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ARTICLE

α-Solasonine and α-Solamargine Contents of Gboma (*Solanum macrocarpon* L.) and Scarlet (*Solanum aethiopicum* L.) Eggplants

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The gboma (*Solanum macrocarpon* L.) and scarlet eggplants (*Solanum aethiopicum* L.), which form part of the traditional sub-Saharan African culture, are two of the many neglected crops with potential for increased cultivation or as a genetic resource for improving agronomic traits of the common eggplant. This work is focused on the analysis of glycoalkaloid levels in *S. macrocarpon* and *S. aethiopicum* to assess their safety. Liquid chromatography−mass spectrometry was used to quantify glycoalkaloids in *S. macrocarpon* and *S. aethiopicum* compared to *Solanum melongena* L. Fruits of *S. aethiopicum* and *S. melongena* contained 0.58−4.56 mg/100 g of α-solamargine and 0.17−1 mg/100 g of α-solasonine, on a wet basis. *S. macrocarpon* fruits had much higher values of α-solamargine (124−197 mg/100 g) and α-solasonine (16−23 mg/100 g). However, the proportions of α-solamargine and α-solasonine of *S. melongena* and *S. macrocarpon* were similar (76−89% of α-solamargine), whereas in *S. aethiopicum* fruit composition was more variable (48−89% of α-solamargine). According to these results, the glycoalkaloid levels of *S. macrocarpon* fruits are 5−10 times higher than the value considered to be safe in foods and might not be considered suitable for human consumption; however, the glycoalkaloid levels of *S. aethiopicum* were similar to those of *S. melongena* (about 14% of values considered as toxic) and could be considered as safe for consumption. The incorporation of the cultivated African *S. aethiopicum* into eggplant breeding programs to develop improved varieties of the common eggplant may represent an alternative to crossing with wild species.

KEYWORDS: Eggplant; glycoalkaloids; α-solasonine; α-solamargine; *Solanum macrocarpon* L.; *Solanum aethiopicum* L.; *Solanum melongena* L.

INTRODUCTION

Eggplant fruits are one of the most widely consumed vegetables in the world. The most popular eggplant species is the common eggplant (*Solanum melongena* L.), which was domesticated in Southeast Asia (*1*). Two other cultivated species of eggplant, namely, the gboma eggplant (*Solanum macrocarpon* L.) and the scarlet eggplant (*Solanum aethiopicum* L.), are native to Africa and are popular in some parts of that continent. *S. macrocarpon* is grown in tropical western Africa for its fruits and leaves, as well as in areas of South America, the Caribbean, and Southeast Asia, where different cultivars are used for food and ornamental purposes. *S. aethiopicum* is widely distributed on the African continent, and there are several cultivars (Gilo, Kumba, and Shum) adapted to a wide range of environments, from humid areas of tropical regions (Gilo and Shum groups) to semidesert regions.

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Neither the gboma nor scarlet eggplant has been subjected to intensive breeding and could be useful genetic resources for improving the common eggplant (*1*). *S. macrocarpon* and *S. aethiopicum* are resistant to several diseases and pests that affect the common eggplant and may also represent a source to improve consumer quality traits of the common eggplant. For example, they have been shown to contain a high level of phenolics (above 110 mg/100 g of fresh weight) and vitamin C (9−22 mg/100 g of fresh weight), compared to common eggplant. In this respect, the stability of vitamin C in cooked eggplant fruits is estimated to be around 60%. Interspecific hybrids between both species and *S. melongena* have shown that they do not present many undesirable traits typical of wild species that have to be discarded in breeding programs and are therefore desirable candidates for eggplant breeding programs (*2*−*6*). Common eggplant lines with resistance to *Fusarium* introgressed from *S. aethiopicum* have been obtained (*7*), and work has been done to introgress resistance to spider mites from *S. macrocarpon* (*8*).

Although *S. aethiopicum* and *S. macrocarpon* are cultivated crops, *S. macrocarpon* has been reported to produce high levels of

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glycoalkaloids compared to the common eggplant (*1*). Solanaceae glycoalkaloids (based on a steroid structure bonded to different sugars) may play an important role in the defense of the plant against pests, but they also may have harmful effects for the health of humans. More than 75 naturally occurring glycoalkaloids have been isolated from 350 species. Generally, the glycoalkaloids are referred to as α-compounds; the cleavage (by acid or enzymatic hydrolysis) of the individual sugars of the glycoside leads to *β*-, *γ*-, or *δ*-compounds, depending on the number of sugars in the side chains.

