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Effect of Selenate Supplementation on Glycoalkaloid Content of Potato (*Solanum tuberosum* L.)

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Potatoes (*Solanum tuberosum* L.) supplemented with increasing amounts of sodium selenate were analyzed for glycoalkaloid (GA) content. GAs were extracted with 5% acetic acid from freeze-dried tubers of two potato cultivars, Satu and Sini, harvested 10 weeks after planting as immature. The GAs α-solanine and α-chaconine were quantified by reverse-phase high-performance liquid chromatography (RP-HPLC) with diode array detection. Two independent experiments were performed. In the first experiment, the total GA concentration ± standard error of the tubers ranged between 105 ± 9 and 124 ± 10 mg kg^{-1} fresh weight in Satu and between 194 ± 26 and 228 ± 10 mg kg^{-1} fresh weight in Sini. The ratio of α-solanine to α-chaconine was 0.2 in Satu and 0.5−0.6 in Sini. In the second experiment, the total GA concentration ± standard error was 75 ± 4 to 96 ± 11 mg kg^{-1} fresh weight, and the ratio of α-solanine to α-chaconine was 0.3−0.4 in Satu. A high sodium selenate supplementation (0.9 mg of Se kg^{-1} quartz sand) slightly decreased the GA content in Satu, but this decrease was not statistically significant. Furthermore, at this addition level the Se concentration increased to a very high level of 20 μg g^{-1} dry weight, which cannot be recommended for human consumption. In both experiments, the Se concentration in tubers increased with increasing sodium selenate application levels. Our results show that acceptable application levels of selenate did not have an effect on the GA concentration in immature potato tubers.

KEYWORDS: Glycoalkaloid; selenium; HPLC

INTRODUCTION

Potato (*Solanum tuberosum* L.) tubers contain glycoalkaloids (GA), which are natural metabolites protecting plants against stresses. Of these GAs, α-solanine and α-chaconine (**Figure 1**) are the most abundant in cultivated potatoes. GAs are toxic to mammals causing various symptoms at the levels higher than 5 mg kg^{-1} body weight (*1*). Generally, the recommended upper limit for total GAs is 200 mg kg^{-1} fresh weight (*1*, *2*). The amount of GAs in potato tubers is dependent on growing and storage conditions and on the genetic background of the cultivar. Factors such as exposure to light, mechanical damage, or delayed maturation in cool climate conditions can result in high GA concentration (*3*−*5*). Genetic background also causes variation in the sensitivity of potato cultivars to accumulate GAs under inducing conditions (*6*). Generally, GAs are synthesized during early tuber development and their concentrations decrease during maturation (*7*, *8*). A potential risk for excessively high GA concentrations occurs in Scandinavian diets, where consumption of immature potato tubers, so-called new potatoes, is popular in early summer. Therefore, efforts to minimize GA concentration in potato tubers through breeding or management practices are important.

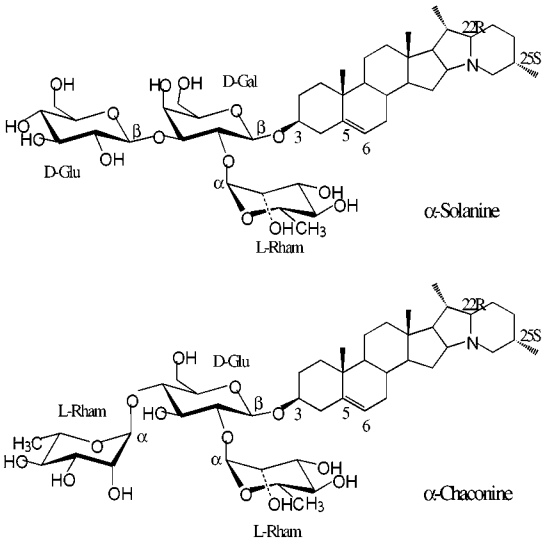

Figure 1. Structural formulas of α-solanine and α-chaconine, which have the same aglycon, solanidine, but different sugar moieties.

