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Transcriptional Expression of Genes Involved in Steroidal Glycoalkaloid Biosynthesis in Three Wild Solanum Species

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ABSTRACT

Plants are used as a source of pharmaceutical due to presence of certain chemicals/ ingredients inside of their body and they know as medicinal plants, these are marked as a prime and effective source of primary health care in rural areas. Secondary metabolites of the plants are performing role to treat disorders and its types and concentration is variable among the plants. In solanum, steroidal glycoalkaloids (SGAs) are a family of secondary metabolites that are present in a wide range of plant species, including the Solanaceae. The goal of this study was to evaluate the relationship between different plant species based on expression of HMG1 (gene coding for hydroxy methyl glutaryl coenzyme A reductase), SGT1 (solanidine galactosyl transferase), and SGT2 (solanidine glucosyltransferase) genes in *Solanum nigrum*, *S. lycopersicum* and *S. tuberosum*. And determine the best source for steroidal glycoalkaloids to save time and cost in pharmaceutical industry based on Relative expression of three putative steroidal glycoalkaloid's HMG1, SGT1, and SGT2 genes. Result indicated high level of expression of HMG1 gene in S. tuberosum, SGT1 in *S. nigrum* and SGT 2 in *S. lycopersicum*. These findings provide an important step toward understanding TGA variation in Solanum species then selecting the best source in pharmaceutical industries and drug preparation.

Keywords
Solanum, HMGR, SGT1, SGT2, RT-PCR, gene expression.

Introduction

The Solanaceae family contains a diverse variety of secondary metabolites, making them extremely valuable for economic, agricultural, and medicinal purposes. (Zadra et al., 2012, Mohasana et al., 2020). Steroidal glycoalkaloids (SGAs) are a type of secondary metabolite found in many plant species, including Solanaceae and some commercially valuable crops, such as potatoes (S. tuberosum) and tomatoes (S. lycopersicum). SGAs are the primary component of some medicines with anti-inflammatory effects against herpes virus and cancer cells, such as solamargine (Cham, 2000; Lawson, 2003; Carter and Lake, 2004; Lee et al., 2004; T Cui et al., 2014). Steroidal glycoalkaloids (GAs) are important plant protection compounds in Solanaceae plants, like potato, which have a neurotoxic impact on animal feeders(S. tuberosum), tomato (S. lycopersicum), and aubergine (S. melongena) (Piasecka et al., 2015; Okamoto et al., 2020). One of the key factors in the aggregation of GAs in potato tubers is genetic heritage, and modern varieties have been bred for their comparatively low GA accumulation(Hellenas et al., 1995). Furthermore, environmental factors such as sun, drought, temperature, and wounding have been shown to cause steroidal GAs in potato tubers. (Sinden et al., 1984; Griffiths et al., 1997; Friedman and McDonald, 1999; Bejarano et al., 2000) including in modern commercial varieties (Grunenfelder et al., 2006; Nahar et al., 2017). GAs were generated in the cytoplasm through the mevalonate pathway from a common primary metabolite, acetyl coA. Enzymes are conserved across eukaryotes and this is the main pathway for cholesterol biosynthesis. (Desmond and Gribaldo, 2009). Despite the importance of GAs, research on their biosynthetic mechanism and the factors that control

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GA levels was limited until recently(Ginzburg et al 2011).

The primary metabolic activities of the mevalonic acid, isoprenoid condensation, and sterol pathways contribute to the biosynthesis of various terpenoids including SGAs (Fig. 1). Although the intermediate products of these pathways are precursors in common with a variety of terpenoid products, some of the steps are catalyzed by isoenzymes that are associated with specific terpenoid products. For instance, the enzyme 3-hydroxy- 3-methylglutaryl coenzyme A reductase (HMGr) catalyzes the formation of mevalonic acid. The specific isoenzymes HMG2 and HMG3 are associated with the production of sesquiterpenoid phytoalexin defense compounds, while HMG1 has been associated with SGA accumulation after wounding (Choi et al. 1992, 1994; Krits et al. 2007). Other steps of these pathways are represented by gene families including the oxidation of squalene to 2,3-oxidosqualene, catalyzed by squalene epoxidase (SQe) in the isoprenoid condensation stage of triterpene–sterol formation. Inhibition of this enzyme activity decreased sterol biosynthesis (Wentzinger et al. 2002). Cholesterol is the precursor of SGAs (Arnqvist 2007); hence, SQe may have a regulatory role in SGA biosynthesis.