Solanine and chaconine (with the solanidine aglycone), the major glycoalkaloids in potatoes, are highly toxic and have been responsible for some serious intoxications in humans due to their ability to bind membrane sterols, disrupt cell membranes, inhibit acetylcholinesterase, and perturb acid−base equilibrium in vivo. At this time informal guidelines limiting the total glycoalkaloids concentration in potatoes to 200 mg/kg of fresh weight have been established as a safe value; however, this level has to be re-evaluated for glycoalkaloids different from solanine, because the activity of each compound can vary from the others and also because synergistic effects between different glycoalkaloids can occur (*9*).

The triglycosides α-solamargine and α-solasonine are the major glycoalkaloids of the common eggplant fruits (*10*). They contain the same aglycone (solasodine), bound to the trisaccharide chacotriose [α-L-rhamnose 1→4 (α-L-rhamnose 1→2)*β*-D-glucose] for α-solamargine, and solatriose [*β*-D-glucose 1→3 (α-L-rhamnose 1→2) *β*-D-galactose] for α-solasonine. Solasodine-derived alkaloids do not have a marked toxicity as do solanidine-derived compounds (*11*), although they may have some estrogenic effect that may affect male fertility and disrupt cell membrane, resulting in alterations in the liver and central nervous system. A estimated LD_{50} value in rodents (about 1.75 mg/kg of body weight) has been found to be similar to that of solanine (*12*,*13*). Some beneficial and medicinal properties have been described for α-solamargine and α-solasonine such as inducing apoptosis in human cancer cells (*14*), cytolysis of the parasite *Trypanosoma cruzii* (*15*), and treating herpes viruses (*16*).

Different methodologies have been proposed for the analysis of Solanaceae glycoalkaloids in foodstuffs and plant materials, including colorimetric, chromatographic, or immunoassay methods (*6*, *17*, *18*). Gas chromatography requires a previous hydrolysis of the glycoside to aglycones, so it is not useful for the analysis of α-solamargine and SS. The most common method to analyze glycoalkaloids is HPLC-UV; however, due to the lack of a strong UV chromophore, 200−210 nm is used as working wavelength, and many interfering substances may be observed. HPLC−mass spectrometric (LC-MS) methods can result in better sensitivity and specificity for glycoalkaloid analysis, because they give much specific structural information about each compound, avoiding interferences, simplifying the pretreatment of the sample, and having high sensitivity (*19*, *20*).

The complex nature of glycoalkaloid−dietary relationships suggests the need for accurate methods to measure the content of each individual glycoalkaloid in foods as well as in body fluids (*10*). Furthermore, for an efficient development of eggplant breeding programs, it is necessary to collect information concerning the characteristics and diversity of eggplant genetic resources (*9*). The use *S. aethiopicum* and *S. macrocarpon* and other neglected eggplant species to develop new cultivars of the common eggplant may result in the introduction of unacceptable levels of glycoalkaloids in the breeding lines. The necessity of determining the glycoalkaloid content of new cultivars of Solanaceae before they can be released for commercial use is recognized (*10*). To the authors' knowledge, very few studies reporting quantitative data of α-solamargine and α-solasonine levels in *S. macrocarpon* and *S. aethiopicum* fruits have been published (*6*).

For that reason, this work is focused on the quantification of separate contents of the most important glycoalkaloids in eggplants (α-solamargine and α-solasonine), in the fruits of different accessions of *S. macrocarpon* and *S. aethiopicum*, compared to *S. melongena* fruits. This will provide relevant information to improve the utilization of these species both as crops for human consumption and also as sources of variation for the economically more important common eggplant (*S. melongena*).

MATERIALS AND METHODS

Acetic acid, butanol, and HPLC solvents were obtained from Merck (Darmstadt, Germany). Standards of α-solamargine and α-solasonine were generously provided by Dr. M. Friedman and Carol Levine, Western Regional Research Center, U.S. Department of Agriculture, Albany, CA.

Plant Material. Fruits from different accessions of *S. macrocarpon* (gboma eggplant), BBS-196, BBS-178, RNL-371, and RNL-374; and *S. aethiopicum* (scarlet eggplant), BBS-116, BBS-157, and RNL-187, were used. The *S. aethiopicum* accessions respectively belong to the Shum, Gilo, and Kumba cultivar groups. Three commercial accessions of common eggplant *S. melongena* were also selected and analyzed for comparison purposes: IVIA-371 and CS-16 (varieties for fresh consumption) and H-11 (variety produced for pickles). The differences in morphology, color, and size are shown in **Figure 1** and **Table 1**.

Twenty plants of each of these accessions were grown in open field conditions in an experimental plot of the Polytechnical University of Valencia (Valencia, Spain) using the common cultivation practices of the area. Fruits were harvested in September−October 2006, and three representative samples of each accession, each containing a minimum of 1 kg of fruit, were used for the analyses. All of the samples were peeled with the exception of BBS-116, due to its extremely small size (**Table 1**). Flesh samples were homogenized and analyzed for dry matter by drying at 105 ± 2 °C or were freeze-dried, ground to a fine powder, and preserved in a dark and dry environment until analyzed for glycoalkaloids.

