S. pinnatisectum* 3GT cDNA (designated as Sp3GT) consists of a 1406 bp fragment with the translation start site of the major ORF at 13 and the TGA stop site a nucleotide 1359. Comparison of the length of the Sp3GT with the ORF of other 3GT gene indicates that Sp3GT cDNA shared high sequence homology with many known plant flavonoid 3-o-gulcosyltransferases. The nucleotide sequence analysis by GenBank blastn revealed that Sp3GT has the highest identities with the 3GT from *Solanum melongena* and *P. hybrida*. The ORF encoding a protein of 448 amino acid residues with calculated molecular weight of about 50 kDa. Comparison of the deduced 3GT protein sequence with those in the GenBank database revealed a significant sequence similarity with 3GTs of S. melongena (76%), P. hybrida (76%). There are two domains in the C-terminus that were well conserved among all these glucosyltransferases. These domains are the common motifs found in the family of UDP-glucose-dependent glucosyltransferases, responsible for UDP-glucose binding (Yamazaki et al., 2002). All these signatures are found in the Sp3GT (data not shown).

These clones of SpCHS, SpF3H, SpDFR, and Sp3GT have been deposited in Genbank (http://www.

Table 1. Comparison of the deduced proteins of anthocyanin biosynthesis from *S. pinnatisectum* with other related proteins.

Gene	Species	Deduced proteins		Accession number
		Length*	Identity (%)	
CHS	*S. pinnatisectum*	389	100	AY954033 (this study)
	S. tuberosum	389	93	Q43163
	Lycopersicon esculentum	389	92	P23418
	Nicotiana tabacum	389	89	Q93XP8
	Petunia hybrida	389	89	P08894
	Camellia sinensis	389	87	P48387
	Perilla frutescens	389	85	O04111
	Ipomoea batatas	389	83	Q9MB38
	Pueraria lobata	389	82	P23569
	Pisum sativum	389	80	Q01288
F3H	*S. pinnatisectum*	358	100	AY954032 (this study)
	S. tuberosum	358	96	Q8L8C8
	Petunia hybrida	366	88	O22530
	Nierembergia	367	85	Q8LP21
	Ipomoea batatas	368	82	Q9ZQS8
	Citrus sinensis	362	81	Q9ZWR0
	Fragaria ananassa	364	79	Q66ME9
	Callistephus chinensis	356	77	Q05963
	Perilla frutescens	372	74	O04112
	Zea mays	372	73	Q43262
	Ginkgo biloba	357	67	Q5XPX2
DFR	*S. pinnatisectum*	382	100	AY954035 (this study)
	S. tuberosum	382	95	Q6WG03
	Lycopersicon esculentum	379	92	P51107
	Petunia hybrida	380	86	P14720
	Nierembergia	374	85	Q8LP24
	Gerbera hybrida	366	76	P51105
	Ipomoea purpurea	386	73	Q9ZNV0
	Zea mays	353	60	O82104
	Oryza sativa	353	58	Q5VNT1
3GT	*S. pinnatisectum*	448	100	DQ087526 (this study)
	Petunia hybrida	448	76	Q9SBQ3
	Solanum melongena	433	76	Q43641
	Forsythia intermedia	454	50	Q9XF16
	Perilla frutescens	447	49	O04114
	Gentiana triflora	453	48	Q96493
	Vitis vinifera	456	47	Q9AQV0
	Hordeum vulgare	455	34	P14726
	Zea mays	471	33	Q8W1D2

*Amino acid residues.

ncbi.nlm.nih.gov) under the accession numbers AY954033, AY954032, AY954035, and DQ087526, respectively.

