JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Glycoalkaloid and Calystegine Levels in Table Potato Cultivars Subjected to Wounding, Light, and Heat Treatments

Erik V. Petersson,[†,‡] Usman Arif,[†,‡] Vera Schulzova,[§] Veronika Krtková,[§] Jana Hajšlová,[§] Johan Meijer,[‡] Hans Christer Andersson,[∥] Lisbeth Jonsson,[⊥] and Folke Sitbon[‡,*]

[‡]Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences, and Linnean Centre for Plant Biology, P.O. Box 7080, 750 07 Uppsala, Sweden

[§]Institute of Chemical Technology, Department of Food Analysis and Nutrition, Technická 3, 166 28 Prague 6, Czech Republic

[∥]National Food Agency, P.O. Box 622, 751 26 Uppsala, Sweden

[⊥]Department of Ecology, Environment and Plant Sciences, Stockholm University, 106 91 Stockholm, Sweden

S Supporting Information

ABSTRACT: Potato tubers naturally contain a number of defense substances, some of which are of major concern for food safety. Among these substances are the glycoalkaloids and calystegines. We have here analyzed levels of glycoalkaloids (α-chaconine and α-solanine) and calystegines (A_3, B_2, and B_4) in potato tubers subjected to mechanical wounding, light exposure, or elevated temperature: stress treatments that are known or anticipated to induce glycoalkaloid levels. Basal glycoalkaloid levels in tubers varied between potato cultivars. Wounding and light exposure, but not heat, increased tuber glycoalkaloid levels, and the relative response differed among the cultivars. Also, calystegine levels varied between cultivars, with calystegine B_4 showing the most marked variation. However, the total calystegine level was not affected by wounding or light exposure. The results demonstrate a strong variation among potato cultivars with regard to postharvest glycoalkaloid increases, and they suggest that the biosynthesis of glycoalkaloids and calystegines occurs independently of each other.

KEYWORDS: *potato (Solanum tuberosum group Tuberosum), alkaloid metabolism, chaconine, food safety, solanine, plant stress response*

INTRODUCTION

Steroidal glycoalkaloids and calystegine alkaloids are naturally occurring toxic secondary metabolites present in some members of the Solanaceae, including important crop species such as potato, tomato, and eggplant.[1−4] In the potato, glycoalkaloids occur in all parts of the plant. The highest levels have been reported in flowers, berries, sprouts, and some other actively growing tissues, whereas levels in tubers are lower. The potato glycoalkaloids contain a steroidal skeleton to which one to three sugar molecules are bound (Figure 1). In cultivated potatoes, over 95% of the total glycoalkaloid content consists of two main forms: α-chaconine (**1**) and α-solanine (**2**). These are derived from the same aglycone, solanidine, but differ in their trisaccharide carbohydrate moiety.[5,6] The composition of the carbohydrate moiety as well as the aglycone is of vital importance for bioactivity.[7] Mild symptoms of glycoalkaloid toxicity include headache, nausea, and diarrhea, but more severe and even fatal poisonings have been reported.[8] For safety reasons, a maximum level of 200 mg total glycoalkaloid/kg fresh weight (f.w.) of unpeeled raw potato tuber aimed for consumption is widely recommended. In some countries, e.g. Sweden, this maximum level is legally binding.[9] The total glycoalkaloid level in tubers of table potatoes generally ranges between 20 and 100 mg/kg f.w., resulting in only a small safety margin for consumers at normal levels of potato consumption. Unfortunately, it is not rare that the upper safe level of total glycoalkaloids in potato tubers is surpassed, and cultivars such as Lenape, Magnum Bonum, and Ulster Chieftain have been withdrawn from the U.S. and Swedish markets due to frequently exceeding the upper safe limit for total glycoalkaloid content.[10,11]

The total glycoalkaloid content in healthy unstressed tubers strongly depends on genotype. In addition, stress of growing tubers, for example by wounding, mechanical injury, light exposure, nitrogen fertilization, inappropriate storage, or extreme temperature, may result in considerably increased glycoalkaloid contents compared to the basal level.[12−14] The sum of various pre- and postharvest stresses can thus increase the glycoalkaloid level several-fold, and this constitutes a problem of major concern to the entire food chain, from producers to consumers.

