Comparative Expression of Key Genes Involved in Steroidal Glycoalkaloid Biosynthesis in Tubers of Two Potato Cultivars, Atlantic and Haryoung

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ABSTRACT Quantification of potato glycoalkaloids (PGA) exerting toxicity to humans has some limits if applied to potato breeding populations on a large scale due to its high cost and time consumption. The aim of this study was to investigate key genes involved in PGA biosynthesis and their tuber expression patterns in two potato cultivars, Atlantic with low PGA content (18.6 mg/100g FW) and Haryoung with high PGA content (40.1 mg/100g FW), and to test the utility of these PGA gene transcript levels as selectable markers in potato breeding program. Pot grown potato plants of both 'Atlantic' and 'Haryoung' were exposed to drought stress where the transcript accumulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*Hmg1*) and squalene synthase 1 (*Pss1*) gene was doubled in the tubers of 'Haryoung' at 5 days after stress (DAS). The abundance of *Hmg1*, *Pss1*, solanidine galactosyl transferase, solanidine glucosyl transferase and rhamnosyl transferase gene transcripts increased at 10 DAS. Especially, the ratio of *Hmg1* and *Pss1* transcripts in tubers of 'Haryoung' to those in tubers of 'Atlantic' was higher at 5 DAS compared to that at 10 DAS. The results suggest that drought treatment for 5 days can make a distinct difference in the transcript levels of *Hmg1* and *Pss1* correlated to PGA levels in tubers of 'Atlantic' and 'Haryoung', and the mRNA level of *Hmg1* and *Pss1* can be used as selection markers for breeding potato cultivars with low PGA levels.

Keywords 3-Hydroxy-3-methylglutaryl coenzyme A reductase 1, Gene expression marker, Potato glycoalkaloids, Squalene synthase 1

INTRODUCTION

Steroidal glycoalkaloids are the secondary metabolites present in Solanaceae to serve defense response in plants (Fewell and Roddick 1997; Percival *et al.* 1994). In potato, 95% of the total glycoalkaloids are α -solanine and α -chaconine which are triglycosylated products of the same aglycone, solanidine, but differ in their sugar moieties (Friedman and McDonald 1997). Potato glycoalkaloid (PGA) majorly functions to serve for plant defense against bacterial, fungal diseases and against pests (Lachman *et al.* 2001; Didier *et al.* 2003; Miller *et al.* 2003). Also, PGA is toxic to human when it exceeds a safer limit of 20 mg/100g FW in tuber and PGA poisoning includes severe gastrointestinal disorders, hallucination, partial paralysis to

convulsions, coma and even death (Smith *et al.* 1996). This is caused by inhibiting acetyl choline esterase and impairing membrane integrity (Roddick 1989; Bouarab *et al.* 2002). PGA is not destroyed during cooking and frying (Smith *et al.* 1996; Valkonen *et al.* 1996; Peksa *et al.* 2006). This needs to be taken as an important consideration as the alteration in the glycoalkaloid level is of major concern for food safety.

Various factors affect PGA accumulation which exerts toxicity to humans. Recently, wild species have been used extensively in potato breeding programs, particularly as a genetic resource of disease and pest resistance due to a narrow genetic diversity of the cultivated potato. The wild species tend to show high level of PGA, which means that potato cultivars derived from crosses with wild germplasm

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are at increased risk of exceeding acceptable limit of PGA contents. Sinden *et al.* (1984) reported that the cultivar Lenape, developed through introgression of the wild species *S. chacoense* Bitter, had to be withdrawn from the market due to high average content of 29 mg/100g FW over 10 locations in the United States. In addition, PGA accumulation is caused by development and environmental conditions. The effect of environmental factor on PGA accumulation is supported by the result of Hellenäs *et al.* (1995) that ‘Magnum Bonum’ initially having low content of PGA accumulated hazardous level in the variety grown in Sweden. PGA accumulation in major potato cultivars is due to external factors. Such factors including high temperature (Lafta and Lorenzen 2000), light exposure (Dale *et al.* 1995), light quality (Lin *et al.* 2010; Tian *et al.* 2010) and wounding (Bergenstrahle *et al.* 1992; Choi *et al.* 1994) increase tuber glycoalkaloid content. In Republic of Korea, potato cultivated in spring is sensitive to drought stress (Bejarano *et al.* 2000) which alters PGA accumulation. Considering these factors involved in PGA accumulation,

the risk of exceeding acceptable limit of PGA contents could increase more highly than usual.

