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Steroidal glycoalkaloid profiling and structures of glycoalkaloids in wild tomato fruit

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ABSTRACT

Steroidal glycoalkaloids (SGAs) constitute one of the main groups of secondary metabolites in tomato fruit. However, the detailed composition of SGAs other than α-tomatine, dehydrotomatine and esculeoside A, remains unclear. Comparative SGA profiling was performed in eight tomato accessions, including wild tomato species by HPLC-Fourier transform ion cyclotron resonance mass spectrometry (HPLC-FTICR/MS). On the basis of molecular formulae obtained from accurate *m*/*z* and fragmentation patterns by multistage MS/ MS (MS^n), 123 glycoalkaloids in total were screened. Detailed MS^n analysis showed that the observed structural diversity was derived from various chemical modifications, such as glycosylation, acetylation, hydroxylation and isomerization. Total SGA content in each tomato accession was in the range of 121–1986 nmol/g fr. wt. Furthermore, the compositional variety of SGA structures was distinctive in some tomato accessions. While most tomato accessions were basically categorized as α-tomatine-rich or esculeoside A-rich group, other specific SGAs also accumulated at high levels in wild tomato. Here, five such SGAs were isolated and their structures were determined by NMR spectroscopic analysis, indicating three of them were presumably synthesized during α-tomatine metabolism.

1. Introduction

Steroidal glycoalkaloids (SGAs) are characteristic secondary metabolites in plants of the Solanaceae, and are stored in all plant tissues, including leaves, roots, flowers, fruits (cf. tomato and eggplant) and tubers (cf. potato) (Friedman, 2002, 2006; Kozukue et al., 2008; Mennella et al., 2010). Various combinations of the steroidal alkaloid aglycone and sugar moieties generate considerable structural diversity of SGAs. In addition, their chemical structures determine their biological activities, for example, toxicity to animals, anti-cancer properties, and anti-microbial activities (Blankemeyer et al., 1997, 1998; Friedman et al., 2009; Ikeda et al., 2000; Milner et al., 2011).

In tomato plants, α-tomatine (**8**), which consists of tomatidine (**15**) and lycotetraose (**17**) (4-*O*-(2-*O*-*β*-D-glucopyranosyl-3-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-*β*-D-galactopyranoside), is well known as the main SGA predominantly found in leaves and immature fruits (Cataldi et al., 2005; Friedman, 2002; Kozukue et al., 2004) (see Fig. 1). On the other hand, esculeoside A (**1**) and B (**9**) are stored in the ripe fruit of cultivated tomatoes, in particular, the content of esculeoside A (**1**) is comparable to or higher than that of lycopene, the main tomato carotenoid (Fujiwara et al., 2004; Nohara et al., 2010). Recently, it was reported that its content increases during fruit ripening in contrast to a decrease in α-tomatine (**8**) (Iijima et al., 2008; Mintz-Oron et al., 2008; Yamanaka et al., 2008). Furthermore, it was reported that ethylene production or signaling during ripening affects these changes, suggesting that esculeoside A (**1**) is synthesized from α-tomatine (**8**) as a precursor (Iijima et al., 2009).

Tomatoes originate from South America, and many varieties of wild tomato have been collected in the C.M. Rick Tomato Genetics Resource Center at the University of California, Davis (TGRC, URL: http://tgrc.ucdavis.edu/). The morphology of wild tomatoes is unique, and the fruit of each species presents a distinctive size, shape, smell and color. From the viewpoint of tomato plant evolution, genomic diversity determined by DNA polymorphism in wild

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tomato species is of considerable interest, and several groups have evaluated it based on genome sequence using markers and single nucleotide polymorphisms (SNPs) (Frary et al., 2005; Jimenez-Gomez and Maloof, 2009; Miller and Tanksley, 1990; Roselius et al., 2005; Spooner et al., 2005; Tanksley and McCouch, 1997). On the other hand, the composition of metabolites, such as primary metabolites (Schauer et al., 2005), carotenoids (Melendez-Martinez et al., 2010), phenolics (Antonious et al., 2003; Melendez-Martinez et al., 2010), volatiles (Antonious and Kochhar, 2003; Tieman et al., 2010) and minerals (Fernandez-Ruiz et al., 2011), has been reported for wild tomato fruits. Furthermore, the harnessing of wild tomato species has recently arisen in relation to molecular breeding and understanding tomato biodiversity. In particular, quantitative trait locus (QTL) analysis using crossed lines of cultivated and wild tomato species identified candidate gene loci involved in the biosynthesis of metabolites, such as sugars (Fridman et al., 2000), primary metabolites (Bermudez et al., 2008; Schauer et al., 2006, 2008), vitamins (Stevens et al., 2007), and volatiles (Mathieu et al., 2009). These approaches suggest the potential benefits of using wild tomato species to clarify the biosynthesis of various metabolites. Nevertheless, while SGAs are characteristic metabolites in tomato, their metabolic profiles have not yet been fully elucidated in wild tomato fruits.

In this study, comprehensive profiling of SGAs was performed using fruits of wild and cultivated tomato species by HPLC-Fourier transform ion cyclotron resonance mass spectrometry (HPLC-FTICR/MS) analysis. Accurate m/z and MS^n fragmentation analysis allowed screening of more than 120 SGAs from eight tomato accessions. In addition, the structures of five characteristic SGAs other than α-tomatine (**8**), dehydrotomatine (**6**) and esculeoside A (**1**), were determined by NMR and MS. Herein, the SGA structural diversity and metabolism in tomato using detected SGAs is discussed.