Calystegines constitute another form of alkaloids in Solanaceous food plants, including the potato, where common calystegine forms are calystegines A_3 (**3**), B_2 (**4**), and B_4 (**5**).[3,4] In contrast to the glycoalkaloids, which were detected in potatoes almost two hundred years ago and have since then been studied for their toxicity for a considerable period of time, the calystegines were described in the late 1980s and toxicity studies are largely lacking. However, experimental studies have shown that the calystegines can inhibit various glycosidases and that the inhibiting activity on some enzymes increases with the

Received: January 23, 2013
Revised: May 21, 2013
Accepted: May 22, 2013
Published: May 22, 2013

© 2013 American Chemical Society

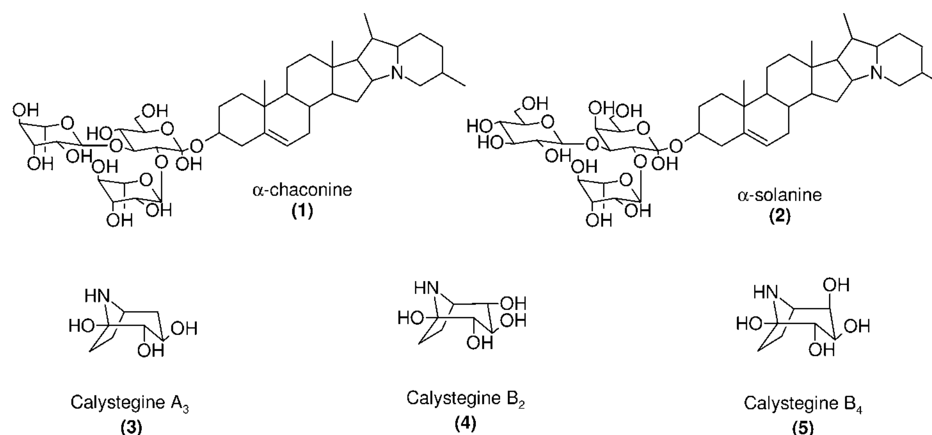

Figure 1. Chemical structures of analyzed glycoalkaloids and calystegines in the present investigation.

degree of hydroxylation of the molecule.[15] Although no cases of human intoxication specifically due to calystegine exposure have been reported, there is evidence supporting that the occurrence of glycosidase inhibitors in specific plants may contribute to toxic effects in experimental and farm animals grazing or fed on these plants. The affected animals show conditions resembling lysosomal storage disease,[3,4] which are rare inborn errors of metabolism affecting the function of the lysosomes. A number of inborn lysosomal storage disorders, such as Gaucher's and Fabry's diseases, have been identified in humans,[16] and similar disorders are known to affect animals. At present, too little information is available about the calystegine levels in potato and their mechanism of action in humans to speculate whether their intake might constitute a risk for the consumer.

The quality assessment of new potato cultivars generally includes analysis for glycoalkaloids but not for calystegines. Furthermore, it has been more common to evaluate the basal glycoalkaloid level in tubers at harvest, and not in tubers subjected to different types of stress, or in the products that reach the consumer. There is a lack of knowledge concerning the influence of genotype (cultivar dependency) and environment on the levels of glycoalkaloids and other metabolites after different types of stress. This hampers a safe and cost-efficient postharvest treatment of potatoes and is the incitement for the present studies.

The metabolic origin of glycoalkaloids is only partially understood. The sterol cholesterol has been suggested as a likely metabolic precursor,[17] although firm evidence is lacking. In line with a precursor role for cholesterol is that potato plants genetically engineered to contain lower cholesterol levels also displayed lower glycoalkaloid levels.[18] Calystegines, on the other hand, are derived from putrescine,[19] which most likely originates from the amino acid ornithine. However, it is unknown to what extent biosyntheses of these two alkaloid classes are correlated. In a previous study,[20] the glycoalkaloid and calystegine A_3 (**3**) and B_2 (**4**) levels in unstressed tubers from eight potato cultivars were monitored. This revealed a considerable variation in the level of both types of alkaloid, and the different glycoalkaloid/calystegine ratios between cultivars led the authors to suggest that synthesis of these two classes of secondary metabolites might be under separate genetic control.