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Selenium (Se) is a trace element whose biological functions in plants have been discovered only recently (*9*−*12*). One of the reported effects of sodium selenite (Na_2SeO_3) is decreased nitrate and GA content in mature potato tubers (*13*, *14*), suggesting that selenite may affect the activities of enzymes involved in the synthesis of GAs (*13*). The Se content of agricultural products depends heavily on Se availability in soils. Low-Se soils can be found, for example, in Scandinavian countries, New Zealand, and certain parts of China (*15*, *16*). Especially in acid soils, such as those found in Finland, selenite is poorly available to plants because it is strongly adsorbed by iron and aluminum oxides and clay minerals, whereas selenate has lower tendency to be adsorbed by soil components (*17*). In plants, selenite is nonenzymatically reduced to selenide and readily incorporated into amino acids, while selenate is first reduced to selenite in leaves before being incorporated into selenoamino acids, selenomethionine, and selenocysteine (*18*−*20*). Several selenoenzymes in mammalian cells having selenocysteine in their active site have been identified (*21*, *22*). In plants, however, incorporation of selenocysteine into selenoproteins or selenoenzymes has not been reported (*20*, *23*). In Finland, fertilizers have been supplemented with sodium selenate since 1984 to provide adequate dietary Se intake by humans and livestock (*24*).

The aim of this study was to investigate the effect of sodium selenate supplementation on GA content of immature potato tubers.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Two independent experiments were performed. In the first experiment, two potato (*S. tuberosum* L.) cultivars, Satu (the middle early variety) and Sini (the middle late variety), were grown in a greenhouse under a 16-h photoperiod at 20 to 28 °C/16 °C (day/night), with a photon flux density of 220 to 250 μmol m^{-2} s^{-1} (400 W high-pressure sodium lamps, Lucalox, LU 400/HO/T/40 NG, Hungary). In the second experiment, the potato cultivar Satu was grown in a greenhouse under a 16-h photoperiod at 20 °C/16 °C (day/night), with a photon flux density of 220−250 μmol m^{-2} s^{-1} (400 W high-pressure sodium lamps). Plants were grown in individual pots (diameter 23 cm, 7.5 L) containing 7.5 kg of quartz sand (grain size 0.1−0.6 mm, SP-Minerals Oy Ab, Nilsiän kvartsi, Finland). Before planting, modified Hoagland nutrient solution and 5 g of dolomite per pot (Saxo Mineral Oy, Loukolampi, Finland) were mixed into the quartz sand. The plants were fertilized a second time 8 weeks after planting by adding modified Hoagland solution in amounts corresponding to half that given at the first fertilization. The total amounts of nutrients added per pot were 2.25 g of N, 0.75 g of P, 3.60 g of K, 1.35 g of Ca, 1.14 g of S, 0.015 g of Fe, 0.10 g of Mg, 6.22 mg of Na, 2.02 mg of B, 0.83 mg of Mn, 0.60 mg of Zn, 0.22 mg of Ni, and 0.38 mg of Mo.

Selenium in the form of sodium selenate, Na_2SeO_4 (Aldrich Chemical Co., Milwaukee, WI), was dissolved in deionized water and applied three times at 1-week intervals after shoot emergence. In the first experiment, quantities corresponding to a total addition of 0.01 or 0.075 mg of Se kg^{-1} quartz sand were used. In the second experiment, the supplementation levels were 0.0035, 0.01, 0.075, or 0.9 mg of Se kg^{-1} quartz sand. In both experiments, the control plants were treated with water.

The first experiment was carried out with both varieties using a completely randomized block design, with 8 plants per treatment and 3 replicates. Potato tubers were harvested 10 weeks after planting and were stored at 10 °C at 85% humidity for 1 week before analysis. In the second experiment, a completely randomized design was used with 8 plants per treatment. Potato tubers were harvested 10 weeks after planting and were stored at 5 °C at 85% humidity for 5 weeks before analysis. In both experiments, tubers were harvested immature as so-called new potatoes. In the first experiment, one sample from each experimental unit (subplot with 8 plants) was taken for GA analysis (three samples per cultivar). Two subsamples of ca. 100 g fresh weight (four tubers) were taken for freeze-drying and further extraction. In the second experiment, potato tubers, ca. 130 g fresh weight (three tubers), were used for freeze-drying. Four samples from each Se level were taken. For freeze-drying, potato tubers were chopped and weighed accurately. Samples were freeze-dried (Heto Dry Winner, System FD 8−85, Heto-Holten Lab Equipment A/S, Allerød, Denmark) and ground into fine powder with a mill (Cyclotec 1093 sample mill, Foss Tecator, Höganäs, Sweden).