Because of the close relationship between SGA content and food protection, research into the biosynthetic pathway of SGAs in plants has gotten a lot of attention since the 1970s. (Zitnak and Johnston., 1970). According to research, the expression of hmg1 is related to the behaviour of PSS1 (Zook & Kuc 1991; Yoshioka et al. 1999), implying that HMGR and PSS1 are controlled in concert. High SGA levels have been linked to high transcript levels of HMGR1- and PSS1-encoding genes (hmg1 and pss1, respectively) in potato tubers(Krits et al. 2007).

These findings suggested that the dedicated stage of the mevalonate pathway is involved in the control of SGA biosynthesis. In an in vivo sample, the developmental stage of potato tubers and light-regulated regulation of HMGR occurred post-translationally, affecting the tuber's SGA content(Korth et al. 2000). SGA accumulation varies depending on developmental and environmental conditions in all areas of the plant(Distl and Wink 2009; Friedman and Dao 1992; Friedman and McDonald 1997; Kolbe and StephanBeckmann 1997; Mweetwa 2009; Nema et al. 2008).

The expression level of glycoalkaloid varied organs in the same plant so variability among different species would be significant to characterize plant of high glycoalkaloid expression level which will be with great medicinal value.

The aim of the study was to evaluate the expression level of HMG2, SGT1, and SGT2 genes in S. nigrum, S. lycopersicum and S. tuberosum and determine variability of gene expression level between the three species. At which the expression level of glycoalkaloid varied organs in thesameplantsovariability among different species would be significant to characterize plant of high glycoalkaloid expression level which will be with great medicinal value.

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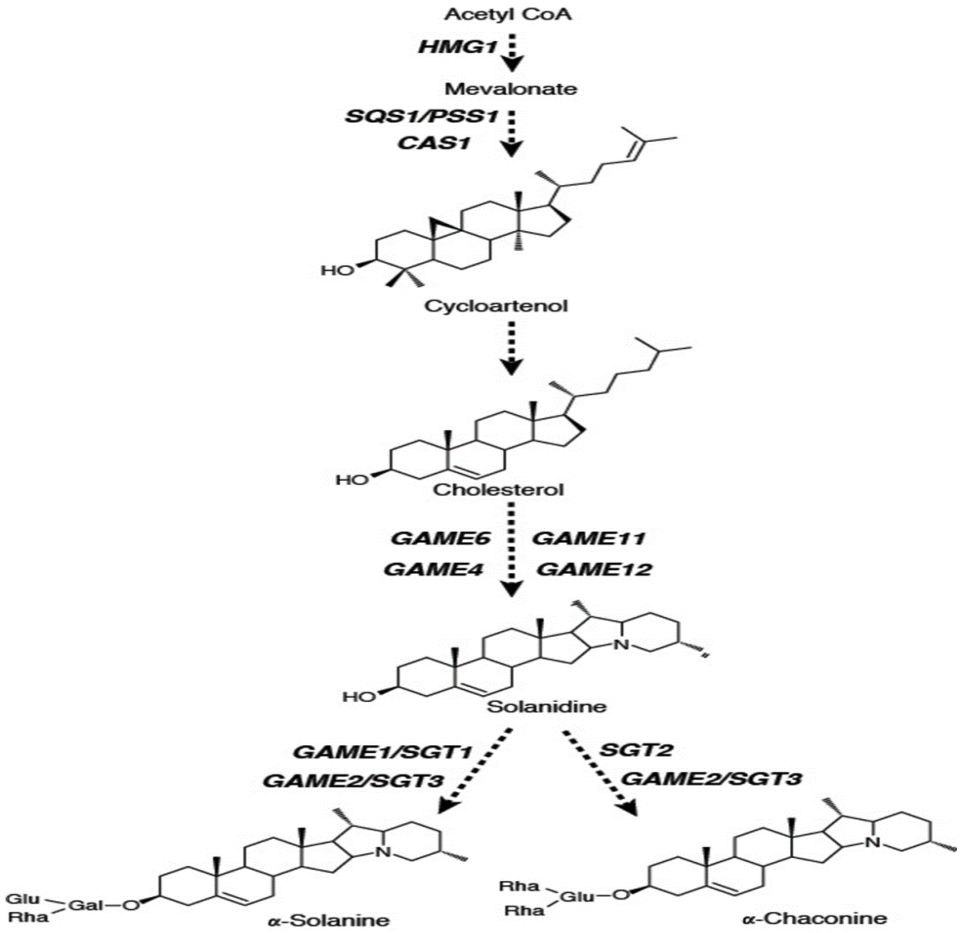