To increase our understanding of alkaloid metabolism in potato plants, in particular the interplay between glycoalkaloid and calystegine levels in postharvest tubers, we have here surveyed the levels of glycoalkaloids and calystegines in a large number of potato cultivars during conditions known to promote glycoalkaloid synthesis. Cultivar-specific metabolic responses were evaluated, as well as the proneness for exceeding the glycoalkaloid maximum level of 200 mg/kg f.w.

MATERIALS AND METHODS

Plant Materials. Tubers of 21 common table potato cultivars in Sweden were obtained from certified producers or in some cases purchased from retail stores. For some cultivars, tubers from two or three independent suppliers were used. The cultivars included both early and late developing cultivars and cultivars used in conventional as well as organic farming. The chosen cultivars accounted for over 75% of the total potato production in central Sweden in the year 2005 (A. Magnusson, Svegro AB, personal communication).

Tubers from all cultivars were on the same day in June 2009 planted in black plastic, 12 L pots filled with fertilized peat. Care was taken to select tubers of similar weight (50 ± 10 g) and to place them at the same depth in the pots. Six tubers were planted for each cultivar or replicate of cultivar. Pots were randomized and grown under outdoor conditions (Uppsala, Sweden), for 4 months until harvest. During cultivation, pots received regular watering and fertilization according to common practice. At harvest, tubers from the six pots in each batch were pooled, rinsed with water, blotted dry, and stored in dark paper bags for 5 months at +8 °C in the dark.

Tuber Stress Treatments and Sampling. After five months of storage, the tubers were on the same occasion treated, to simulate wounding or exposure to light or heat. Care was taken to use tubers of similar weight (42 ± 13 g) and free of any visible damage or greening. Wounding was simulated by cutting four 5 mm thick transversal discs from the central region of tubers, two of which were either immediately frozen in liquid nitrogen as control samples or incubated on moist filter papers in a Petri dish for 48 h before freezing in liquid nitrogen. Exposure to light was administered by placing tubers for 8 d in a growth cabinet kept at 22 °C and supplying a constant white fluorescent light with a quantum flux density of 110 μmol/(m^2 s). Heat was supplied by incubating tubers in the dark at 34 °C for 7 d. At the end of the light and heat treatments, tubers were cut transversally, in the same way as when tubers were wounded, and immediately frozen in liquid nitrogen. Duplicate treatments were performed for some tuber batches. All samples were stored at −70 °C until analyses. For all analyses, one slice from each of three tubers was pooled into one sample for extraction. For calystegine analyses, samples from a representative subset of the potato cultivars that were studied for glycoalkaloid content were transported on dry ice by speed courier service from Uppsala, Sweden, to the Institute of Chemical Technology in Prague, Czech Republic, where the calystegine determinations were performed. Hence, glycoalkaloid and calystegine analyses were performed on parallel samples from the same set of three pooled tubers.

Glycoalkaloid Extraction and Analysis. Frozen tuber discs (10.0 g), were homogenized in a food processor using 40.0 g of an acidic extraction buffer consisting of water/acetic acid/sodium bisulphite 100:5:0.5 v/v/w. A part of the homogenate (15.0 g) was supplied with 200 μg of solamargine (>98%, Glycomix Ltd., Reading, U.K.) as an internal standard and further homogenized with an UltraTurrax homogenizer. The extract was cleared by centrifugation, and the supernatant was subjected to a solid phase extraction clean-up step, similar to published methods.[21,22] The solid phase consisted of a Sep-Pak C18 500 mg 3 cc column (Waters, Milford, MA, USA) that was activated with 5 mL of HPLC-grade acetonitrile (Sigma-Aldrich, Schnelldorf, Germany), conditioned with 5 mL of the extraction buffer, after which 10 mL of the sample was added. The column was washed with 4 mL of 15% acetonitrile in water, and glycoalkaloids were eluted with 4 mL of 50% acetonitrile in 10 mM phosphate buffer, pH 7.6, and then quantitated using LC-UV.[21,22] The LC-UV equipment was a HP1100 series (Hewlett-Packard, Waldbronn, Germany) with a G1312A binary pump and a G1314A UV-detector set to 202 nm. Separation was achieved with a 150 mm × 2.1 mm i.d., 5 μm, Hypersil Gold C18 column (Thermo Scientific, Waltham, MA, USA) using a mobile phase consisting of 10 mM phosphate buffer (pH 7.6) with 36% acetonitrile, at a flow rate of 0.5 mL/min. Reference standards used for the LC-analyses were α-chaconine 95% (**1**) (Sigma-Aldrich, Schnelldorf, Germany) and α-solanine 95% (**2**) (MP Biomedicals, Solon, OH, USA).