Glycoalkaloid Extraction and Analysis. Several methods of extraction based on previous literature have been evaluated, and a method based on that Friedman and co-workers (*9*, *17*) was selected. Approximately 50 mg of freeze-dried eggplant flesh was mixed with 25 mL of 5% acetic acid and extracted twice by ultrasonic bath for 10 min followed by centrifugation at 10000*g* for 10 min. The supernatants were combined, filtered, and adjusted with 10 mL of 29% NaOH to pH 10−11. The filtrate was extracted twice with 20 mL of water-saturated butanol in a separatory funnel. The butanol fractions were combined, and the solvent was evaporated under a N_2 stream, using a evaporator (Organomation, Ontario, Canada). The dried residue was dissolved with 1 mL of a mixture of methanol/acetonitrile/water (10:55:35) and filtered through a 0.45 *μ*m membrane (HA, Millipore, Billerica, MA), and 20 *μ*L was injected into the chromatographic system. The following chromatographic conditions were used: carbohydrate column (Alltech, Fresno, CA), 250 × 4.6 mm, 5 *μ*m particle size; acetonitrile/water (70:30) as mobile phase; flow rate = 0.5 mL/min. The LC system (Surveyor system including a LC pump, autosampler, and PDA, Thermo Finnigan, San Jose, CA) was connected to a Finnigan LCQ quadrupole ion trap mass spectrometer (Thermo Finnigan, Inc., Waltham, MA) through an electrospray ionization source (ESI). The optimized operating parameters of the ESI-MS interface were as follows: capillary temperature, 280 °C; capillary voltage, 25 V; sheath N_2 gas flow, 80 arbitrary (instrument) units; tube lens offset, 10 V; and ion collection time, 200 ms. The MS experiment was performed first in full scanning mode over a mass range of *m*/*z* 840−1100, and those peaks that are relevant to glycoalkaloids were further investigated by performing product ion scanning (PIS). In PIS, masses for molecular ions of glycoalkaloids were selected and fragmented with collision energy ramped from 20 to 60 [unit, NCE (%)], and the fragments were scanned over the range of *m*/*z* 350−900. The collision energy was adjusted as 30 [unit, NCE (%)] for all glycoalkaloids and used for the glycoalkaloid analysis in the samples where the most unambiguous fragmentation patterns are acquired. The HPLC-MS chromatograms of a typical eggplant fruit extract are shown in **Figure 2**. Calibration curves were generated using a mixture of