Thus, it is essential to screen commercial cultivars which contain low PGA content in tubers. Usually, PGA content has been analyzed in tubers of breeding clones through PGA assays using high performance liquid chromatography (HPLC) and gas chromatographic/mass spectrometry (Hellenäs *et al.* 1995; Laurila *et al.* 1999), which is time-consuming, expensive and laborious. Potato cultivars with low PGA content can be efficiently selected by using marker-assisted selection (MAS) approaches. Until recently, such markers have been identified in potato and high resolution mapping has been made by using genome-wide association study (GWAS) (Sorensen *et al.* 2008; Stich *et al.* 2013). The problem of such approaches is that the cultivated potato, *Solanum tuberosum* L., is an autotetraploid species that displays tetrasomic inheritance (Bradshaw and Mackay 1994; Slepner and Poehlman 2006). To use marker-assisted selection (MAS) in potato breeding program, we need more studies related to quantitative trait loci (QTLs) for

Fig. 1. Steroidal glycoalkaloid biosynthetic pathway in potato plants. Dashed arrows indicate multiple enzymatic steps. 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*Hmg1*), squalene synthase 1 (*Pss1*), vetispiradiene/sesquiterpene synthase 1 (*Pvs1*), sterol C24-methyltransferase 1 (*Smt1*) solanidine galactosyl transferase (*Sgt1*), solanidine glucosyl transferase (*Sgt2*) and rhamnosyl transferase (*Sgt3*). Five enzymes that are used in this study are *Hmg1*, *Pss1*, *Sgt1*, *Sgt2* and *Sgt3*.

PGA content and markers involved in this trait. For this reason it would be relatively easy to apply PGA-biosynthetic gene expression to potato breeding program compared to PGA assay and MAS.

For effective breeding of potato cultivars with low PGA content, there is a need to understand the expression pattern of PGA-biosynthetic genes in the cultivated potato. PGA is bio-synthesized by the sterol branch of mevalonic acid/isoprenoid pathway (Fig. 1). Many of the enzymes involved in the PGA pathway have been identified, including 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*Hmg1*) and squalene synthase 1 (*Pss1*) of the mevalonic acid/isoprenoid pathway and solanidine galactosyl transferase (*Sgt1*), solanidine glucosyl transferase (*Sgt2*) and rhamnosyl transferase (*Sgt3*) for PGA glycosylation (Clouse and Sasse 1998; Moehs *et al.* 1997; Benveniste 2004; McCue *et al.* 2005; Krits *et al.* 2007; McCue *et al.* 2007). PGA accumulation appears to be regulated at different steps in the bio-synthetic pathway in response to environmental conditions. Krits *et al.* (2007) reported that increased transcript levels of two PGA-biosynthetic genes, *Hmg1* and *Pss1*, were correlated to PGA content.

The aim of this study was to use transcript levels of PGA-biosynthetic genes as selection markers which could be applied to breeding potato cultivars with low PGA content. We investigated expression pattern of PGA-biosynthetic genes in the edible tuber of two commercial cultivars, 'Atlantic' and 'Haryoung', which showed low and high level of PGA, respectively. Our results suggested that *Hmg1* and *Pss1* involved in PGA biosynthetic pathway can be selected to use the transcript levels of these genes for selection of potato cultivars with low PGA content. This research article gives an efficient selection method of potato cultivars with low PGA content for conventional breeding program.