Calystegine Extraction and Analysis. HPLC-grade acetonitrile and ammonium acetate were supplied from Sigma−Aldrich (St. Louis, MO, USA), and methanol from Merck (Darmstadt, Germany). As calystegine standards were not commercially available, total calystegines were isolated from potato sprouts, and the individual calystegines A_3 (**3**), B_2 (**4**), and B_4 (**5**) were separated by liquid chromatography. Individual calystegines were identified and their purity established by NMR.

Frozen tuber samples were homogenized in a tissue grinder. Five grams of homogenate were extracted by shaking with 80 mL of a methanol−water mixture 1:1; v/v for 30 min. The extract was filtered through a Büchner funnel and transferred to a 100 mL volumetric funnel that was filled up by the extraction mixture. Before LC-MS/MS analysis, the filtrate was diluted 50−100 times with 90% acetonitrile in water, and an aliquot was passed through a 0.22 μm polytetrafluoroethylene membrane filter.

High Performance Liquid Chromatography/Tandem Mass Spectrometry Analysis of Calystegines. The analyses were performed using an Acquity Ultra-Performance LC system (Waters, Milford, MA, USA) equipped with an 100 mm × 3 mm i.d., 3 μm, Atlantis HILIC column (Waters, USA) maintained at 35 °C. The mobile phase consisted of 0.020 M ammonium acetate, pH 5.3 in Milli-Q water; buffer A. At the start, the mobile phase composition was 10% buffer A and 90% acetonitrile using a flow rate of 0.45 mL/min, but from 0.5 min it changed linearly during 4.5 min to 60% buffer A + 40% acetonitrile, and was then held constant during 1 min. The sample volume was 2 μL, and the autosampler temperature was maintained at 4 °C. The LC system was connected to a 5500 QTRAP tandem mass spectrometer (AB SCIEX, Concord, ON, Canada), equipped with a Turbo VTM ion source operated in positive ion mode. The ion source parameters were as follows: needle voltage 4300 V, curtain gas 275 kPa, nebulizer and Turbo gas 410 kPa, temperature of Turbo gas 600 °C. Declustering potential, collision potential ,and collision cell exit potential were optimized during infusion of a mixture of the analytes (10−100 ng/mL) employing the Analyst 1.5 software (AB SCIEX, Concord, ON, Canada).

RESULTS AND DISCUSSION

Evaluation of Glycoalkaloid and Calystegine Analyses. The limit of detection (LOD) values for glycoalkaloids were estimated in potato samples, providing a signal-to-noise ratio (S/N) higher than 3. The LOD values for the individual glycoalkaloids α-chaconine (**1**) and α-solanine (**2**) in potato tubers were 3.0 and 2.1 mg/kg, respectively. Repeatability, expressed as relative standard deviation, was below 1% for the targeted glycoalkaloids. Similarly, the LOD for each calystegine was estimated as the lowest amount of calibration standard, which provided a S/N higher than 3. The LOD values for individual calystegines A_3 (**3**), B_2 (**4**), and B_4 (**5**) in potato tubers were 0.4 mg/kg, 0.6 mg/kg, and 0.6 mg/kg, respectively. Repeatability, expressed as relative standard deviation, was 3% for calystegines A_3 and B_2, and 4% for calystegine B_4. Representative chromatograms of glycoalkaloids and calystegines extracted from potato tubers are shown in Figure 2.

