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ORIGINAL RESEARCH

Expression Profiles of Genes Involved in the Carotenoid Biosynthetic Pathway in Yellow-Fleshed Potato Cultivars (*Solanum tuberosum* L.) from South Korea

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Abstract Efforts are being made to identify a parental cultivar suitable for metabolic engineering of potato (*Solanum tuberosum* L.) that will elevate total carotenoid content or produce a specific type of carotenoid. As an initial step in this search, we performed high performance liquid chromatography analyses and comparisons among gene expression profiles for several cultivars domesticated or bred in South Korea. Here, the dark yellow-fleshed "Golden Valley" contained the highest level of total carotenoids (23.8 μg g^{-1} dry weight (DW)), which was 1.7- to 3.7-fold higher than those measured in other cultivars. The predominant carotenoids in "Golden Valley" were lutein (40.3% of the total), violaxanthin (29.8%), and β-carotene (8.8%), with only a trace amount of zeaxanthin (0.02%) being detected. Levels of lutein and β-carotene in that cultivar were significantly higher than in the others. Interestingly, relatively high amounts of violaxanthin were accumulated in all cultivars, ranging from 15.9% (1.0 μg g^{-1} DW in "Jowon") to 61.7% (8.2 μg g^{-1} DW in "Dejima"). In accordance with the relatively high content of total carotenoids in "Golden Valley", remarkably elevated transcripts were also accumulated for most of the genes involved in the carotenoid biosynthetic pathway. In particular, genes encoding enzymes for the first three steps of carotenogenesis—phytoene synthase, phytoene desaturase, and ζ-carotene desaturase—were most actively expressed. A relatively high level of transcript for the carotene hydroxylase (*Chy2*) gene was detected in all cultivars, including "Jowon", which had accumulated the lowest amount of total carotenoids. In contrast, almost no transcripts were detected for carotene isomerase (*CrtIso*) and *Chy1* in any of these cultivars. Our preliminary results suggest that "Golden Valley" is an excellent candidate for metabolic engineering that further increases its content of specific carotenoids, e.g., β-carotene and astaxanthin.

Keywords β-Carotene · Carotenogenesis-related genes · Carotenoids · Lutein · Potato · Violaxanthin

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Introduction

In 2006, nearly 126,215 M/T of potato (*Solanum tuberosum* L.) were produced on approximately 24,000 ha in South Korea, where the annual per capita consumption was 15.0 kg (http://www.kosis.kr). However, this level of consumption is far lower than in countries where potatoes are cultivated as a

major staple food (average consumption per capita of 75.0 kg). For the last few decades, national production in Korea has depended mainly on two cultivars: "Superior" and "Dejima" (over 90%). Recently, several other cultivars have been bred in Korea because consumers, especially in younger generations, tend to prefer various color-fleshed potatoes. For instances, cultivars Golden Valley and Haryoung have dark- or light-yellow (citrine) flesh, respectively. "Golden Valley" was registered as a new potato variety (no. 1342) in July, 2005, being named for its color (http://www.potatovalley.com). "Haryoung" was submitted for registry in January, 2006, at the Rural Development Administration (RDA). Based on breeder descriptions, both are resistant to several potato diseases, including late blight and viral infection (http://www.seed.go.kr). Another light yellow-fleshed cultivar, Jowon, also was registered as domesticated in 2001.

This yellow or orange coloring is due to the accumulation and composition of carotenoids. For instance, the flesh turns orange as the zeaxanthin content rises [7], whereas yellow flesh becomes dominant when lutein or its derivatives, e.g., lutein-5,6-epoxide, increases and levels of zeaxanthin are much lower [27]. In other tuber or root crops, their orange to red colors result from the accumulation of β-carotene, a major carotenoid in sweet potato [4] and carrot taproots [35]. White-fleshed potato cultivars, which mainly belong to *S. tuberosum*, generally contain much lower levels of total carotenoids compared with the yellow-fleshed *Solanum phureja* and *Solanum stenotomum* [5].