MATERIALS AND METHODS

Plant material

The experiments were carried out with *Solanum tuberosum* L. cultivar Atlantic and Haryoung. 'Atlantic' was the introduced cultivar with low PGA level, whereas 'Haryoung'

was the indigenous cultivar with high PGA level bred at Highland Agriculture Research Center, Rural Development Administration (RDA) in Pyeongchang, Korea. Certified seed-grade potatoes were planted on April 15 and harvested on August 5 in 2013 in the area of Jinbu-myeon (altitude 576m) in Pyeongchang-gun of Republic of Korea. To quantify PGA, the skin was peeled by hand from the tuber flesh. For each sample, three biological replicates were prepared, each with the skin and the tuber flesh pooled from five plants. The samples were stored in -70°C deep freezer and freeze-dried.

Extraction and determination of PGA in tubers of 'Atlantic' and 'Haryoung' by HPLC

PGA content in potato tubers was analyzed by HPLC according to the method described in Friedman *et al.* (2003). One gram of freeze-dried potato tuber powder was taken in 50 mL Falcon tube and shaken with 20 ml acetic acid (5%) for 2 hrs. The sample was filtered using filter paper (No. 42, Whatman International, Maidstone, UK) and then extracted with ammonium hydroxide (NH₄OH) at 70°C for 50 min. The extract was placed at 4°C refrigerator for 24 hrs, and then centrifuged at 13,000 rpm at 4°C for 10 min. The precipitate was dissolved in methanol, and then extracted with the same method. The supernatants were filtered using 0.45 µm PVDF syringe filter (PALL, USA), and then subjected to HPLC analysis (2695 Alliance HPLC, Waters Co., MA, USA).

The samples were injected onto Inertsil NH₂ column (5µm, 4.0×250 mm, GL Science, Tokyo, Japan) using acetonitrile/20mM KH₂PO₄ (80:20, v/v) at a flow rate of 0.7 mL/min. PGA were identified by their retention time and by absorption spectra at 208 nm using a photodiode array detector (2996 PDA detector, Waters Co., MA, USA). For quantification of PGA, α-chaconine (Extrasynthase Co., Genay cedex, France) and α-solanine (Sigma-Aldrich Co., Mo, USA) were dissolved in methanol. Four working calibration solutions ranging from 3.1 to 25.0 mg/L were prepared by diluting stock solutions of the external standards.

Drought treatment

Two sets of plants from 'Atlantic' and 'Haryoung' were grown in pots filled with perlite soil-less medium in

containment conditions. Plants were well watered until 70 days after planting. Drought stress was imposed by withholding water to one set of plants from 70 days after planting which is the tuber initiation stage. The other set of plants were irrigated and maintained as unstressed control plants. Leaf and tuber sample was collected from both control and stress plants at 5, 10 and 20 days after imposing drought stress (DAS). Tuber samples were washed, cut into pieces of 3 cm diameter and frozen in liquid nitrogen.

Quantitative Real Time PCR

Total RNA was isolated from the tuber sample of both control and drought stress imposed plants of ‘Atlantic’ and ‘Haryoung’. RNA from leaves was isolated using RNeasy Plant Mini kit (Qiagen Sciences, Germantown, MD, USA) and from tuber using PureLink® Plant RNA Reagent (Life technologies Korea LLC, Seoul, Korea). Two micrograms of total RNA was reverse-transcribed into cDNA using Superscript reverse transcriptase enzyme (Invitrogen, Life technologies Korea LLC, Seoul, Korea). Five key genes were selected in PGA pathway and their expression pattern was studied after treating both ‘Atlantic’ and ‘Haryoung’ with drought stress. The expression pattern of these genes was analyzed by Quantitative Real-Time PCR using gene specific primers (Table 1) and Thunderbird™ SYBR® qPCR Mix (Toyobo Biotechnologies Co., Osaka, Japan) according to manufacturer’s protocols. Each PCR was performed with three technical replicates and three sample replicates. Relative abundance was calculated with the Δ

C_T method (Livak and Schmittgen 2001) using 18s rRNA for template normalization. The average C_T standard was set around 0.4. The graphs were represented as fold increase in the level of expression over the respective control sample.