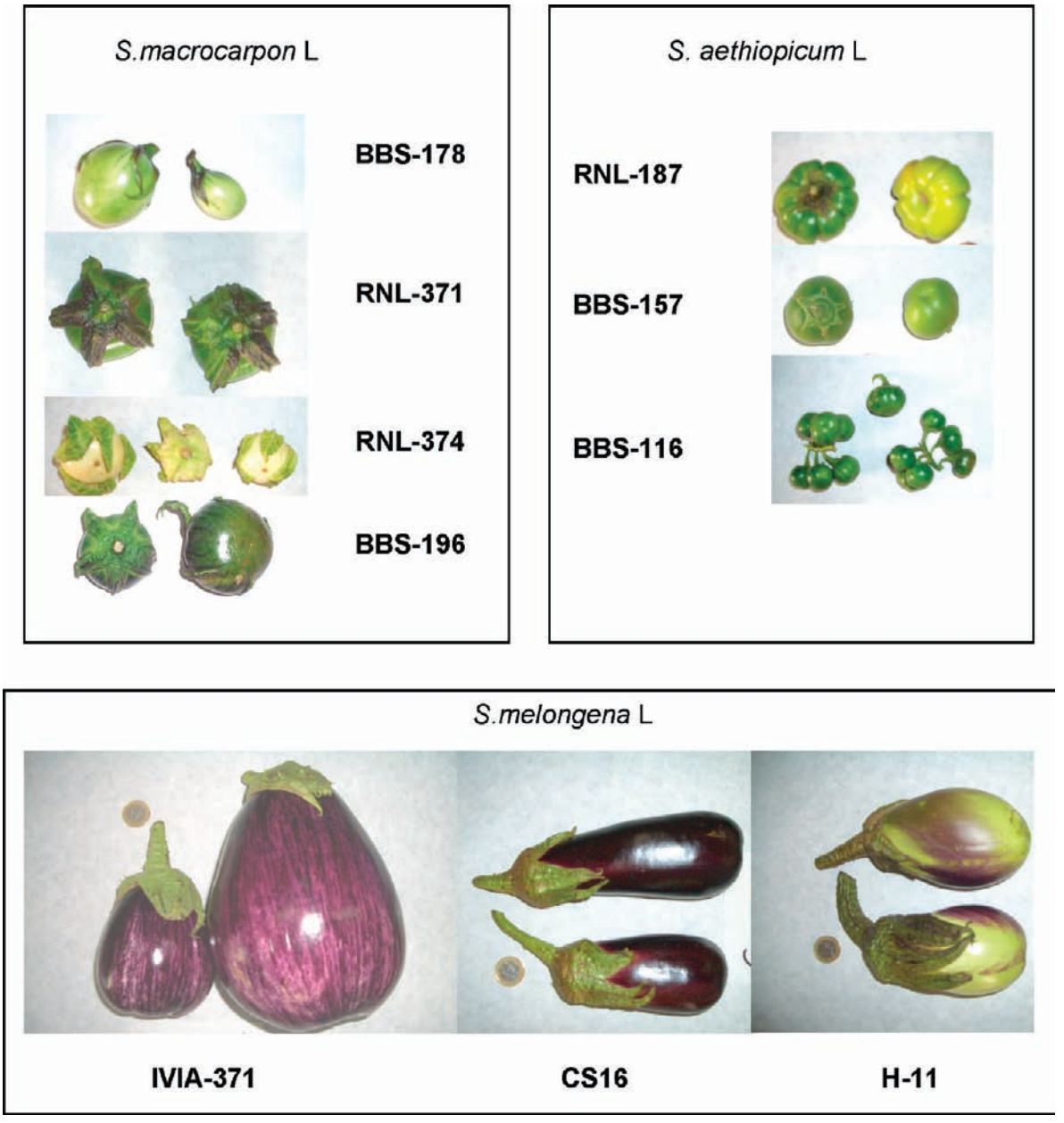

Figure 1. Morphology of the common (*S. melongena*), scarlet (*S. aethiopicum*), and gboma (*S. macrocarpon*) eggplant fruits analyzed.

α-solamargine and α-solasonine standards in the ranges from 0 to 100 μg/mL and from 0 to 30 μg/mL, respectively (R^2 = 0.99). All glycoalkaloid analyses were made in triplicate.

RESULTS AND DISCUSSION

Ultrasonication in 5% acetic acid was found to be the most efficient condition, in agreement with Friedman et al. (*9*), followed by liquid/liquid extraction was preferred in agreement with previous authors (*17*−*21*), because other methods showed poorer reproducibility and recoveries. Some authors (*9*, *21*) reported that problems with peak separation and resolution may occur in the HPLC analysis of glycoalkaloids, either with C18 or amino columns with different elution conditions compatible with evaporative light scattering detector or mass spectrometry detector (nonvolatile compounds such as phosphates are incompatible with both detection systems). To overcome these difficulties in the separation of glycoalkaloids with the same aglycone, mass spectrometry detection with electrospray ionization (ESI) has been previously applied to glycoalkaloids analysis and permits the identification and quantification of coeluting compounds by performing MS experiment in selective ion monitoring (SIM) mode (*19*, *20*). This method was used for our samples. The accuracy of the method was evaluated by spiking *S. macrocarpon* fruits with known amounts of mixed standards of α-solamargine and α-solasonine in triplicate. It was found that the average recovery amounts were 97−101%, with RSD of < 5%; both parameters are in agreement with the recommendations of the AOAC (*22*) (recovery of 90−107% and relative standard deviation below 5.3%) for analytes found in samples in concentration near 100 mg/100 g.

Solamargine and solasonine were found in all samples including the common eggplant *S. melongena*. Other glycoalkaloids evaluated (solanine, chaconine, tomatine) were not found in the extracts. The identification was made at first by their molecular weights acquired in MS experiment and further confirmed by fragmentation patterns obtained by MS/MS experiment in PIS mode, comparing the fragmentation patterns with standards of α-solamargine and α-solasonine under the same conditions of analysis, as well as with those reported by the literature (*19*, *23*). PIS at

Table 1. Mean Weight and Dry Matter Content in the Analyzed Fruits

species and accesion	origin	mean fruit weight[a] (g)	dry matter[a] (%)
S. macrocarpon L.			
BBS-178	Ivory Coast	26.22 ± 5.34	14.01 ± 0.46
RNL-371	Ghana	38.36 ± 4.63	13.06 ± 0.54
RNL-374	Ghana	29.43 ± 3.27	11.74 ± 0.54
BBS-196	Ivory Coast	81.57 ± 4.68	12.47 ± 0.42
S. aethiopicum L.			
RNL-187	Burkina Faso	19.33 ± 1.68	13.58 ± 0.27
BBS-157	Ivory Coast	21.95 ± 2.93	12.44 ± 0.55
BBS-116	Ivory Coast	2.92 ± 0.82	14.2 ± 0.71
S. melongena L.			
IVIA-371	Spain	317.20 ± 23.50	7.13 ± 0.17
CS-16	Spain	188.72 ± 36.11	7.22 ± 0.80
H-11	Spain	192.09 ± 25.46	7.76 ± 0.04