Potato is a popular nutritious food because it has high-quality carbohydrates, proteins, vitamins, and minerals [36]. Much attention also has been focused on its significant amounts of antioxidant compounds, such as polyphenols and vitamin C [6, 24]. In addition, Roberts et al. [32] have reported that the consumption of unpeeled cooked potatoes significantly increases lipid metabolism and antioxidant activity in cholesterol-fed rats. Potato extracts also significantly inhibit the proliferation of human breast cancer cells, via MCF-7, in a dose-dependent manner [26]. Although potato is not known as a source of provitamin A, e.g., β-carotene and β-cryptoxanthin [37], some yellow-fleshed cultivars contain a noticeable amount of lutein or zeaxanthin. These xanthophylls, oxygenated derivatives of carotenes, are synthesized through the branched pathway of carotenoid biosynthesis [12, 29]. Lutein and zeaxanthin have been extensively characterized as protective agents against age-related macular degeneration that causes blindness [1]. Epidemiological studies also have shown that certain types of carotenoids in a diet can prevent the onset of some cancers and cardiovascular diseases [15]. Therefore, metabolic engineering approaches are being taken to address how the accumulation of those particular carotenoids is induced, either by up- or downregulation of the genes involved in carotenoid biosynthetic pathways.

Condensation of two geranylgeranyl pyrophosphate to produce 15-*cis* phytoene is the first committed step in a carotenoid pathway. It is catalyzed by phytoene synthase (PSY). This is a rate-limiting step in diverse organs from various species, including tomato [16], canola [34], and marigold flowers [28]. Phytoene is converted to lycopene by three enzymes: phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), and carotene isomerase (CRTISO). This step is unique to plants; the detailed mechanism for CRTISO is still under investigation [23]. Cyclization of lycopene, the linear form of C_{40}, is carried out by two different enzymes, resulting in a branched pathway. One is lycopene β-cyclase (LCY-B) for the production of β-carotene and the other is lycopene ε-cyclase (LCY-E) for α-carotene. β-Carotene can be converted into β-cryptoxanthin and zeaxanthin by β-carotene hydroxylase (CHY). Zeaxanthin is then further modified to antheraxanthin and violaxanthin by zeaxanthin epoxidase (ZEP). α-Carotene is also further modified to lutein, lutein epoxide, and neoxanthin by α-carotene hydroxylase (LUT1; see reviews by Hirschberg [21] and Wang et al. [40]). In fruits of pepper (*Capsicum annuum* L.), antheraxanthin and violaxanthin are further modified to capsanthin and capsorubin, respectively, by capsanthin–capsorubin synthase [19].

Most carotenogenesis-related genes have been extensively characterized in plants and microorganisms. Moreover, metabolic engineering with those available gene sources from diverse species is now being actively pursued to elevate their total carotenoid contents as well as to accumulate specific carotenes in major crops, including rice [41]. In potato, overexpression of the *Erwinia uredovora crtB* gene encoding phytoene synthase increases total carotenoids up to five- to sevenfold compared with wild-type cultivars; lutein and β-carotene also are dramatically elevated [12]. A silencing approach that utilizes the zeaxanthin epoxidase gene also leads to significantly elevated amounts of zeaxanthin and total carotenoids [33]. Downregulation of lycopene epsilon cyclase (*LCY-e*) and *Chy* also increases the level of β-carotene [9, 10].

These previous results suggest that a parental potato cultivar is a critical factor in achieving the accumulation of specific carotene types. Here, we used yellow-fleshed Korean potato cultivars to examine their carotenoid composition as well as the expression profiles of carotenogenesis-related genes. This is an initial step in our metabolic engineering approach toward developing a transgenic potato that accumulates a high level of valuable carotenoids in its tubers.

Materials and Methods

Plant Materials and In Vitro Propagation

Tubers of *S. tuberosum* cv. Golden Valley were kindly provided by Dr. Lim, Hak-Tai, School of Biotechnology,

Kangwon National University (Chuncheon, South Korea). Those of cultivar Haryoung were also kindly given by the National Institute of Highland Agriculture, RDA, South Korea. Upon their arrival, some of these tubers were immediately frozen with liquid N_2 for carotenoid analyses and reverse transcriptase polymerase chain reaction (RT-PCR). Others were maintained for in vitro propagation, in which the sprout meristem tissues were initially cultured in a liquid medium (4.4 g L^{-1} of basic Murashige and Skoog salts and 30 g L^{-1} of sucrose), then subcultured on a solid medium (8 g L^{-1} of phyto agar in liquid media) [18, 25, 39]. Three other cultivars—Jowon, Superior, and Dejima—were obtained from the National Institute of Highland Agriculture, RDA, South Korea in 2005 and have since then been maintained in vitro. They were planted in Spring 2007 at the experimental station of JinJu National University. Their harvested tubers (3.0 cm in diameter) were also immediately frozen in liquid N_2 and stored at −80°C.