Isolation of *Sgt1*, *Sgt2* and *Sgt3*

Total RNA was extracted from the leaves of ‘Atlantic’ and ‘Haryoung’ plants using RNeasy plant mini kit (Qiagen). cDNA was synthesized using Superscript™ Reverse Transcriptase enzyme (Invitrogen) and OligodT primer (Enzynomics co Ltd, Daejeon, Korea). Specific primers were designed for *Sgt1* (U82367), *Sgt2* (DQ218276) and *Sgt3* (DQ266437) resulting to respective fragments of about 1,441bp, 1,434 bp and 1,467 bp. Primers were designed for the complete coding sequence of *Sgt1*, whereas two halves with fragment sizes of 753 bp and 804 bp were designed in the case of *Sgt2* and 749 bp and 750 bp for *Sgt3*.

The gene of interest was amplified using the cDNA template from ‘Atlantic’ and ‘Haryoung’ with the gene-specific primers. The amplified products were column purified using gel elution kit (Qiagen) and cloned into pTOP vector using TOPcloner TA/Blunt kit for PCR cloning (Enzynomics co Ltd., Daejeon, Korea) and sequenced. Homology search was done for deduced amino acid sequence similarity / identity (www.ncbi.nlm.nih.gov/blastp). Sequences were aligned with the existing gene in the database for the gene identity and analyzed for any substitutions using CLC Main Workbench 5.7.1 software.

Table 1. Primer sequence of one housekeeping gene and PGA-biosynthetic genes used for qRT-PCR analysis.

Gene ²⁾	Primer forward (5' → 3')	Primer reverse (5' → 3')
<i>Hmg1</i>	GGGTACAGTGGGTGGTGAAC	CACCAGCAAGAACAGAACC
<i>Pss1</i>	CTTGCAGAGACTCGGGAACCTTG	CGGTTGCCAGAAAGTTGTGC
<i>Sgt1</i>	GGTTTACAGACCTCACAAGCAGCC	CAATGCCATAGCTTCGTTCTCCG
<i>Sgt2</i>	GCAGTCGGAGGATTCATGACACAC	TCGATATCTCAGCACCTCCGTTGG
<i>Sgt3</i>	GTTTATCATCCAAGGCTGGGCACC	CCAATAGCAAGGCCAAGTGTCTCC
18s rRNA	ATCCTGCCAGTAGTCATATGC	CTACGGTTATTTCGAGTAGTAG

²⁾3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*Hmg1*), squalene synthase 1 (*Pss1*), vetispiradiene/sesquiterpene synthase 1 (*Pvs1*), sterol C24-methyltransferase 1 (*Smt1*) solanidine galactosyl transferase (*Sgt1*), solanidine glucosyl transferase (*Sgt2*) and rhamnosyl transferase (*Sgt3*).

RESULTS

Characteristics and PGA contents of two potato cultivars, Atlantic and Haryoung

In a previous study (Kim *et al.* 2014), we showed the total PGA contents of the potato tubers were 16.5 in Goun, 19.7 in Atlantic, 21.2 in Hongyoung, 45.7 in Jayoung, 46.2 in Haryoung and 47.7 mg/100g FW in Superior. As expected, contents of α -solanine, α -chaconine and total PGA which were determined in field-grown tubers by HPLC were higher in tuber peel and flesh of 'Haryoung' compared with those of 'Atlantic' (Fig. 2). In addition, 'Haryoung' showed similar maturity with 'Atlantic' (Table 1) which exhibited low PGA level. Considering PGA contents and maturity of two potato cultivars, we investigated expression pattern of PGA-biosynthetic genes in the edible tuber of the above mentioned cultivars.