Figure 1. steroidal glycoalkaloid biosynthetic pathway. HMGR 3-hydroxy-3-methylglutaryl coenzyme A reductase, SGT1 solanidine galactosyltransferase and SGT2 solanidine glucosyltransferase.

Methods

Plant Material collection

The whole S. nigrum plant was collected in april 2020, S. lycopersicum plant was collected in January 2020 and S.tuberosum were collected in April 2020. The collected species of were washed thoroughly with tap water, followed by distilled water then young, fresh leaves stored in a refrigerator for further use.

RNA extraction and SYBR green rt-PCR

To protect RNA from degradation, a double volume (1 ml) of the RNA protect Reagent (Qiagen, Germany, GmbH), was added to one volume (0.5 ml) of the broth of the harvested plant, the mix was then vortexed and incubated for 5 min. at room temp., then centrifuged for 10 min. at 8000

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rpm. The supernatant was decanted. Then 200 µl of TE buffer containing 1 mg/ml Lysozyme (Biochemica, Applichem) was added to the pellet. Also, 700 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml was added. Then 500 µl of 100% ethanol was added, and the steps were completed according to the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). Primers used were supplied from Metabion (Germany) are listed in table (1).

Primers were utilized in a 25- µl reaction containing 12.5 µl of the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25 µl of RevertAid Reverse Transcriptase (200 U/µL) (Thermo Fisher), 0.5 µl of each primer of 20 pmol concentration, 8.25 µl of water, and 3 µl of RNA template. The reaction was performed in one step plus real time PCR machine.

Analysis of the SYBR green rt-PCR results

Amplification curves and CT values were determined by the strata gene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the positive control group according to the "ΔCt" method stated by Yuan et al., 2006 using the following ratio: ΔCt = Ct housekeeping – Ct target

Table 1. Primers sequences, target genes and cycling conditions for SYBR green rt-PCR.

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Reference
				Secondary denaturation	Annealing	Extension	
HMG1	f-CTTCTCGTAAGGTGGCGTGA r-AACCCAATCCCAAAGAAACC	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec.	72˚C 30 sec.	T Cui et al., 2014
SGT1	F-AAGCCACAATCCTCACTACCC r-AGGCAACCCAACTTCAGCAG	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec.	72˚C 30 sec.	T Cui et al., 2014
SGT2	F-TACCCACCACAATGCTTCC r- ATCCCTTCAGGCAACCCA	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec	72˚C 30 sec.	T Cui et al., 2014
B-actin	f-CTCAACCCCAAGGCTAACAG r-GGCATGAGGAAGGGCATAA	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec	72˚C 30 sec.	Iskandar et al. 2004

Results

Glycoalkaloids have been described as toxic. Since GAs are critical for food safety, understanding the expression and regulation of genes involved in the GA biosynthetic pathway in Solanum may provide important insight into GA prediction and control(Fridman et al. 1992; Mariot et al., 2016).