HPLC Analyses for Carotenoid Composition

Triplicate potato tubers were peeled and freeze-dried. After lyophilization, 250-mg samples were homogenized in a chilled mortar and a pestle with 5 mL of acetone (0.01% butylated hydroxytoluene, BHT), sea sand, Na_2SO_4, and $NaHCO_3$. Acetone extractions were repeated until the tuber matrix was colorless. The extract was centrifuged at 5,000 rpm for 5 min and the supernatant was dried under N_2 gas flow. The residue was saponified at room temperature for 3 h with 2 mL of 10% (*w*/*v*) methanolic potassium hydroxide solution to free the esterified carotenoids [20]. These carotenoids were then extracted three times from that phase, using 4 mL of diethyl ether containing 0.01% BHT and saturated sodium chloride solution. The combined diethyl ether fraction was washed with water to remove the remaining alkali and concentrated to dryness under a stream of nitrogen. After the residue was dissolved in acetone, the sample solution was filtered through a 0.45 μm membrane (Whatman, PTFE, 13 mm) prior to high performance liquid chromatography (HPLC) analysis. All extraction procedures were performed under subdued light to avoid degradation loss of the pigments.

Our Agilent 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) comprised a temperature-controlled autosampler, column oven, and binary pump. A 10-μL volume of standard or sample solution was directly injected on a YMC C_{30} carotenoid column (3 μm, 4.6×250 mm; Japan) with solvent A [methanol/*tert*-butylmethyl ether/water (81:15:4, *v*/*v*)] and solvent B [methanol/*tert*-butylmethyl ether/water (6:90:4, *v*/*v*)], using a step-gradient elution of 100% A for the first 15 min, then 100% A to 100% B for 35 min (flow rate, 0.7 mL min^{-1}; column temperature, 22°C). A conditioning

Table 1 Primers used for semiquantitative RT-PCR analyses of carotenogenesis-related genes in potato tubers

Gene	Sequence	Length (bp)	Accession No.
Psy1 F	CGGTCTGCTATTGTTGCTACTCC	141	TC122598
*Psy1*R	CAGGAACAGGTATGTCTGGCTTC		
Psy2 F	AGCTTTAGATAGGTGGGAGGCA	162	L23424
Psy2 R	CAAGTCCATACGCATTCCTTCAA		
PDS F	AGAGACTTTGCATGCCGATTGT	151	AY484445
PDS R	AAAGCATCGCCCTCAACTGT		
ZDS F	TTGCCATGTCAAAGGCCA	141	TC114158
ZDS R	ACAGGCACTCCGACCAATTT		
CrtIso F	TGGCAGCAGTAGGACGTAAAC	151	TC117194
CrtIso R	TCCCTTCCTTTTCATGTGGAA		
Lcy-e F	GCAAAATGGATGTGGCAG	151	AF321537
Lcy-e R	CAATGTTGCACCAGTAGGATCAG		
Lut1 F	CTTCTCCGCCCAAAAAAC	140	TC117729
Lut1 R	TTGGCCTAAAGTAAGTGACCTGG		
Lcy-b F	ATGGGTGGTCCACTTCCAGTA	76	X86452
Lcy-b R	GGATGGATGAACCATGCCAG		
Chy1 F	TTTGCTGTCTCGAAGAAAGCC	152	TC36005
Chy1 R	CCTCAAATTGAGGTTTCAGCTTCT		
Chy2 F	TTTTGCTGTCTCGAAGAAAGCC	148	TC32024
Chy2 R	AGCCAACAGGCAGCTAAACTCT		
ZEP F	TCATGAATGCTGGCTGCATC	151	EST724320 (K278242)
ZEP R	TGCTGCAAAGTCATGCGG		

Information about nucleotide sequences, expected lengths of amplified fragments, and GenBank accession numbers were originally reported by Diretto et al. [9]