Spatial expression patterns of the four anthocyanin biosynthetic genes

In order to examine the expression pattern of CHS, F3H, DFR and 3GT genes in various tissues or organs of *S.*

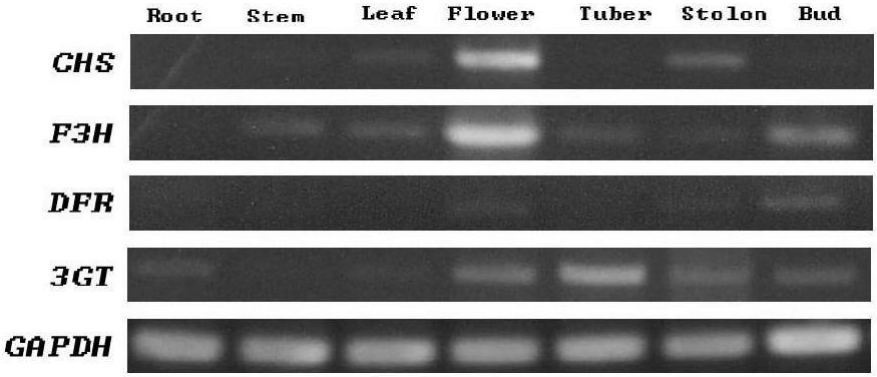

Figure 2. Expression analysis of SpCHS, SpF3H, SpDFR and Sp3GT in various tissues or organs by RT-PCR. The PCR products were separated in 1% agarose gel and visualized under UV light by ethidium bromide staining. The loading mRNA amount was standardized by comparison with PCR product of GAPDH, which generated a 380 bp PCR product.

pinnatisectum plants, total RNA from roots, stems, leaves, flowers, tubers, stolons and terminal buds were analyzed by semi-quantitative RT-PCR with the specific primers (Figure 2). The expression analysis indicated that these genes were preferentially expressed in flowers, stolons and terminal buds, and their transcripts could not detected in roots except of 3GT. The expression level of CHS in flowers and stolons was much higher than that in stems, leaves, and terminal buds; however, it was not detected in roots and tubers. The expression level of F3H was prominent in flowers and terminal buds, and declined in stems, leaves, tubers and stolons. The expression level of DFR was low in all tissues, although it could be detected in more amounts in flowers and termial buds. The transcripts of 3GT could be detected in all tissues, and they in tubers and flower were more than that in others.

Induction of anthocyanin biosynthetic genes in tuber skins by white light

The expression of the genes involved in anthocyanin biosynthesis has been shown to be induced by UVB light and white light (Sparvoli et al., 1994; Pelletier et al., 1997; Gong et al., 1997; Kim et al., 2003). We investigated the response of the four genes expression by RT-PCR analysis in *S. pinnatisectum* tubers to white light. Tuber skin tissues of different light duration at 0, 3, 10, 20 days were used for analysis of both gene expression and anthocyanin accumulation. All the transcripts were induced in the tuber skin tissues at 3 days after light exposure (DALE) (Figure 3B). Non-detectable level of the CHS transcripts was observed at 0 DALE, and increased rapidly at 3 DALE, reaching the maximum level of expression at 10 DALE and decreased slightly at 20 DALE. Both F3H and DFR transcripts increased gradually with increasing exposure time, and reached the saturation level after 20 DALE. The expression of 3GT stayed at a lower stable level from 0 DALE to 10 DALE and increased slightly at 20 DALE. Anthocyanin contents were determined at different DALE. Anthocyanin accumulation in tuber skin tissues increased rapidly from 0.027A541/surface area at 0 DALE to 2.483 A541/surface area at 20 DALE (Figure 3C). This reveals that the expression level of anthocyanin biosynthetic genes induced by white light may be closely related to the anthocyanin pigments accumulated in the tuber skin tissues by white light.