Assuming that the optimum PCR performance is 100%, since the entire target cDNA is duplicated at every PCR loop during the exponential process, the efficiency values obtained were deemed satisfactory. As a result, the amplification products of each reaction were identical. Reverse transcription qPCR was used to examine the expression of HMG1, SGT1, and SGT2 (RT-qPCR)to verify differences in the expression pattern in the three Solanum species, tested genes were constitutively expressed in all plants with different expression patterns indicating various responses. Relative expressions (fold-change or fold-difference ofexpression levels) of the representative genes were compared to thosewithin the control of Beta actin. In particular, the entire set of Hmg1 genes was found to be down regulated in both of S.nigrum and S.

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lycoperiscum but up regulated in S. tuberosum while Sgt1 down regulated in S. lycoperiscum and S. tuberosum and finally Sgt2 not show remarkablevariation in their fold changes. This elucidated that S. nigrum as a medicinal plant is an important source for alpha solanine.

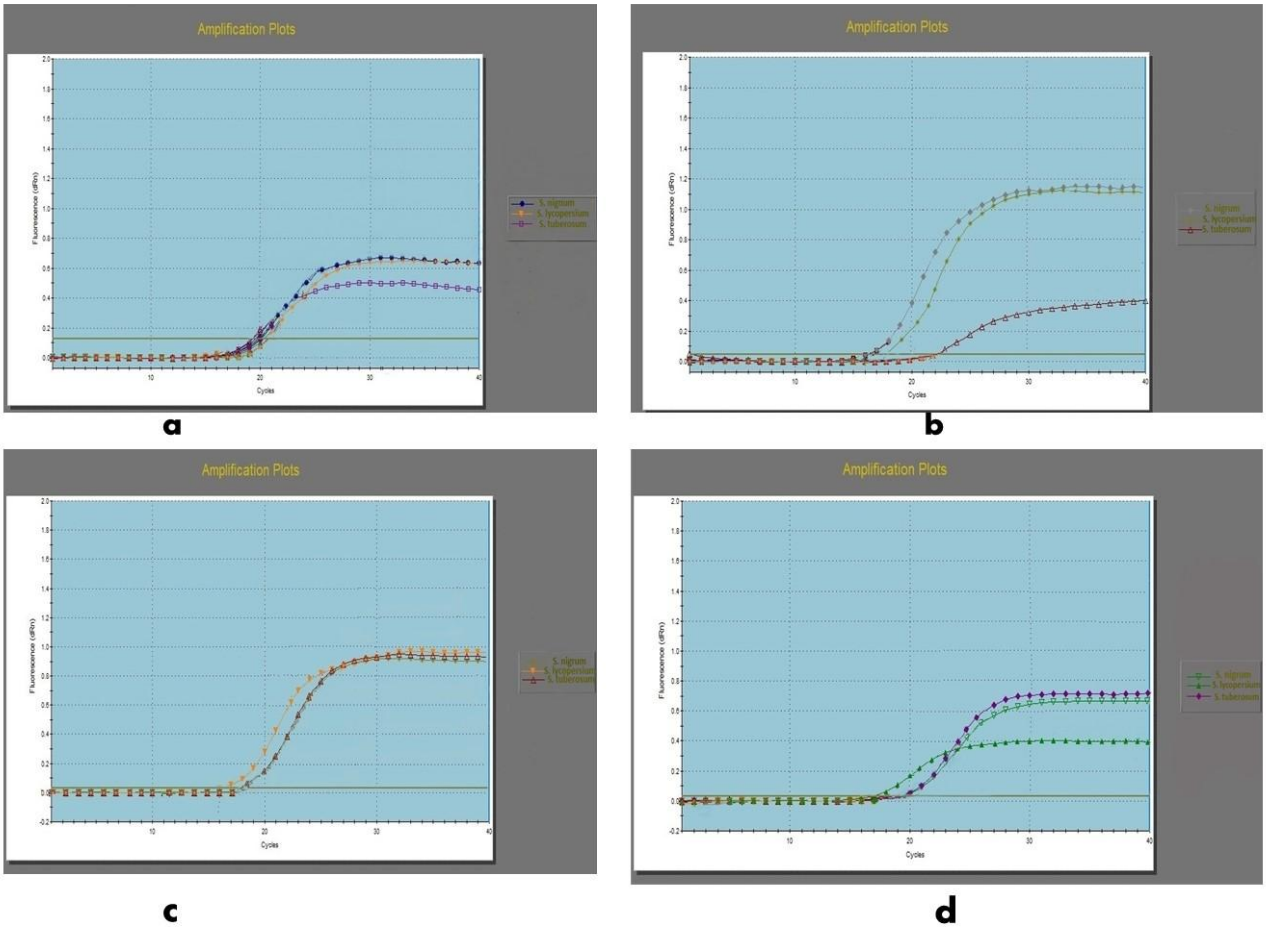

Figure 2. Data sheet of RT-PCR showing amplification curves of (a) b-actin structural genes (b) HMGR 3-hydroxy-3-methylglutaryl coenzyme A reductase, (c) SGT1 solanidinegalactosyltransferase and (d) SGT2 solanidine glucosyltransferase.

Table 2. Fold changes in expression of b-actin structural genes, HMGR 3-hydroxy-3-methylglutaryl coenzyme A reductase, SGT1 solanidine galactosyltransferase and (d) SGT2 solanidine glucosyltransferase on three wild Solanum species

Sample	B-actin	HMGR		SGT1		SGT2	
	CT	CT	ΔCt	CT	ΔCt	CT	ΔCt
S. nigrum	19.34	16.71	-2.63	20.40	1.06	19.64	0.3

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S. lycopersicum	18.65	17.92	-0.73	17.80	-0.85	16.87	1.78
S. tuberosum	19.12	22.14	3.02	17.77	-1.35	19.59	0.47