2. Results

2.1. Screening and annotation of SGAs from tomato fruit extracts by HPLC-FTICR/MS

Fruit of eight tomato accessions, including 3 accessions of *Solanum lycopersicum* were harvested (Table 1). The fruit of all three *S. lycopersicum* accessions (LA1090, LA2213, LA3911) is similar to that of common cultivated red tomatoes, except for size. The fruit of LA2213 (*S. lycopersicum* var. *cerasiforme*) was reported as bitter tasting, with a high α-tomatine (**8**) content (Rick et al., 1994). Therefore, it was included as a sample in this research. All *S. lycopersicum* and LA 1589 (*Solanum pimpinellifolium*) fruits turned red after ripening, whereas LA0716 (*Solanum pennellii*), LA1777 (*Solanum habrochaites*), and LA2133 (*Solanum neorickii*) remained green, and LA1414 (*Solanum cheesmaniae*) ripened yellow (Table 1).

The frozen pericarp of each sample was used to extract SGAs with methanol, and each extract was analyzed by HPLC-FTICR/MS analysis using an electron spray ionization (ESI) method in the positive ion mode. Total ion chromatograms in the range of m/z 800–1500 are shown in Fig. 2. Accurate m/z values of detected peaks, which indicate the quasi-molecular ions ($[M+H]^+$) of the metabolites, were used for calculation of molecular formulae. Estimation of molecular formulae from detected peaks was performed using a previously described procedure (Iijima et al., 2008). First, the detected peak ions in the data from each sample were obtained, then exported to text files and compared at an accurate molecular mass level using analytical tools previously developed (Power Get; http://www.kazusa.or.jp/komics/software/PowerGet/, MatchedIonsFinder; (Yamamoto et al., 2012)). Detailed analytical settings are described in the Section 5. Next, peaks commonly observed in the data for the same tomato species ($n = 3$), were selected with accurate m/z values used to calculate molecular formulae (Sakurai et al., 2013). MS^2 fragment ions targeted to quasi-molecular ions also aided in distinguishing peaks of SGAs (Cahill et al., 2010; Cataldi et al., 2005; Iijima et al., 2009). The combined information of molecular formulae and these fragment ions allowed identification of peaks representing 123 distinct SGAs in the tomato samples (Supplemental Table 1). The structural variety of the 123 SGAs is summarized in the Supplemental Table 1. The *M*r of the detected SGAs ranged between 901.5 and 1431.6, and SGAs with *M*r values greater than 1200 being detected frequently in LA1414 (*S. cheesmaniae*), LA1589 (*S. pimpinellifolium*), and LA3911 (*S. lycopersicum* cv. Micro-Tom), respectively.

α-Tomatine (**8**) was one of the few SGAs commonly detected in all tomato samples (Peak No. 8 in Fig. 2, Peak No. 34 in Supplemental Table 1), while other SGAs were detected in specific accessions. The main factor contributing to the structural diversity of SGAs in tomato fruit was the presence of many isomers. For example, peaks representing the formula $C_{50}H_{83}NO_{22}$ were detected as eight isomers. In order to investigate the structure of each SGA in detail, MS^n analysis was performed. The key fragment ions used for determination of partial structures are shown in Table 2. Sufficient MS^n analytical data was acquired from 108 of the 123 SGAs detected (Supplemental Table 1). Initial MS/MS analysis for 39 SGAs showed a strong fragment ion derived from H_2O removal ($-m/z$ 18) (Table 2). From the MS^n analysis of 25 SGAs, fragment ions corresponding to neutral loss of an acetic acid group ($[M+H-CH_3COOH]^+$) were detected. The cleaved acetoxy moiety as acetic acid is easily detected as a nominal mass of 60 Da by MS^n analysis (Table 2). Previously identified acetoxy SGAs in tomato fruit were lycoperoside A (**10**), B (**11**) and C (**7**), and esculeoside A (**1**) (Fujiwara et al., 2004; Yahara et al., 1996). Most other acetylated SGAs detected are reported for the first time.

With respect to glycosidically bound metabolites such as SGAs, triterpene saponins and flavonoids, MS^2 fragment ions are informative to estimate varieties of the bound oligosaccharide (Cataldi et al., 2005; Huhman and Sumner, 2002; Iijima et al., 2008; Suzuki et al., 2008). Fragment ions indicating glycosidic cleavages were gained, and the composition of sugar moiety was elucidated by calculation of neutral loss in MS^n data (Table 2). Most of these SGAs were considered to result from conjugation of lycotetraose (**17**).

Table 1
Description and fruit characteristics of the fruits of wild and cultivated tomato species used in this study.

Accession	Taxon	Color at ripeness
LA0716	*S. pennellii*	Green
LA1090	*S. lycopersicum* cv. Rutgers	Red
LA1414	*S. cheesmaniae*	Yellow
LA1589	*S. pimpinellifolium*	Red
LA1777	*S. habrochaites*	Green
LA2133	*S. neorickii*	Green
LA2213	*S. lycopersicum* var. *cerasiforme*	Red
LA3911	*S. lycopersicum* cv. Micro-Tom	Red

Table 2
Estimation of partial structures of detected SGAs using MS^n fragmentation in positive ion mode.

Detected ions (m/z)	Partial structures contained
$[M+H]^+-162$	Glucose
$[M+H]^+-132$	Xylose
$[M+H]^+-294$	Glucose + xylose
$[M+H]^+-456$	Glucose + xylose + glucose
$[M+H]^+-618$	Lycotetraose
$[M+H]^+-18$	Hydroxy group
$[M+H]^+-60$	Acetoxy group
273, 255	Cholestan-3-ol derivatives
271, 253	Cholesten-3-ol derivatives