Table 2 Carotenoid composition in mature tubers of various potato cultivars

Cultivar	Carotenoids (μg g^{-1})								
	Vio	Neo	Ant	Lut	Zea	α-car	β-car	9Z-β-car	Total
Golden Valley	7.1±0.2	1.1±0.3	0.5±0.1	9.6±0.4	0.4±0.0	1.7±0.1	2.1±0.2	1.3±0.1	23.8±1.4
Haryoung	5.4±0.7	0.3±0.1	1.9±0.1	1.0±0.1	0.7±0.1	0.6±0.1	0.5±0.1	0.9±0.1	11.3±1.4
Dejima	8.2±0.2	0.3±0.1	0.1±0.0	2.2±0.1	0.2±0.0	1.2±0.1	1.0±0.1	0.1±0.0	13.3±0.6
Superior	3.9±0.2	0.1±0.0	0.2±0.0	0.6±0.1	0.2±0.0	1.6±0.2	1.4±0.0	0.3±0.0	8.3±0.5
Jowon	1.0±0.1	0.1±0.0	0.1±0.0	0.8±0.2	0.2±0.0	1.7±0.4	1.5±0.3	0.1±0.0	6.5±1.0

Data are means (average contents per dry weight) and standard deviations from three independent experiments
Vio violaxanthin, *Neo* neoxanthin, *Ant* antheraxanthin, *Lut* lutein, *Zea* zeaxanthin, *α-car* α-carotene, *β-car* all-*trans*-β-carotene, *9Z-β-car* 9Z-β-carotene

phase (50 to 60 min) was then used to return the column to the initial state. The eluent was detected with a UV–visible DAD detector (at 360, 450, and 472 nm). Chemstation software (Hewlett-Packard, Avondale, CA, USA) was used to operate this HPLC-DAD system.

Carotenoids were quantified at 450 nm per the external calibration method. Each 1-mg standard sample was dissolved in 10 mL of dichloromethane that contained 0.1% BHT. Working calibration solutions (0.1 to 50.0 μg mL^{-1}) were prepared by diluting the stock solution of the external standard. Standards of violaxanthin, antheraxanthin, neoxanthin, lutein, zeaxanthin, β-carotene (all-*trans*-β-carotene), and 9Z-β-carotene were purchased from CaroteNature (GmbH, Lupsingen, Switzerland). α-Carotene was obtained from Sigma as a 1:2 mixture with β-carotene. The methanol, water, and *tert*-butylmethyl ether were all HPLC grade and other chemicals in our carotenoid analysis were extra grade. Under these chromatographic conditions, standard carotenoids gave peaks at t_R (min): 11.5 for violaxanthin, 13.4 for neoxanthin, 18.2 for antheraxanthin, 23.9 for lutein, 26.9 for zeaxanthin, 37.2 for α-carotene, 39.1 for β-carotene, and 40.5 for 9Z-β-carotene.

Nucleic Acid Purification

Genomic DNA was extracted with cetyltrimethylammonium bromide buffer as described by Doyle and Doyle [11]. It was subsequently cleaned up by several steps of phenol/chloroform/isoamylalcohol and treated with ribonuclease A (Sigma) prior to PCR amplification.

Total RNA was isolated with Trizol reagent (Invitrogen Co., USA), with slight modification from the manufacturer's directions. Briefly, 3.0-cm-diameter tuber samples (50 to 100 mg) were powdered with liquid N_2 and mixed with 1.0 mL of Trizol reagent. This RNA was further purified with chloroform and isopropanol extraction steps. The final RNA pellet was dissolved in diethylpyrocarbonate-treated ddH_2O prior to RT-PCR.

Semiquantitative Reverse Transcriptase PCR

RT-PCR was conducted with a One-Step RT-PCR PreMix Kit (iNtRON Biotech.) according to the manufacturer's directions. Total RNA (20 ng) was mixed with 8 μL of premix solution and a gene-specific primer in 20 μL of total reaction volume. We performed one cycle of reverse transcription at 45°C for 30 min then denatured the RNA/cDNA hybrid at 94°C for 5 min. This was followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C