Analytical Methods. The reverse-phase high-performance liquid chromatography (RP-HPLC) method of Kuronen et al. (*25*) was used for GA analysis. The Agilent Technologies 1100A liquid chromatographic system (Waldbronn, Germany) with an autoinjector and a diode-array detector was computer-controlled with Chemstation software (release 08.03[847]). All results were calculated from the peak areas. The chromatographic column Zorbax-Rx 250 mm × 4.6 mm i.d. of 5 μm particle size (Agilent Technologies) was operated at 50 °C. The elution with acetonitrile−triethylammonium phosphate (TEAP) buffer followed a stepwise gradient of 20%, 25%, 35%, 45%, and 65% acetonitrile at times 0, 12, 15, 17, and 25 min. The effluent from the column was monitored at 205 nm. The injection volume was 10 μL. Distilled water was purified with the Milli-Q apparatus from Millipore. Aqueous 1.0 M TEAP buffer was obtained from Fluka Biochemika (Fluka Chemie AG, Buchs, Switzerland) and further diluted to 25 mmol TEAP eluent with pH 3.0. HPLC-grade S acetonitrile was obtained from Rathburn (Walkerburn, Scotland). All eluents were filtered through a 0.45-μm poly(vinylidene difluoride) membrane disk filter (Durapore Membranes Disks, HVLP 04700, Millipore, Carrigtwohill, Ireland), and the samples were filtered before injection with 0.45-μm syringe filters from Agilent Technologies. Stock solutions of 1.0 mg mL^{-1} α-solanine, 1.0 mg mL^{-1} α-chaconine, and 1.0 mg mL^{-1} solanidine (Sigma, St. Louis, MO) were prepared separately in methanol (HPLC-grade, Rathburn Chem. Ltd, Walkerburn, Scotland). The external calibration standards with five levels of α-solanine were 5, 10, 25, 50, and 100 μg mL^{-1}, and of α-chaconine, 10, 25, 50, 100, 150, 250, 350, and 500 μg mL^{-1}.

The α-solanine and α-chaconine contents were determined from freeze-dried tuber materials with increasing Se concentrations. In this study, the extraction solution was 5% acetic acid prepared from 99.5% acetic acid (Merck & Co., Inc., Darmstadt, Germany). Each sample of 10 g of freeze-dried potato tuber material was mixed with 60 mL of 5% acetic acid and homogenized with Ultra Turrax for 2 min (Ultra Turrax T25, Janke & Kunkel GmbH & Co, IKA Labortechnik, Staufen, Germany). The suspension was further ultrasonicated (Branson 2510E-MTH, Branson Ultrasonics Corp., Danbury, CT) for 5 min. The suspension was transferred to a centrifuge tube and centrifuged at 4 °C for 10 min (5000 rpm, 2988 *g*) (Sorvall RC 5C, Global Medical Instrumentation Inc., Albertville, AL). The supernatant was filtered through filter paper (Whatman 4) into a 100 mL flask. The residue was further mixed and shaken with 20 mL of 5% acetic acid and centrifuged for 10 min, and the supernatant was filtered into the flask. The flask was then topped off with 5% acetic acid to 100 mL. The extracts were stored at 4 °C until analysis.

The solid-phase extraction (SPE) method with Sep-Pak Vac 3 cm^3 C_{18} (500 mg) SPE cartridges from Waters Corp. (Milford, MA) was validated and used to clean up and concentrate the tuber extracts. SPE was aided by the vacuum manifold (Waters Corp.). The preconditioning/equilibration of the SPE cartridges were done with 5 mL of methanol and 5 mL of 5% acetic acid. The preconditioned cartridges were loaded with 5 mL of the extracts and washed with 5 mL of 5% aqueous methanol. The GAs were eluted with 4 mL of methanol. The effluent was evaporated to dryness (SpeedVac SVC 100, Savant Instruments, Farmingdale, NY), and the residue was dissolved in 0.5 mL of methanol to obtain the sample for HPLC analysis. The capacity of the sorbent was tested with a potato extract (cv. Sini), by analyzing the loading and washing fractions and also by re-elution. In summary, the GA loads in this study were between 0.02 and 0.3 mg/100 mg of sorbent.

One of the extracts (cv. Sini) from the first experiment was chosen for the repeatability test of SPE. Three cartridges were used for extraction of 5-mL aliquots. Spiked sample recoveries were performed by adding the mixtures of 30 μg of α-solanine and 150 μg of

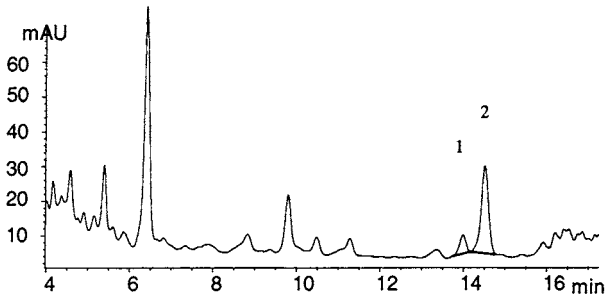

Figure 2. HPLC chromatogram of a potato tuber extract prepared with Sep-Pak C_{18}. Identified compounds were (1) α-solanine and (2) α-chaconine.