[a] Values expressed as mean value (n = 3) ± standard error.

m/z 868.5 ([α-solamargine + H]$^+$) and 884.4 ([α-solasonine + H]$^+$) followed by daughter ion scanning between m/z 350 and 900 resulted in one peak at 6.68 min, and its spectrum indicates the coelution of α-solamargine and α-solasonine (**Figure 2**). The positive ionization mode was chosen over negative ionization mode because it provided more structural information in agreement with Chen et al. (*23*). The interpretation of the positive ion MS/MS fragmentation spectrum of α-solasonine is explained in **Figure 3** and was based on that of Chen et al. (*23*), Domon and Costello (*24*), and Costello and Vath (*25*). The fragments obtained in this study were coincident with those obtained by these authors. Some dehydratation fragments were also obtained on the basis of the mechanisms proposed by Stobiecki et al. (*19*) and Väänänen et al. (*20*).

There were large differences in the glycoalkaloid content among the three different species (**Table 2**). The literature also reports a wide range of variation in the glycoalkaloid contents of different species of the *Solanum* genus, but to the authors' knowledge, very few studies reporting quantitative data of total glycoalkaloids content, and none about levels of α-solamargine and α-solasonine in *S. macrocarpon* and *S. aethiopicum* fruits, have been published (*6*).

The levels of total glycoalkaloids in all accessions of the scarlet eggplant *S. aethiopicum* (with the exception of BBS-116) and the common eggplant *S. melongena* were less than or about 2 mg/100 g on a wet basis (**Table 2**). The highest levels of glycoalkaloids in *S. melongena* were found in the pickling variety H-11, which contained almost double levels of the other accessions. Of the *S. aethiopicum* varieties, BBS-116 had the highest values of glycoalkaloids and might be attributed to the presence of peels in the samples analyzed for this accession. However, although it is well-known that tuberous Solanaceous crops such as potatoes have higher glycoalkaloid levels in the peels than in the flesh (*10*), the location of glycoalkaloids in fruits is not always the same as in the tubers. Aubert et al. (*26*) have shown that solasodine-derived glycoalkaloids are mainly located in the flesh of eggplant fruits; these authors found the highest amount of total glycoalkaloids (7−38 mg/100 g) in the mesocarp including seeds, compared to the mesocarp without seeds (1−4 mg/100 g), and only trace amounts in the peels. For that reason, the fact that the unpeeled accesion BBS-116, belonging to *S. aethiopicum*, showed higher values of glycoalkaloids than the other varieties of the same species cannot be attributed only to the fact that peels are included.

Total eggplant glycoalkaloid contents reported by those authors agree with the data presented in our study for *S. melongena* and *S. aethiopicum*, which were also lower than the levels found in most of the fruits of different species of genus *Solanum* (*27*). *S. macrocarpon* fruits clearly contained a much higher glycoalkaloid content than *S. aethiopicum* or *S. melongena* (**Table 2**). Total