DISCUSSION

Many structural genes and regulators of anthocyanin biosynthesis have been isolated from the solanaceous species, and sequence analysis indicated that most of anthocyanin biosynthetic structural genes were quite conserved in the Solanaceae (De Jong et al., 2004). We have reported here cDNA clones of CHS, F3H, DFR and 3GT were isolated by RT-PCR with degenerate primers from *S. pinnatisectum* for the first time. Sequence comparison showed that they share 76-96% identities with each corresponding solanaceous gene reported previously (Table 1). These degenerate primers which contain the OFR of each gene were designed from the relatively conserved regions of corresponding gene of other Solanaceae plants, i.e. both the amino acid and nucleotide sequence of *S. tuberosum*, *Lycopersicon esculentum*, *Petunia hybrida*, *S.melongena, Nicotiana tabacum* and capsicum spp. In CHS-subfamily enzymes, Cys162, Phe213, Phe263, His301, and Asn338 (numbered in SpCHS and data not shown) are involved in the reactions and are strictly conserved (Suh et al., 2000). SpCHS also contains all five amino acids (370GFGPG) and this is another highly conserved family signature sequence that is common to CHS-subfamily

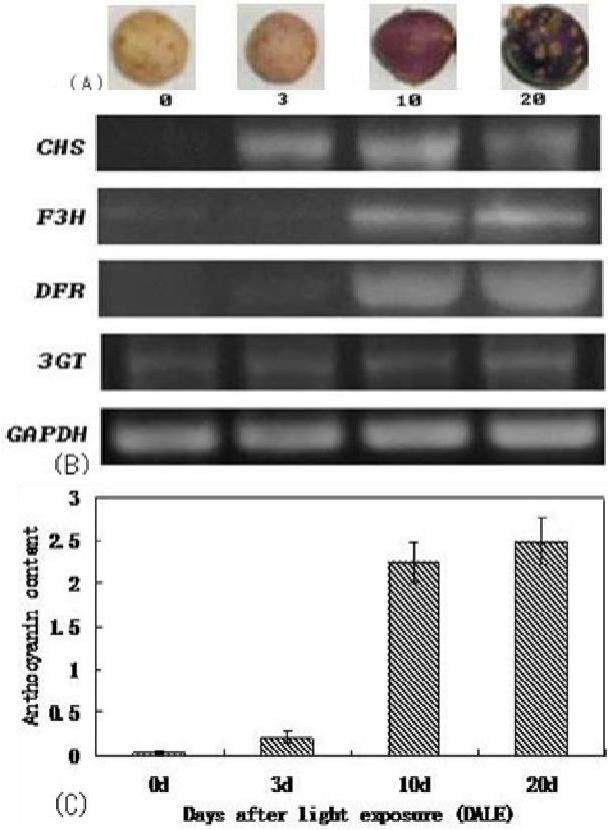

Figure 3. Temporal expression of anthocyanin biosynthetic genes and quantification of anthocyanin pigments. (A) the colour of S. pinnatisectum tuber skins change at different days after light exposure (DALE). (B) Induction of the CHS, F3H, DFR and 3GT genes in the tuber skin tissues at different DALE. Total RNA extraction from the tuber skin tissues, and the RT product was used as template, 26 cycles with specific primers for semi-quantitative RT-PCR analysis. The PCR products were separated in 1% agarose gel and visualized under UV light by ethidium bromide staining. The loading mRNA amount was standardized by comparison with PCR product of GAPDH, which generated a 380 bp PCR product. (C) Total anthocyanins content expressed as content per given surface area of tuber skins. Values are the mean ± S.D of three independent experiments.

enzymes (Suh et al., 2000). For F3H, all the amino residues that were absolutely conserved in other F3Hs were found in SpF3H (data not shown), including the five motifs in which some amino acids were conserved in the 2-oxoglutarate-dependent dioxygenase family. Three histidines, which are possibly responsible for the iron-binding function of -oxoglutarate-dependent dioxygenases, are conserved (Britsch et al., 1993; Lukacin and Britsch, 1997). Domain swapping experiments between Gerbera and Petunia have shown that the ability of DFR to metabolize dihydrokaempferol resides in the first 170 amino acids (170L) corresponding to SpDFR position 181 (181L). Moreover, altering a single amino acid at position 134 has been shown to affect substrate specificity. Gerbera amino acid position 134 (134D) corresponds to SpDFR position 145 (145D) (De Jong et al., 2003; Johnson et al., 2001). The 3GT responsible for the glucosylation of anthocyanidins to produce stable molecules in the anthocyanin biosynthetic pathway, and leading to anthocyanidin 3-glucoside is well conservative and extensively characterized (Holton and Cornish, 1995; Dooner and Robins, 1991; Yamazaki et al., 2002).