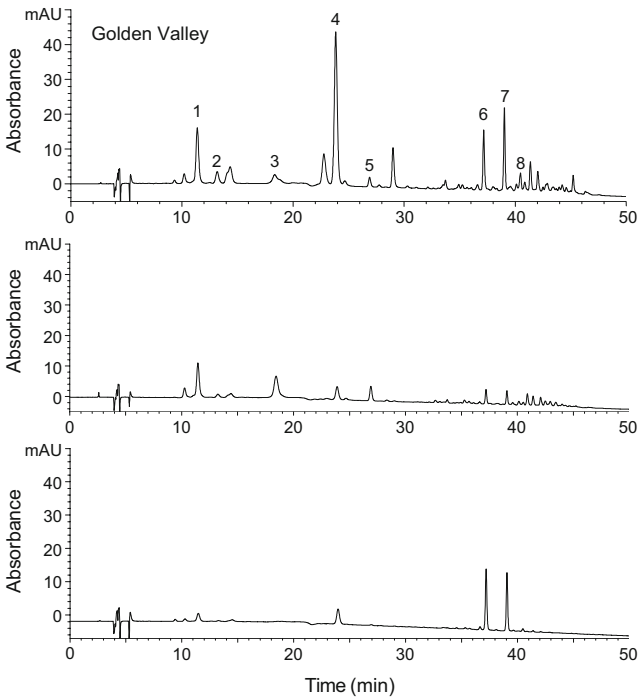

Fig. 1 Representative HPLC chromatograms recorded at 450 nm for measurements of carotenoid composition in tuber tissues. Standard carotenoids: *1* violaxanthin, *2* neoxanthin, *3* antheraxanthin, *4* lutein, *5* zeaxanthin, *6* α-carotene, *7* all-*trans*-β-carotene, and *8* 9Z-β-carotene. Several unidentified peaks were observed in "Golden Valley" compared with "Jowon"

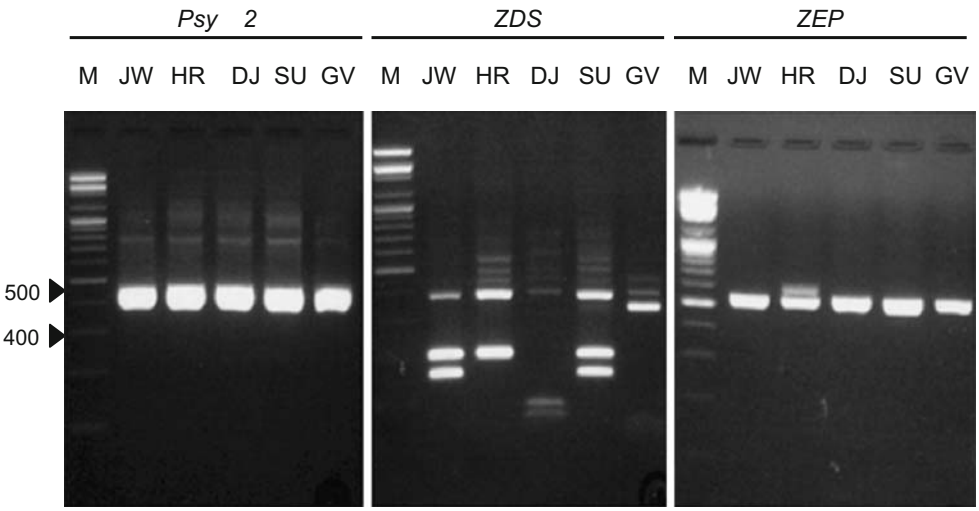

Fig. 2 Polymorphism of *Zds* and *Zep* in potato cultivars. Genomic PCR was conducted (primer sets in Table 1) with genomic DNAs isolated from "Jowon" (*JW*), "Haryoung" (*HR*), "Dejima" (*DJ*), "Superior" (*SU*), and "Golden Valley" (*GV*). Only results from *Psy 2*, *Zds*, and *Zep* are presented because these either produced larger-than-expected fragments based on nucleotide sequence information (*Psy 2*) or exhibited polymorphism with multiple bands (*Zds* and *Zep*)

for 1 min. The final extension was run at 72°C for 5 min prior to fractionation on a 2.0% agarose gel. Relative band intensities were calculated by using the Image J program (Research Service Branch, http://rsb.info.nih.gov). Primer sets for 11 carotenogenesis-related genes are listed in Table 1 [9]. The gene-specific primer set (forward, 5′-GGCTGGATT TGCTGGTGATG-3′ and reverse, 5′-CCGCCTGAATAG CAACATAC-3′) for the actin gene was designed from the nucleotide sequence information for soybean actin cDNA (GenBank accession no. J01298).