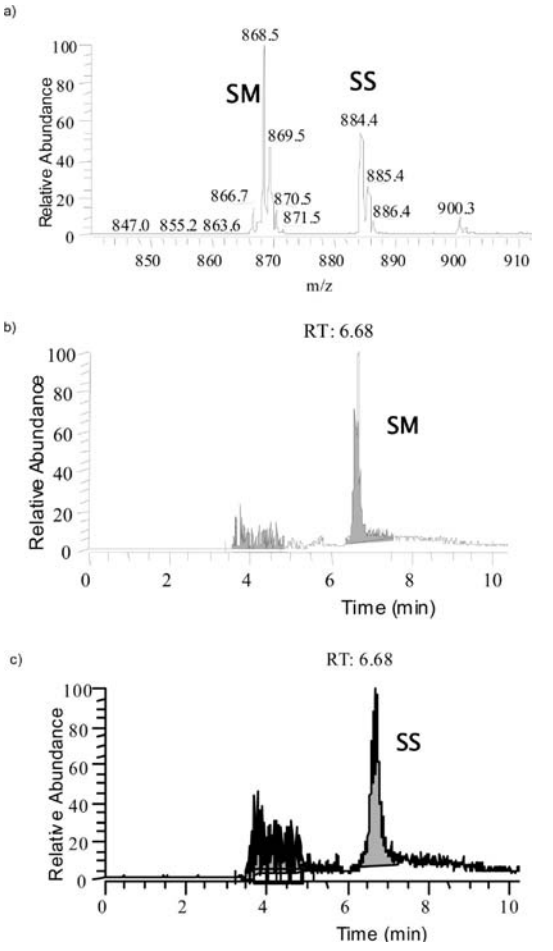

Figure 2. LC-ESI-MS analysis of eggplant fruit extracts: (**a**) spectrum acquired in full scanning mode (m/z 840−1100) at time 6.57−6.68 min; (**b**) MS chromatogram acquired by parent ion scan (parent ion, m/z 868.5; product ions, m/z 350−900) (SM = α-solamargine); (**c**) MS chromatogram acquired by parent ion scan (parent ion, m/z 884.5; product ions, m/z 350−900) (SS = α-solasonine).

levels of glycoalkaloids were above 100 mg/100 g and reached 221 mg/100 g in RNL-371. The α-solamargine content ranged from 124 to 197 mg/100 g. The glycoalkaloid contents found in our study exceed the amounts reported by Aubert et al. (*6*) for *S. macrocarpon* fruits (12.5 mg/100 g on wet basis) and may be an indication that important intraspecific variation exists for this trait in this species. Levels of glycoalkaloids found in our study for *S. macrocarpon* (1.0−1.7% of total glycoalkaloids on dry basis) are probably related to the bitter taste of the fruits of *S. macrocarpon* and were close to those reported in some fruits of wild *Solanum* species. Many of these wild species are considered to be toxic, such as *Solanum persicum* Wild ex Roem & Schuldt (about 1.9% of total glycoalkaloids on a dry basis) or *Solanum sodomaeum* L. (0.22% on a wet basis). Even higher glycoalkaloid contents have been reported in other fruits such as *Solanum linnaeanum* Hepper & PML Jaeger fruits (2.5% of α-solamargine on a dry basis) by Eanes et al. (*21*) or *Solanum khasianum* C.B. Clarke (3.4−4.2% of total glycoalkaloids on a dry basis), as reported by Putalun et al. (*28*).

As in many Solanaceous species, "paired" alkaloids are present (two compounds based on a common aglycone but differing in their carbohydrate moiety) (*11*); in this case α-solamargine was always the major compound of *S. melongena* and *S. macrocarpon*, being 76−85 and 84−89% of total glycoalkaloids, respectively (**Figure 4**). The *S. macrocarpon* accession RNL-371 had much