Table 1. Spiking Test for Sep-Pak C_{18} with Methanol Elution[a]

spiked amount of total GA (mg kg^{-1} FW)	recovery,[b] %		
	α-solanine	α-chaconine	TGA
29	111	81	86
88	125	75	83
	mean = 118 RSD = 8.4	mean = 78 RSD = 5.7	mean = 85 RSD = 2.5

[a] An extract (cv. Satu) was spiked with mixtures of α-solanine and α-chaconine. [b] Mean of three replications.

α-chaconine, as well as 10 and 30 μg, respectively, to 5-mL aliquots of extract. The test was carried out in triplicate.

Selenium was analyzed according to the electrothermal atomic absorption spectrometric method of Kumpulainen et al. (*26*). An in-house reference sample was included for every analytical round to test the accuracy of the analysis.

Data Analysis. The data were tested by an analysis of variance (ANOVA) in the GLM procedure of SAS version 6.12 (SAS Institute Inc., Cary, NC). Significantly different means between treatments were separated with Duncan's multiple range tests.

RESULTS

HPLC chromatograms of the samples showed two GAs, α-solanine and α-chaconine, with retention times of 14.0 and 14.5 min, respectively (**Figure 2**). With the HPLC method used here, also the aglycons and partially hydrolyzed GAs can be detected in the same run as GAs. Solanidine standard eluted with a retention time of 19.4 min, but no solanidine was detected in any of the tuber samples.

Solid-Phase Extraction of Glycoalkaloids. The SPE method showed good repeatability (relative standard deviation, RSD) for α-solanine (4.8%), α-chaconine (7.2%), and total GAs (6.4%). The recovery tests gave acceptable recoveries for α-solanine and α-chaconine (**Table 1**).

GA Content as Related to Se. The Se concentration in tubers increased with increasing sodium selenate application levels in both experiments (**Table 2**). The Se concentration in tubers did not differ between the cultivars (**Table 2**). In the first experiment, the total GA concentration ± standard error of tubers without sodium selenate application was 105 ± 9 mg kg^{-1} fresh weight in Satu, and 228 ± 10 mg kg^{-1} fresh weight in Sini (**Figure 3a**). The ratio of α-solanine to α-chaconine was 0.19 in Satu and 0.50 in Sini (**Figure 3a**). In the second experiment, the total GA concentration ± standard error in Satu without sodium selenate application was 92 mg kg^{-1} fresh weight, and the ratio of α-solanine to α-chaconine was 0.36 (**Figure 3b**). Sodium selenate application did not have a significant effect on the total GA concentration or on the ratio of α-solanine to α-chaconine (**Figure 3a,b**). Although the highest sodium selenate application used in the second experiment (0.9 mg kg^{-1}) slightly decreased the GA concentration in tubers, the decrease was not statistically significant (**Figure 3b**). This dosage increased the Se concentration in tubers to ∼20 μg of Se g^{-1} dry weight (**Table 2**).

Table 2. Selenium Concentrations of Tubers Harvested 10 Weeks after Planting, Supplemented with Various Amounts of Se[a]

Se application (mg kg^{-1} quartz sand)	μg of Se g^{-1} dry weight		
	experiment 1		experiment 2
	Sini	Satu	Satu
0	0.01	0.01	0.01
0.0035			0.07
0.01	0.15	0.16	0.30
0.075	1.14	1.18	2.00
0.9			∼20
r^2 [b]	0.96	0.99	0.99

[a] Each determination was done in triplicate. [b] Regression coefficient describing the relation of Se application level to Se concentration in tubers.