Expression pattern of PGA-biosynthetic genes in tubers of 'Atlantic' and 'Haryoung' under water-deficit compared with well-watered condition

Bejarano *et al.* (2000) reported that PGA accumulation was induced under drought conditions. Thus, we used drought treatment in 'Atlantic' and 'Haryoung', for the selection of genes which could be applied for screening potato cultivars with high PGA content. In the present study, the expression pattern of PGA-biosynthetic genes was determined in the tuber of 'Atlantic' and 'Haryoung' plants exposed to drought stress for various time intervals 5, 10 and 20 days after drought stress (DAS). Gene expression analysis of PGA-biosynthetic genes in tubers of 'Atlantic' and 'Haryoung' exposed for 5 DAS revealed similar transcript levels in tubers of 'Atlantic' with well-watered condition whereas tubers of 'Haryoung' exhibited increased transcript levels of two genes. The genes, *Hmg1* and *Pss1*,

Fig. 2. Comparison of potato glycoalkaloid (PGA) contents in tuber peel (A) and flesh (B) of two potato cultivars, Atlantic and Haryoung. For each sample, three biological replicates were prepared, each with the skin and the tuber flesh pooled from five plants. The PGA content was quantified by HPLC. PGA values are the means $\pm$ SD from three replications.

in tubers of ‘Haryoung’ increased 2.4- and 2.6-fold, respectively. A significant difference was observed in the transcript levels of five key genes in tubers of ‘Atlantic’ and ‘Haryoung’ exposed to drought stress for 10 days compared to well-watered condition. The transcript levels of *Hmg1*, *Pss1*, *Sgt1*, *Sgt2* and *Sgt3* increased to 2.3-, 2.5-, 5.1-, 3.7- and 4.4-fold in tubers of ‘Atlantic’ and 1.7-, 8.6-, 2.8-, 5.8- and 10.7-fold in tubers of ‘Haryoung’, respectively. At 20 DAS, expressions of the genes in two cultivars were repressed (Table 3). However, the drought treatment did not alter the transcript levels of PGA biosynthetic genes in potato leaves of ‘Haryoung’ and ‘Atlantic’ (data not shown).

Expression ratio of key genes involved in steroidal glycoalkaloid biosynthesis in tuber

A difference in the ratio of five key genes expression between the tubers of ‘Haryoung’ and that of ‘Atlantic’ exposed to drought stress for 5 and 10 days was noted. Higher expression of *Hmg1*, *Pss1*, *Sgt1*, *Sgt2* and *Sgt3* were indicated by the respective higher values, 12-, 3.7-, 1.7-, 3.2- and 2.2-fold compared with those in the tubers of ‘Atlantic’ exposed for 5 DAS. At 10 DAS, *Pss1*, *Sgt2* and

Sgt3 in tubers of ‘Haryoung’ were expressed higher than in the tubers of ‘Atlantic’. Among the genes with increased transcripts, the transcript level of *Pss1* increased by 3.4-fold, which suggests that the expression level of *Pss1* could be used for selecting potato cultivars with low PGA content. In addition, the increase in the transcript abundance of *Sgt1*, *Sgt2* and *Sgt3* at 10 DAS indicated their direct involvement in PGA accumulation (Fig. 3). These results showed that drought treatment made a significant difference in PGA-biosynthetic gene expression in tubers of ‘Atlantic’ and ‘Haryoung’. The PGA-biosynthetic genes, *Hmg1* and *Pss*, involved in PGA accumulation could be used for the selection of potato cultivars with low PGA content easier than PGA assays by analytical instruments.

Comparison of SGT1, SGT2 and SGT3 sequences from ‘Atlantic’ and ‘Haryoung’

Initially, cDNA of the selected key genes in PGA-biosynthetic pathway *Sgt1*, *Sgt2* and *Sgt3* was prepared from the RNA isolated from the leaves of ‘Atlantic’ and ‘Haryoung’. Isolation of *Sgt1* was done by the amplification of cDNA with *Sgt1* specific primers, producing a 1.44 kb

Table 2. Characteristics of Haryoung and Atlantic potato cultivars.