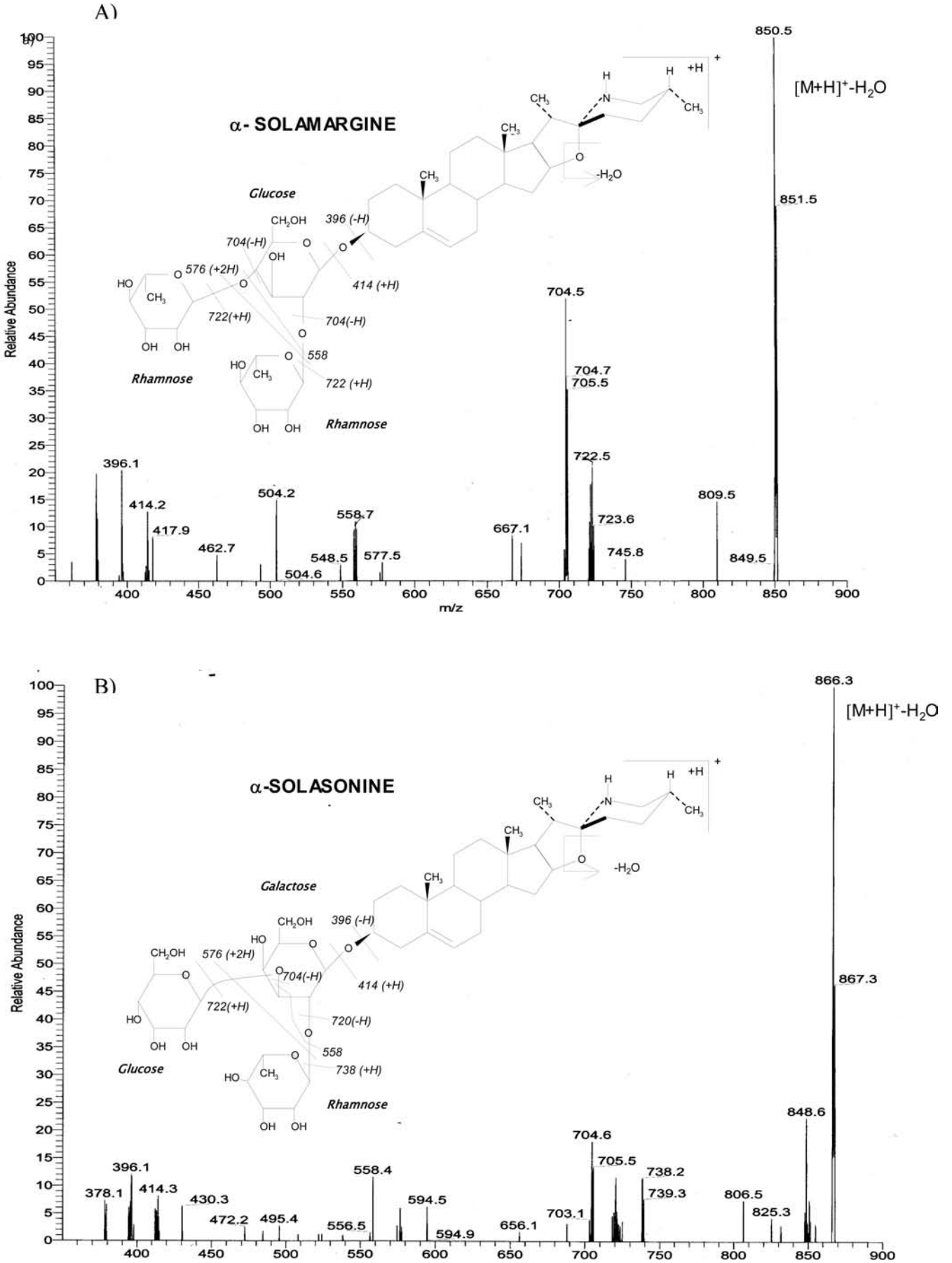

Figure 3. LC-ESI-MS/MS spectrum of α-solamargine (**A**) and α-solasonine (**B**) and interpretation of their major fragments: (**A**) spectrum of product ion scan of *m*/*z* 868.5 (identified as α-solamargine); (**B**) spectrum of product ion scan of *m*/*z* 884.5 (identified as α-solasonine).

higher levels for α-solamargine than the rest of accessions of this species; however, α-solasonine was similar for RNL-371, RNL-374, and BBS-196, being lower for BBS-178. Friedman and McDonald (*29*) postulated that many *Solanum* species including potato and eggplant may produce initially only one glycoalkaloid but, as an adaptative response to acquired immunity of the pathogen over time, later elaborates a second one (by modification of the carbohydrate moiety), which is biologically more active. The predominance of one glycoalkaloid in the fruits of *S. melongena* and *S. macrocarpon* may be due to this adaptation hypothesis.

The proportions of α-solamargine and α-solasonine in *S. aethiopicum* were very variable and different from that in *S. melongena*, with α-solamargine content ranging from 48 to 58% for RNL-187 and BBS-157 and to 89% for BBS-116 (**Figure 4**). Previous authors have also reported very high variability in the ratio of α-solamargine to α-solasonine in other Solanaceous fruits. High intraspecific variability has been reported for fruits of *Solanum laciniatum* L., with ratios of 1−2 (*30*). In our samples, this ratio ranges from 0.94 to 8.6 for *S. aethiopicum*, in contrast with the stability of this parameter in *S. melongena* (3.1−5.8) and *S. macrocarpon* (5.4−8.3).

Table 2. Glycoalkaloids Contents Found in Analyzed Eggplant Fruits (on Wet Basis)

species and accession	α-solamargine[a] (mg/100 g)	α-solasonine[a] (mg/100 g)	total[a] (mg/100 g)
S. macrocarpon L.			
RNL-371	197.5 ± 6.7	23.5 ± 0.06	221.0 ± 8.8
RNL-374	125.1 ± 8.2	21.1 ± 1.67	146.1 ± 6.5
BBS-196	125.5 ± 9.7	22.9 ± 2.54	148.4 ± 12.2
BBS-178	124.1 ± 8.8	16.1 ± 0.75	140.2 ± 9.52
S. aethiopicum L.			
RNL-187	0.94 ± 0.02	1.00 ± 0.02	1.95 ± 0.13
BBS-157	0.58 ± 0.02	0.41 ± 0.03	1.00 ± 0.20
BBS-116	4.86 ± 0.10	0.57 ± 0.03	5.43 ± 0.15
S. melongena L.			
IVIA-371	0.85 ± 0.03	0.27 ± 0.02	1.12 ± 0.02
CS-16	0.98 ± 0.07	0.17 ± 0.02	1.15 ± 0.15
H-11	1.61 ± 0.20	0.40 ± 0.02	2.01 ± 0.16

[a] Values expressed as mean value (n = 3) ± standard error.