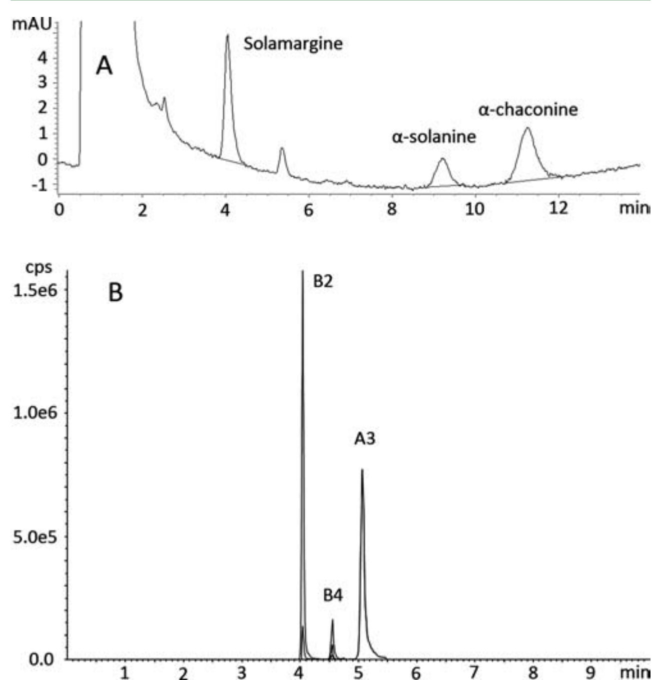

Figure 2. Representative chromatograms of potato tuber extracts analyzed for glycoalkaloids (A) and calystegines (B). Solamargine was added in part A as an internal standard for glycoalkaloid quantitation. The levels of total glycoalkaloids and calystegines in the samples shown were 98 mg/kg f.w. and 181 mg/kg f.w., respectively.

Variation in Potato Cultivars of the Tuber Glycoalkaloid Response to Wounding and Light Exposure. It is well established that the levels of glycoalkaloids in potato tubers are influenced by stress factors such as mechanical injury (wounding) and light exposure.[23−26] In previous studies, the effects of these factors on the glycoalkaloid level have generally been evaluated one at a time, and not always in the same set of cultivars. This makes it difficult to compare the response of cultivars to various types of stress, as well as to compare cultivars regarding their relative stress sensitivity. We have here analyzed the effects of wounding, mild heat treatment, and light exposure on the tuber glycoalkaloid level in 21 cultivars that are common table potatoes in Sweden. As the experiments were designed to reveal cultivar differences, all tubers were planted, grown, harvested, and stored in the same way, and subsequently treated in parallel. Individual cultivars are in the following referred to by name, as well as an entry number reflecting a rank based on the sum of SGA increases for the cultivar after the heat, mechanical wounding, and light treatments (Table 1).

For the 21 potato cultivars investigated, the average glycoalkaloid level in untreated control tubers was 127 ± 65 mg/kg f.w. (Table 1). For most potato cultivars, this basal glycoalkaloid content was below the frequently accepted

Table 1. Steroidal Glycoalkaloid (SGA) Levels in Tubers from 21 Table Potato Cultivars Subjected to Mechanical Wounding, Heat, or Light Exposure[a]