To determine the spatial expression of CHS, F3H, DFR and 3GT genes in *S. pinnatisectum*, semi-quantitative RT-PCR analysis was carried out with total RNA extracted from seven different tissues and organs. The expression analysis indicated that these genes were preferentially expressed in flowers, stolons and terminal buds, and their transcripts could not detected in roots except 3GT. SpCHS was not detected in roots and tubers. The flowers, stolons and buds from *S. pinnatisectum* are lightly purple, while roots and tubers are white. These expression patterns may be involved in the tissue-specific accumulation of anthocyanins. Some similar or different expression patterns have also been reported from *Perilla frutescens* (Gong et al., 1997), pea (Ito et al., 1997), gentian (Nakatsuka et al., 2005) and apple (Kim et al., 2003; Honda et al., 2002).

Environmental factors such as light (lancaster, 1992; sato et al., 1996; kim et al., 2003; frohnmeyer et al., 1992; koes et al., 1989; kubasek et al., 1992; toguri et al., 1993) and temperature (mori et al., 2005; lewis et al., 1999) affect the biosynthetic capacity for anthocyanin. we investigated the response of the four genes expression by RT-PCR analysis in s. pinnatisectum tuber skins to whitelight. the transcripts of these genes were barely present or non-detected in the white tuber skins before light induction, but light treatment increased their expression greatly. expression of spCHS was highest at 3 DALE, and not detected at 0 DALE while tubers were only white before light treatment. this indicate that the transcriptional activity of spCHS basically correlate to anthocyanin production in tuber skins. this result was similar with carrot (daucus carota l) cell cultures (gleitz and seitz, 1989), and different from apple (lancaster, 1992). expression of spF3H and DFR were highest at 10 DALE, while accumulation of the anthocyanin in tuber skins were highest after 20 DALE, and these indicated both enzymes might be stable (kim et al., 2003). expression of sp3GT stayed at a relative stable level, and increased slightly at 20 DALE. this indicate that 3GT was important to catalyze not only the 3-glucosylation of anthocyidins but also other flavonols (yamazaki et al., 2002), and this expression pattern induced by light was different from perilla frutescens (gong et al., 1997) and apple (malus domestica) (kim et al., 2003). Expression of sp3gt stayed at a relative stable level, and increased slightly at 20 dale. this indicate that 3gt was important to catalyze not only the 3-glucosylation of anthocyidins but also other flavonols (yamazaki et al., 2002), and this exp ression pattern induced by light was different from *perilla frutescens* (gong et al., 1997) and apple (*malus domes-*

tica) (kim et al., 2003) where 3gt were induced rapidly and strongly by light. our results suggested that different plant have a different regulation mechanism controlling the expression of structural genes. whether the uv light is responsible for the light quality should be studied further. it needs to be further addressed how regulatory genes control anthocyanin biosynthetic structural genes. recently, it has been suggested that petunia an1 was a basic helix-loop-helix (bhlh) transcriptional regulator of dfr and possible regulator of other structural genes (spelt, 2000). the jaf13 gene encodes a second bhlh regulator of anthocyanin gene expression in petunia (quattrocchio et al., 1998) and the petunia an2 gene encodes a myb domain transcriptional regulator of the anthocyanin pathway (quattrocchio et al., 1999). also, an11 is a wd40 repeat regulator of anthocyanin expression in petunia (vetten et al., 1997). low temperature has been considered to be another factor affecting the accumulation of anthocyanins and structural gene expression under the fixed lighting conditions (mori et al., 2005; lewis et al., 1999; christie et al., 1994).

Our primary study has shown that the tuber from *s. pinnatisectum* exposed to light at low temperature can slightly enhance the accumulation anthocyanin pigments. the major anthocyanins in tubers of *s. pinnatisectum* induced by white light should be further identified.

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