Cultivar	Registration year	Maturity	Total PGA in tubers (mg/100g FW)	Cross combination	Utilization type
Haryoung	2005	Medium	46.2	Atlantic × Superior	Table potato
Atlantic	1995	Medium	19.7	B5141-6 × Wauseon	Potato chip

Table 3. The relative transcript levels of PGA biosynthetic genes in potato tubers of ‘Atlantic’ and ‘Haryoung’ under drought stress compared to well-watered condition.

Potato Cultivar	Days After Stress	Transcript level of PGA-biosynthetic genes ²⁾				
		<i>Hmg1</i>	<i>Pss1</i>	<i>Sgt1</i>	<i>Sgt2</i>	<i>Sgt3</i>
Atlantic	5	0.2±0.1	0.7±0.2	0.4±0.1	0.4±0.1	0.4±0.2
	10	2.3±0.3	2.5±0.2	5.1±1.2	3.7±0.2	4.4±0.3
	20	0.7±0.2	0.3±0.1	0.8±0.3	0.8±0.3	0.8±0.2
Haryoung	5	2.4±0.3	2.6±0.2	0.6±0.1	1.1±0.3	0.9±0.2
	10	1.7±0.4	8.6±1.2	2.8±0.7	5.8±0.4	10.7±1.2
	20	0.2±0.1	0.3±0.1	0.2±0.1	0.5±0.1	0.1±0.1

²⁾The PGA gene transcript levels were quantified by qRT-PCR. The values are the means ± SD from three replications. 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*Hmg1*), squalene synthase 1 (*Pss1*), vetispiradiene/sesquiterpene synthase 1 (*Pvs1*), sterol C24-methyltransferase 1 (*Smt1*) solanidine galactosyl transferase (*Sgt1*), solanidine glucosyl transferase (*Sgt2*) and rhamnosyl transferase (*Sgt3*).

fragment which was then sequenced. Alignment comparison of the amino acid SGT1 sequence of ‘Atlantic’ and ‘Haryoung’ with reference gene, showed a highly similar sequence in ‘Haryoung’ with reference gene whereas ‘Atlantic’ revealed three substitutions. These sequence variations in ‘Atlantic’ were observed at amino acid position 54 (arginine is substituted with lysine), 213 (glutamic acid with leucine) and 309 (alanine with serine) (Fig. 4). There was no difference observed in predicted phosphorylation or glycosylation sites in SGT1 protein from ‘Atlantic’. Isolation of *Sgt2* and *Sgt3* was done by amplifying the coding sequence in two halves which produced the amplicons of 753 bp and 804 bp in *Sgt2* and 749 bp and 750 bp for *Sgt3* which were then sequenced. Amino acid sequence alignment of *Sgt2* and *Sgt3* from ‘Atlantic’ and ‘Haryoung’ with reference gene showed a 100% homology of both genes in ‘Atlantic’ and ‘Haryoung’ (data not shown).

DISCUSSION

The PGA contents in tubers of ‘Haryoung’ were higher than those of ‘Atlantic’ which were grown in the field located in Jinbu-myeon (Fig. 2). These results are supported by the results of Kim *et al.* (2014) that total PGA contents in tubers of ‘Atlantic’ and ‘Haryoung’ among major potato cultivars in Republic of Korea were 19.7 and 46.2 mg/100g FW, respectively. Also, PGA contents in tubers of ‘Haryoung’ were higher compared with those in tubers of ‘Atlantic’ which were grown in Gangneung and Daegwallyeong (data not shown). These results suggested that ‘Atlantic’ could be cultivated safely, whereas special care is needed in cultivation of ‘Haryoung’ with higher PGA content in various cultivation environments. Thus, we selected ‘Atlantic’ and ‘Haryoung’, for investigating the expression pattern of PGA-biosynthetic genes.