cultivar	treatment	α-solanine (mg/kg)	α-chaconine (mg/kg)	SGA (mg/kg)	SGA alteration (mg/kg)	SGA % of control	mean S/C ratio[b]	tuber batches	samples	analyses per sample
1. Juliette	control	46 ± 0	95 ± 4	141 ± 4	0	100%	0.48	1	1	2
	heat	65 ± 2	94 ± 6	159 ± 5	18	113%	0.70			
	wound	199 ± 29	173 ± 7	372 ± 22	231	264%	1.16			
	light	489 ± 7	327 ± 7	816 ± 14	675	580%	1.49			
2. Maris Bard	control	18	28	46	0	100%	0.62	1	1	1
	heat	18	17	35	−11	77%	1.05			
	wound	98	97	195	149	423%	1.01			
	light	108	101	209	163	455%	1.07			
3. Princess	control	47 ± 13	75 ± 19	122 ± 32	0	100%	0.63	1	2	1
	heat	46 ± 8	67 ± 0	112 ± 8	−10	101%	0.69			
	wound	193 ± 54	191 ± 49	385 ± 103	263	361%	1.00			
	light	198 ± 70	240 ± 72	438 ± 142	316	417%	0.81			
4. King Edward	control	37 ± 5	69 ± 20	106 ± 25	0	100%	0.54	3	6	1
	heat	32 ± 6	65 ± 12	97 ± 17	−9	93%	0.49			
	wound	150 ± 17	186 ± 33	336 ± 48	230	341%	0.81			
	light	228 ± 36	207 ± 36	435 ± 71	329	430%	1.10			
5. Superb	control	20	43	63	0	100%	0.46	1	1	1
	heat	22	38	60	−3	95%	0.58			
	wound	91	113	204	141	325%	0.80			
	light	84	110	194	131	309%	0.76			
6. Eloge	control	47 ± 1	100 ± 6	147 ± 7	0	100%	0.49	1	2	2
	heat	47 ± 9	91 ± 30	138 ± 39	−9	90%	0.61			
	wound	165 ± 32	162 ± 41	328 ± 73	181	213%	1.10			
	light	226 ± 35	243 ± 1	469 ± 36	322	322%	0.99			
7. Early Puritan	control	44 ± 6	67 ± 1	111 ± 5	0	100%	0.65	1	2	2
	heat	40 ± 7	69 ± 7	108 ± 14	−3	97%	0.57			
	wound	118 ± 18	123 ± 41	241 ± 58	130	219%	1.02			
	light	192 ± 5	143 ± 21	335 ± 16	224	303%	1.38			
8. Bintje	control	33 ± 7	69 ± 16	102 ± 22	0	100%	0.48	3	6	1
	heat	36 ± 5	71 ± 10	107 ± 13	6	113%	0.51			
	wound	132 ± 25	146 ± 39	278 ± 63	176	275%	0.93			
	light	102 ± 25	120 ± 28	221 ± 51	120	219%	0.85			
9. Marine	control	83 ± 8	124 ± 9	208 ± 2	0	100%	0.68	1	1	2
	heat	52 ± 2	93 ± 15	145 ± 17	−62	70%	0.57			
	wound	158 ± 0	193 ± 26	351 ± 26	143	169%	0.84			
	light	345 ± 50	319 ± 18	665 ± 69	457	320%	1.08			
10. Asterix	control	47 ± 1	103 ± 14	150 ± 20	0	100%	0.47	2	3	1
	heat	54 ± 6	94 ± 6	148 ± 43	−2	102%	0.57			
	wound	123 ± 5	177 ± 16	299 ± 43	150	204%	0.69			
	light	125 ± 8	224 ± 31	358 ± 54	208	244%	0.62			
11. Folva	control	29 ± 2	54 ± 5	84 ± 4	0	100%	0.55	2	2	1
	heat	35 ± 0	66 ± 13	101 ± 13	17	120%	0.54			
	wound	69 ± 9	73 ± 18	142 ± 27	59	169%	0.97			
	light	82 ± 5	110 ± 27	192 ± 31	108	228%	0.78			
12. Amandine	control	45	71	115	0	100%	0.63	1	1	1
	heat	63	105	167	52	145%	0.60			
	wound	95	130	225	109	195%	0.73			
	light	60	137	197	82	171%	0.44			
13. Sava	control	103 ± 26	190 ± 43	293 ± 69	0	100%	0.54	2	2	1
	heat	91 ± 4	173 ± 20	264 ± 24	−29	97%	0.53			
	wound	238 ± 15	300 ± 24	538 ± 39	245	198%	0.79			
	light	248 ± 14	251 ± 9	499 ± 23	206	178%	0.99			
14. Terra Gold	control	18	37	55	0	100%	0.48	1	1	1
	heat	14	18	32	−23	59%	0.78			
	wound	72	109	181	126	329%	0.66			
	light	14	33	46	−8	85%	0.42			