Results and Discussion

To search for a potato cultivar with high carotenoid contents, we initially performed HPLC analyses from matured tubers of cultivars Golden Valley, Haryoung, Dejima, Superior, and Jowon. "Golden Valley" had the highest amount of total carotenoids (23.8 μg g^{-1} dry weight (DW)) compared with the lowest level in "Jowon" (6.5 μg g^{-1} DW). Respective contents in "Dejima", "Haryoung", and "Superior" were 13.3, 11.3, and 8.3 μg g^{-1} DW (Table 2). The predominant components in "Golden Valley" were lutein (9.58 μg g^{-1} DW), violaxanthin (7.1 μg g^{-1} DW), and β-carotene (2.1 μg g^{-1} DW). In particular, this lutein content was significantly higher (4.4- to 16.5-fold) than in the other cultivars. Likewise, the β-carotene level was 1.4- to 4.0-fold greater in "Golden Valley". However, violaxanthin, antheraxanthin, and zeaxanthin were slightly less accumulated by that cultivar than by "Dejima" and "Haryoung". For our other cultivars, violaxanthin was the primary component. In "Haryoung", levels of antheraxanthin (1.9 μg g^{-1} DW) and zeaxanthin (0.7 μg g^{-1} DW) were the highest among all of these cultivars.

Although potato is not known to accumulate β-carotene, 16 of 23 Andean native cultivars have detectable levels of β-carotene, ranging from 0.42 to 2.19 μg g^{-1} [2].

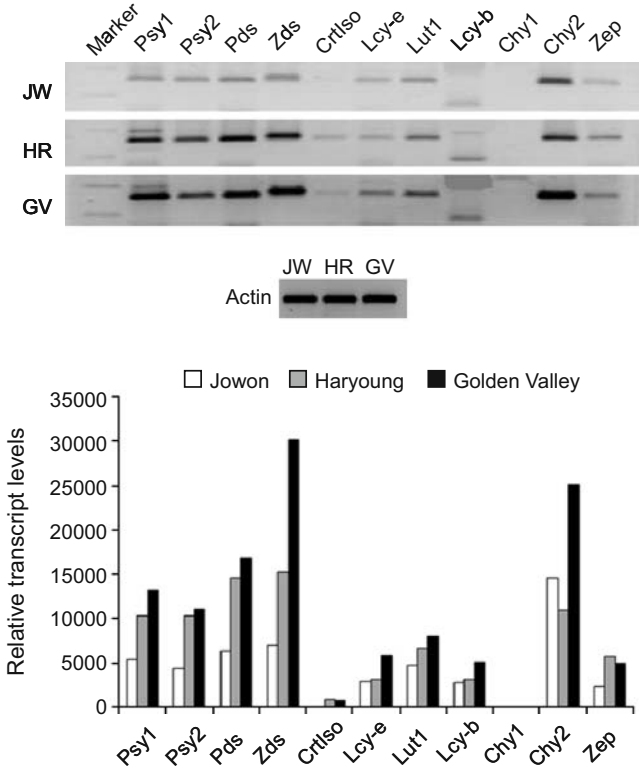

Fig. 3 RT-PCR analyses of carotenogenesis-related genes in mature tubers of "Jowon" (*JW*), "Haryoung" (*HR*), and "Golden Valley" (*GV*). Total RNA was isolated from mature 3-cm-diameter tubers and RT-PCR was performed with primer sets in Table 1. Amplified products were fractionated on agarose gel (*upper panel*). Actin transcripts (*center panel*) were amplified with purified 50 ng of total RNA. Relative band intensities from agarose gel (*lower panel*) were measured with Image J program (Research Service Branch, http://rsb.info.nih.gov). Amounts of total RNAs for RT-PCR were normalized with rRNA; data are means for three replicates. Standard errors were <15.0% of mean values

Nesterenko and Sink [30] also have reported that some breeding lines exhibit a noticeable amount of β-carotene, approximately 0.15 μg g^{-1} DW; however, that level is still too low to recommend them as a dietary food. We also found that significant β-carotene (2.1 μg g^{-1} DW) was accumulated in "Golden Valley". Nevertheless, we cannot exclude the possibilities that errors may have arisen from analyzing procedures used by different facilities, growing conditions, pretreatment of materials, harvesting time, etc.