Fig. 3. Effect of drought stress and treatment time on the expression of PGA genes in tubers of potato cultivars, Atlantic and Haryoung. (A) The ratio of average transcript level in ‘Haryoung’ to that in ‘Atlantic’ under drought stress for five and ten days. 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*Hmg1*), squalene synthase 1 (*Pss1*), solanidine galactosyl transferase (*Sgt1*), solanidine glucosyl transferase (*Sgt2*) and rhamnosyl transferase (*Sgt3*). (B) Phenotype of aerial parts in ‘Atlantic’ and ‘Haryoung’ under 5 and 10 DAS. DAS = days after drought stress

Expression analysis of PGA-biosynthetic genes could be applied as a selection tool for potato cultivars with low PGA content, especially in organisms with autotetraploid genomes such as *S. tuberosum* L. Stress treatment has been proven useful in enhancing gene expression involved in specific biological processes, or that are expressed in unique organs and tissue. Combination of these two techniques would accelerate the screening of genes in cultivated potatoes that are amenable to selection of potato cultivars with low PGA content. The advantage of this technique is that it can be easily tested by drought treatment in the pots and qRT-PCR analysis. We note that the use of gene expression analysis is limited to some extent such that it can only identify components of biological processes that are transcriptionally regulated.

qRT-PCR analysis of the drought-treated potatoes successfully uncovered mRNAs involved in PGA accumulation (Table 3). Two genes, *Hmg1* and *Pss*, involved directly in PGA biosynthesis pathway catalyze the first step in PGA biosynthesis that increases in response to external stress including wounding, plant disease, developmental and

light stimuli (Yang *et al.* 1991; Korth *et al.* 2000). HMGR1 proteins have been shown to be related to PGA content (Choi *et al.* 1994; Dale *et al.* 1995; Percival *et al.* 1994). In addition, *Pss1* is a part of the PGA biosynthetic pathway, and its expression would be expected to be increased in accordance with the changes of PGA. Hence, the use of expression levels of *Hmg1* and *Pss1* under drought stress increases the efficiency of selecting potato clones with low PGA content which can be cultivated in various environmental conditions.

Drought treatment for 5 and 10 days resulted in the higher expression of genes encoding PGA biosynthetic enzymes in tubers of ‘Haryoung’ compared to those in tubers of ‘Atlantic’, indicating that drought treatment made a significant difference in the gene expressions between ‘Haryoung’ and ‘Atlantic’ (Table 3). One exception to this trend was *Hmg1*, which increased 2.3-fold in tubers of ‘Atlantic’ and 1.7-fold in tubers of ‘Haryoung’. Drought treatment for 10 days induced 8.6-fold transcripts of *Pss1* in tubers of ‘Haryoung’, which negatively affected the transcripts of *Hmg1*. Ginzberg *et al.* (2012) showed that

Fig. 4. Comparison of deduced amino acid sequence of SGT1 from potato cultivars, Atlantic and Haryoung with reference sequence. Substitutions in ‘Atlantic’ are shown in the box.

over-expression of *Pss1* causes a reduction of the transcript levels of *Hmg1*, and it seems likely that this gene is regulated by a feedback regulation cycle. Also, post-transcriptional regulation of HMGR activity has been shown through *in vitro* assay, which showed that this protein was inactivated via phosphorylation at position 590 of potato HMGR1 (Mackintosh *et al.* 1992; Dale *et al.* 1995). Although inactivation of HMGR1 protein needs to be considered, our results indicate that the transcript level of *Hmg1* should contribute to PGA synthesis.