Table 1. continued

cultivar	treatment	α-solanine (mg/kg)	α-chaconine (mg/kg)	SGA (mg/kg)	SGA alteration (mg/kg)	SGA % of control	mean S/C ratio[b]	tuber batches	samples	analyses per sample
15. Asparges	control	54	103	157	0	100%	0.52	1	1	1
	heat	52	97	149	−8	95%	0.54			
	wound	100	149	249	93	159%	0.67			
	light	160	166	326	170	208%	0.96			
16. Maritema	control	46	50	96	0	100%	0.92	1	1	1
	heat	32	37	70	−26	73%	0.87			
	wound	141	90	231	135	242%	1.56			
	light	66	74	140	44	146%	0.88			
17. Melody	control	20	26	47	0	100%	0.78	1	1	1
	heat	21	24	45	−2	96%	0.86			
	wound	52	69	122	75	261%	0.75			
	light	20	23	43	−3	93%	0.88			
18. Rocket	control	22 ± 3	41 ± 8	63 ± 4	0	100%	0.57	1	2	1
	heat	9 ± 2	20 ± 12	29 ± 14	−34	45%	0.64			
	wound	99 ± 3	80 ± 23	179 ± 26	116	283%	1.36			
	light	33 ± 6	39 ± 14	72 ± 8	9	113%	1.05			
19. Fontane	control	55	97	152	0	100%	0.56	1	1	1
	heat	55	92	148	−4	97%	0.60			
	wound	152	187	340	188	224%	0.81			
	light	71	100	172	20	113%	0.71			
20. Cherie	control	88	168	255	0	100%	0.52	1	1	1
	heat	89	160	249	−7	97%	0.55			
	wound	167	214	381	125	149%	0.78			
	light	194	249	444	188	174%	0.78			
21. Desiree	control	42	112	155	0	100%	0.38	1	1	1
	heat	43	138	181	26	117%	0.31			
	wound	99	164	263	109	170%	0.61			
	light	60	117	177	22	114%	0.51			
mean ± SD (n = 21 cvs)	control	45 ± 23	82 ± 43	127 ± 65	0	100%	0.57			
	heat	44 ± 22	78 ± 44	121 ± 65	−6	95%	0.63			
	wound	129 ± 47	149 ± 56	278 ± 99	151	246%	0.91			
	light	148 ± 117	159 ± 90	307 ± 202	180	249%	0.88			

[a]Mean value ± range or SD. [b]Mean value of the α-solanine/α-chaconine ratio.

maximum limit of 200 mg/kg f.w. However, three cultivars, Marine (9), Sava (13), and Cherie (20), had basal glycoalkaloid levels at, or above, this maximum limit. As these cultivars also responded strongly to light exposure, we speculate that their high basal glycoalkaloid level may be promoted by a combination of glycoalkaloid induction by light and tuber initiation near the soil surface. Although the position of tuber initiation was not investigated in the present study, the combined effect of shallow tuber initiation and light responsiveness may deserve further attention when characterizing glycoalkaloid profiles of new potato cultivars.

Across the 21 cultivars, wounding and light exposure increased the glycoalkaloid content of tubers significantly ($p < 0.001$; Student's t-test) (Table 1), in line with previous observations.[23−26] However, the heat treatment (34 °C during 7 d) used in the present study had no significant effect on the glycoalkaloid level. It should however be noted that certain cultivars, e.g. Marine (9), Terra Gold (14), and Rocket (18), showed a somewhat lower glycoalkaloid level after the heat stress, whereas a few others, e.g. Amandine (12) and Desiree (21), displayed a slight increase. More analyses are needed to confirm if these changes have a genetic or experimental background. Compared to wounding and light exposure, which both are well-established glycoalkaloid-inducing stress factors, increased temperature has been less well investigated in this aspect. Potatoes grown in a warm climate under field conditions were reported to contain elevated glycoalkaloid levels in tubers,[27] but it is not known to what extent this effect reflected a high temperature as such, or a concomitant drought-related stress. Moreover, a 5-fold increase in glycoalkaloid production by a 4 h treatment at 35 °C has been reported in tubers of the cultivar Atlanta, but not in tubers of the LT7 cultivar.[28] However, as the specificity and accuracy of the colorimetric method used for glycoalkaloid quantitation was not reported in that study, these results should be interpreted with caution. The reasons for choosing a temperature of 34 °C as heat treatment were results from previous studies which showed transcriptomic changes in the tuber periderm of potatoes exposed to a soil temperature of 33 °C and in roots and leaves in potato seedlings treated at 35 °C.[29,30] Unfortunately, glycoalkaloid levels were not measured in these studies, and genes limiting the glycoalkaloid biosynthesis have not been characterized to an extent sufficient to allow interpretation of these potato heat-shock array data with respect to an effect on glycoalkaloid metabolism. Nevertheless, the absence of an influence of increased temperature on the glycoalkaloid content of tubers across the potato cultivars in our study indicates that heat is not a glycoalkaloid-inducing stress factor comparable to wounding and light exposure in postharvest tubers.