Large and often unpredictable variations in TGA levels can arise from differences in genotype, locality, season, cultivation practice, and stress factors,26 resistance to viral and bacterial diseases (Austin et al., 1988; Pehu et al., 1990; Rokka et al., 1990) insect deterrence (Sanford et al., 1992; Sanford et al., 1997) defense response against fungal pathogens, (Fewell and Roddick 1997) and harvest and postharvest treatments, such as drought, (Bejarano et al., 2000) high temperature, (Lafta and Lorenzen 2000) light exposure of the tubers (Dale et al., 1998) and wounding. Bergenstrahle et al., 1992; Choi et al .1994).

High content of glycoalkaloids, such as leptines the acetylatedform of the common Solanum Tuberosum glycoalkaloids, chaconine and solanine—in the foliage is a benefit for the potato plant as it prevents plants from being attacked by Colorado potato beetles (Leptinotarsa decemlineata) (Sinden et al. 1986; Sanford et al. 1998). But an increased amount of SGAs in tubers can reduce the quality (flavour) of the tubers and cause potential food safety concerns. Therefore, one of the future potato breeding goals is to develop genotypes with high SGAs in the leaves to protect the crop from insects or fungal diseases, but a low level of SGAs in the edible tubers. To achieve that goal, SGA biosynthetic genes, the genetic factors affecting the expression levels, as well as the correlation of the SGA content, need to be thoroughly analyzed.

There was a relationship between TGA content and the expression of genes involved in GA biosynthesis in potato tubers. These findings provide an important step toward understanding TGA regulation and variation in potato tubers.

Conclusion

Understanding GA variety in Solanum species and then choosing the best source in pharmaceutical industries and drug preparation is the key goal of these studies, and controlling development of these metabolites is crucial in the future.

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