Although solasodine-derived alkaloids may be less toxic than solanidine-derived compounds, their estimated LD_{50} value in rodents (about 1.75 mg/kg of body weight) has been found to be similar to that of solanine. As there are not official limits for solasodine-derived alkaloid levels in foods, the value of 20 mg/100 g of fresh weight, established as a limit of toxicity for solanidine-derived glycoalkaloids in foods, can be considered as a safe limit also for eggplants (*11*, *14*, *15*). By this criterion the levels of glycoalkaloids found in the fruits of *S. macrocarpon* are 5−10-fold higher than the levels considered to be safe for glycoalkaloids in foods, and for that reason, although the fruits of this species are consumed in some regions of Africa, they might not be considered as suitable for human consumption by present regional standards. However, the fruits of the scarlet eggplant, *S. aethiopicum*, with values similar to those of *S. melongena* commercial accessions, contain about 14% of the advisible toxicity limit, and for that reason, and in the same way that it has been already done with other Solanaceae, may be considered as suitable for human consumption and could be useful to improve several traits of the common eggplant *S. melongena*.

In conclusion, we determined the content of two major glycoalkaloids of eggplants (α-solamargine and α-solasonine) that may be used for the enhancement of these neglected African cultivated species. Our data also showed that intraspecific variability should be taken into account when these species are used in common eggplant breeding programs and that not only the species but also the varieties with safe amounts of glycoalkaloid

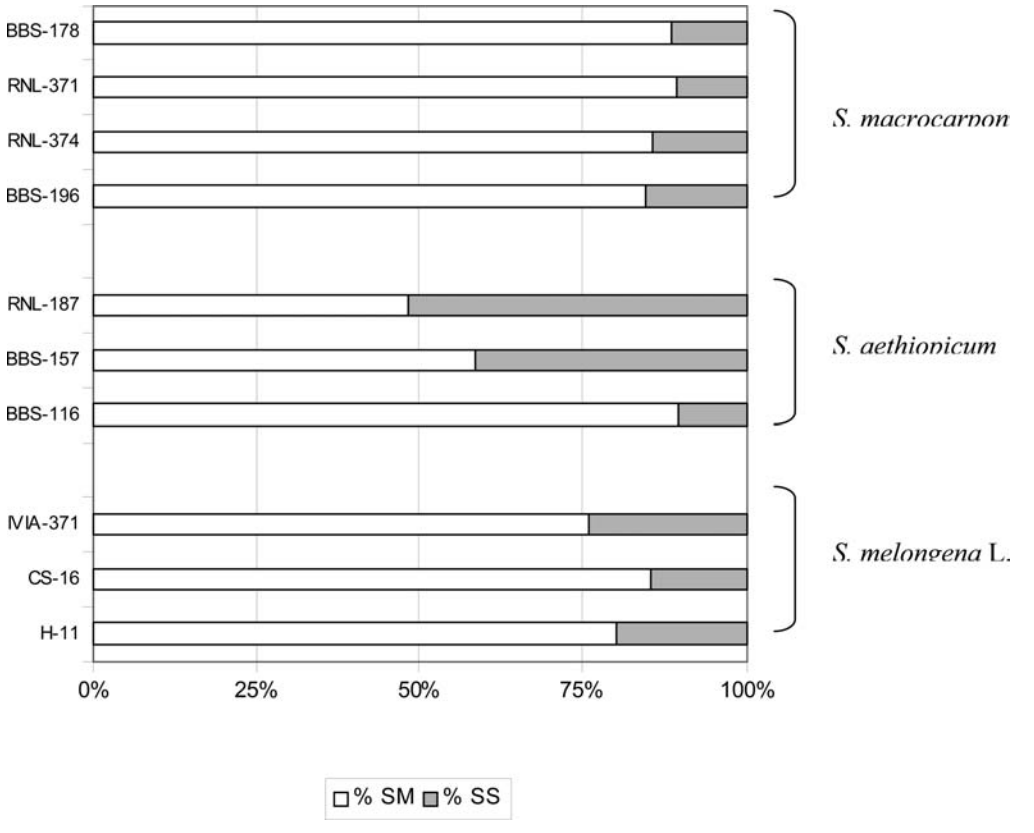

Figure 4. α-Solamargine and α-solasonine proportions in the eggplant fruits analyzed.

contents should be selected. It is also recommended that the analysis of the glycoalkaloid profiles of the hybrids should be performed. Studies of hybrids of wild and cultivated potato cultivars have shown different glycoalkaloid profiles in the hybrids compared to the parental tubers (*20*). The current low level of utilization of the available wild genetic resources may be due to concerns that interspecific hybridization may bring undesirable changes in quality along with the desirable traits of interest into the recipient crop plant. Therefore, this study on eggplant fruits contributes to increase the knowledge about species with desirable but not exploited genetic potential for improving the quality of domestic cultivated eggplant fruits and serves as a first step to study interspecific hybrids.

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