There was a difference observed in ratio of the key gene expression in tubers of 'Haryoung' to those in tubers of 'Atlantic'. At 5 DAS, the relative transcript levels of *Hmg1* and *Pss1* showed 12- and 3.7-fold increase in tubers of 'Haryoung', respectively, compared with those in tubers of 'Atlantic'. Drought stress for 10 days increased 3.4-fold in the relative transcript level of *Pss1* (Fig. 3). This coordinated expression of the isoprenoid key enzymes, *Hmg1* and *Pss1* facilitates precursor flow to PGA biosynthesis. The activities of *Pvs1*, *Pss1* and *Smt1* are the committed steps for the various isoprenoid compounds (Fig. 1). The *Pss1* transcript level, which competes with *pvs1* on the same substrate, farnesyl-PP, increased with *Hmg1* transcript levels at 5 DAS (Fig. 3). These results were comparable with those of Krits *et al.* (2007) where the potato genotypes exhibiting different levels of PGA content showed an association between high PGA levels and high expression of *Hmg1* and *Pss1*. The association of the gene expression with PGA contents was supported by the higher PGA contents in the tubers of 'Haryoung' than 'Atlantic' (Fig. 2). These results suggested that the expression pattern of *Hmg1* and *Pss1* could be used as screening markers to avoid potato cultivars with high PGA content. Especially, the use of *Pss1* as the screening marker is supported by the expression pattern of PGA biosynthetic genes, which showed that the expression level of *Pss1* in tubers of 'Haryoung' was higher than that in tubers of 'Atlantic' at 5 and 10 DAS.

The drought treatment did not alter the transcript levels of PGA biosynthetic enzymes in potato leaves of 'Haryoung' and 'Atlantic' (data not shown). PGA is present in all parts of the plant and higher levels are present in the intensive metabolically active tissues like leaves, stems, fruits and

flowers but their levels are less in tubers comparatively (Peksa *et al.*, 2002). Interestingly, we failed to find significant increase in the transcript levels of key genes involved in PGA biosynthesis in drought-treated leaves of 'Haryoung' and 'Atlantic'. This may be due to an increase in expression of PGA biosynthetic genes before 5 DAS in leaves of 'Haryoung' and 'Atlantic'.

In addition, increase in the transcript abundance of *Sgt1*, *Sgt2* and *Sgt3* at 10 DAS indicated their direct involvement in glycoalkaloid accumulation. Increased transcript levels of *Hmg1* and *Pss1* in the upstream of the pathway under drought stress showed that they might play a role in the regulation of *Sgt1*, *Sgt2* and *Sgt3* levels in the downstream of the pathway. The expression levels of *Sgt1* and *Sgt2* were relatively associated with the content of solanine to chaconine ratio respectively in tuber (Fig. 4). Thus, we isolated three key genes that are directly involved in the formation of PGA; *Sgt1*, *Sgt2* and *Sgt3* to check for any sequence variations which determine the varied accumulation of PGA in two low and high PGA exhibiting cultivars. The deduced amino acid sequence alignments of *Sgt2* and *Sgt3* between 'Atlantic' and 'Haryoung' showed highly homologous sequence with 100% similarity, whereas amino acid alignment of *Sgt1* showed three substitutions in 'Atlantic'. However, there was no variation in the predicted phosphorylation and glycosylation sites in SGT1 protein from 'Atlantic'. Manrique-Carpintero *et al.* (2013) observed the similar kind of synonymous and non-synonymous substitutions in HMGR1 and PSS1 in various potato cultivars, but it was unclear whether those substitutions attributed for the varied accumulation of PGA. Hence, it shows that the nucleotide sequence of *Sgt2* and *Sgt3* and the amino acid substitutions encoded in the cDNA for *Sgt1* in 'Atlantic' may not contribute to the difference in the glycoalkaloid biosynthetic efficiencies of two cultivars.

In conclusion, our results suggest that (i) drought treatment for 5 days can make a distinct difference in the *Hmg1* and *Pss1* transcript levels correlated to PGA levels in tubers of 'Atlantic' and 'Haryoung' and (ii) mRNA level of *Hmg1* and *Pss1* can be used as selection markers for breeding potato cultivars that have low PGA levels. In future, the expression pattern of the selected genes, *Hmg1* and *Pss1* will be applied to investigate the association of

gene expression level with PGA accumulation in 24 potato cultivars bred in RDA, Korea.

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