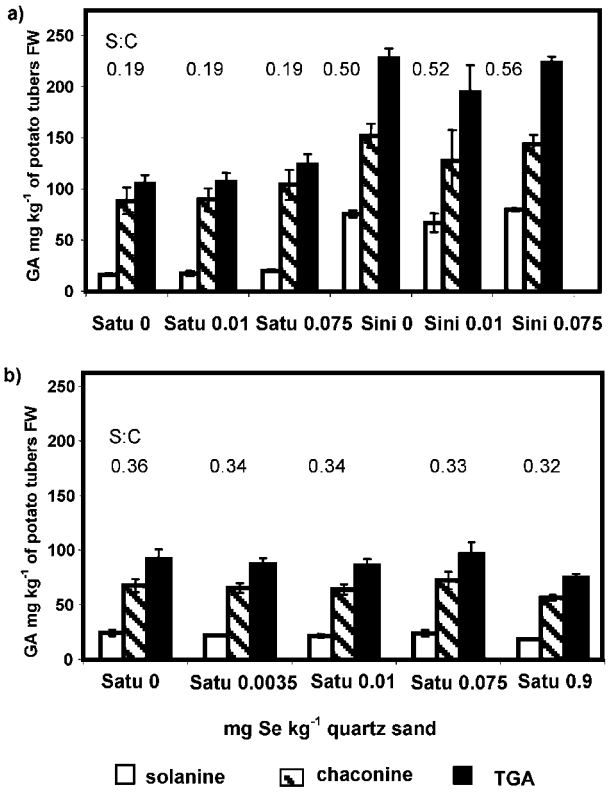

Figure 3. Glycoalkaloid concentration (milligrams per kilogram fresh weight) ± standard error and ratio of α-solanine to α-chaconine (S:C) in immature potato tubers as determined in (a) the first experiment (cv. Satu and Sini) and (b) the second experiment (cv. Satu) at the indicated Se application levels.

DISCUSSION

Sample Preparation. For the most part, solid-phase extraction (SPE) has displaced liquid−liquid extraction and the ammonia precipitation method used earlier for the preparation of GA samples. Silica-based C_{18} sorbents seem to be used most often in protocols for GA quantification. Good recoveries for the GAs have been obtained with the methods reported earlier (*27*−*29*). Saito et al. (*30*) also reported good recoveries between 82% and 97% obtained with Sep-Pak C_{18} and methanol elution. Edwards and Cobb (*31*) compared end-capped and non-end-

capped C_{18} cartridges, with methanol−0.1 M hydrogen chloride as the elution solvent. In their study, the Sep-Pak C_{18} gave the lowest recovery (28%) for α-solanine and α-chaconine. The lack of retention of GAs in the C_{18} SPE sorbents reported by Simonovska and Vovk (*32*) was not detected in this study, where GAs were loaded into the sorbent in 5% acetic acid without an ion-pairing reagent. According to our studies, Sep-Pak C_{18} sorbent can be applied reliably for GA quantification since good repeatability and acceptable recoveries for α-solanine and α-chaconine were obtained.

Glycoalkaloid Contents of the Tubers. In this study, the cultivar Sini exceeded the recommended upper limit for total GAs (200 mg kg^{-1} fresh weight). It is known that a risk of excessively high GA concentration is a characteristic feature of Sini, whereas Satu has a natural lower tendency to accumulate GAs (*33*). As far as the toxicity of the GAs is concerned, the ratio of α-solanine to α-chaconine is as important as the total GA concentration in potato. According to literature (*34* and references therein), α-chaconine is the most toxic of the GAs. In addition, α-solanine and α-chaconine act synergistically; that is, the combined activity of α-solanine and α-chaconine is greater than the sum of their individual activities. A typical ratio of α-solanine to α-chaconine is between 0.2 and 0.6 in cultivated potato plants. In the first experiment the tempreature was as high as 28 °C for 3−4 weeks at the end of the growing period. This could explain the difference in α-solanine and α-chaconine ratios obtained in the first and second experiments for Satu. Studies concerning the effect of storage temperature on the ratio have been published (*35*), but the effect of temperature fluctuation during the growth period has not been studied comprehensively.

In the first experiment, the sodium selenate application levels of 0.01 and 0.075 mg Se kg^{-1} quartz sand were too low to exert any statistically significant effect on the GA concentration in immature potato tubers in both cultivars. The second experiment revealed that an Se dosage as high as 0.9 mg kg^{-1} was needed to produce some indication of decreased GA concentration in the Satu. However, with this application level the Se concentration in the tubers (∼20 μg of Se g^{-1} dry weight) exceeded the level safe for human consumption, which is 55−70 μg of Se per day according to the Recommended Dietary Allowance (RDA) (*36*). In previous studies, sodium selenite application levels of 0.7−3.4 mg of Se kg^{-1} soil have been reported to decrease GA concentration in mature tubers (*13*, *14*), even though at levels higher than 1.1 mg of Se kg^{-1} soil no further decreases in the GA concentration in tubers have been found (*14*). It is noteworthy that when sodium selenite has been used, the Se concentration of tubers has not exceeded 2.8 μg of Se g^{-1} dry weight, although the highest application level used was 3.4 mg of Se kg^{-1} loam soil (*14*). Our previous studies have shown that in the selenate-supplemented potato plants Se accumulation in tubers continues during maturation (data not shown) (*37*). In contrast, in the selenite-supplied plants, no further Se accumulation in tubers has been observed at application levels higher than 2.3 mg kg^{-1} (*38*). According to our results and the literature, the effect of Se on GA content depends on maturity of the tubers and on the chemical form of Se applied.

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