In addition to identifiable carotenoid components, "Golden Valley" showed several other peaks, at around 23 and 29 min and also after 40 min retention time. This suggests that many other unknown carotenoid derivatives were accumulated. In contrast, no extra peaks were obtained from our other cultivars, except for several minor peaks after 40 min on the HPLC chromatogram of "Haryoung" (Fig. 1).

Because lutein and β-carotene were the major components in "Golden Valley", we examined the expression profiles for genes involved in the carotenoid biosynthetic pathway. Our objective was to correlate those results with those of the carotenoid compositional analyses and to compare them with trends for "Haryoung" (middle level of total carotenoid content) and "Jowon" (lowest level). Primer sets for 11 genes were prepared from previous nucleotide sequence information. To verify the gene specificity of each set, we initially conducted PCR analyses with genomic DNA isolated from five cultivars. All sets amplified their expected size of DNA fragments except those for *Psy2*, *Zds*, and *Zep* (Fig. 2). For example, an extra band was amplified from the genomic DNA of "Haryoung" for *Zep*, which indicated that another isoform might be encoding ZEP. For *Zds*, much more diverse polymorphism was observed among cultivars with regard to their genomic structure. In all cultivars, a larger-than-expected fragment for *Psy2* was amplified by the intron.

To examine transcript levels for carotenogenesis-related genes, we performed semiquantitative RT-PCR after normalizing the densities of total RNA with rRNA. This was achieved on a denatured agarose gel with RT-PCR, using the actin gene as an internal control. As expected, in "Jowon" tubers, very low (basal) levels were found for most of those genes. In contrast, the dark yellow-fleshed "Golden Valley" had significantly elevated transcripts for most carotenogenesis-related genes while moderate levels were detected from "Haryoung" (Fig. 3). In particular, transcripts for *Psy1*, *Psy2*, *Pds*, and *Zds* in "Golden Valley" were markedly increased, 2.5- to 4.4-fold, compared with those of "Jowon".

The high levels of total carotenoids were correlated with these increases in transcripts for carotenogenesis-related genes. However, we must still verify their enzyme activities. Interestingly, the first four genes in the carotenoid biosynthetic pathway—*Psy1*, *Psy2*, *Pds*, and *Zds*—were strongly expressed in "Golden Valley". We did not expect that both *Psy 1* and *Psy2* would be strongly expressed in those tubers because they are believed to be differentially regulated in a tissue- or organ-specific manner. For instance, in tomato, *Psy1* is fruit- and flower-specific whereas *Psy2* is a green tissue-specific form [3, 16, 31]. Morris et al. [29] also have reported that only *Psy2* is strongly upregulated in the tubers of *S. phureja* DB375\1, a yellow potato cultivar containing high total carotenoids.

Here, transcripts for *Lcy-e*, *Lut1*, and *Chy2* in "Golden Valley" were moderately increased, approximately 1.7- to 2.0-fold, over those measured from "Jowon". Very low (basal) levels for *Lcy-b* and *Zep* were detected in "Golden Valley" and "Haryoung", although these were slightly higher than in "Jowon"; transcripts for *CrtIso* and *Chy1* were nearly undetectable in any of these cultivars. Therefore, our results suggest that overexpression of *Lcy-b* might be a good strategy for enhancing the accumulation of β-carotene.

Interestingly, significantly high levels of *Chy2* transcript were detected in "Golden Valley", "Haryoung", and "Jowon". This result was correlated with the relatively high content of violaxanthin in all three because the serial hydroxylation of β-carotene by CHY further modifies to generate zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin [14, 22]. Overexpression of *Chy* in *Arabidopsis* leaves causes an elevation in zeaxanthin and neoxanthin of two- to threefold while the level of β-carotene decreases [8]. In *Arabidopsis*, two types of that gene have been identified: nonheme hydroxylases (*Chy1* and *Chy2*) and a cytochrome P450 (*LUT5*) [13, 38]; *Chy1* is preferentially expressed in the leaves versus *Chy2* in the flowers [17]. This is consistent with our result that *Chy1* seemed not to be expressed while *Chy2* was actively transcribed in potato tubers from all tested cultivars.

In summary, our preliminary studies with a dark yellow-fleshed potato cultivar present several intriguing questions and warrant a detailed investigation regarding the regulation mechanism for carotenogenesis in potato tuber tissues. In particular, we should focus on the accumulation of a specific carotenoid component in an individual cultivar.

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