When the total glycoalkaloid level in tubers was increased as a result of exposure to stress, we found a higher increase in the level of α-solanine (**2**) than that of α-chaconine (**1**) in the

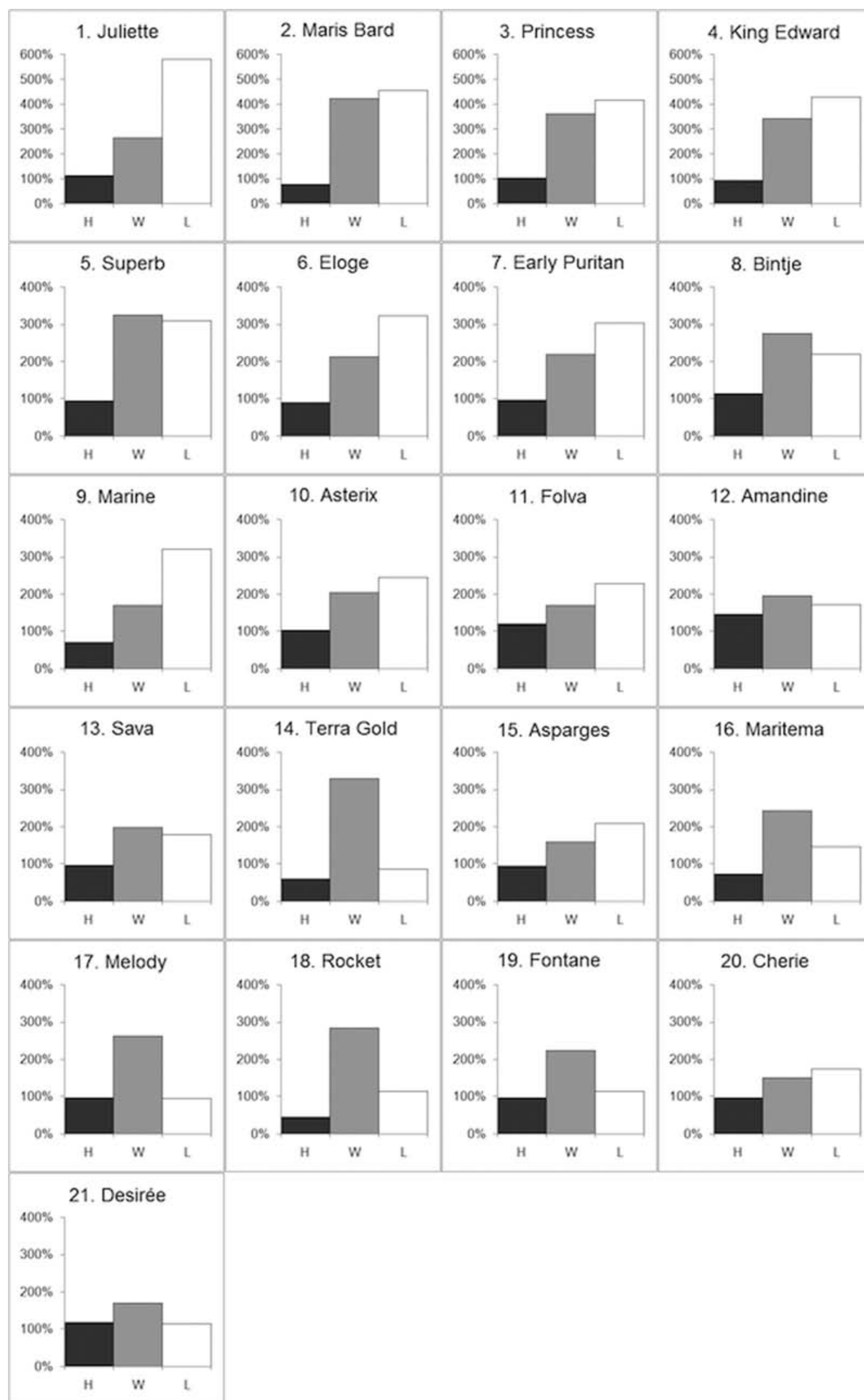

Figure 3. Relative glycoalkaloid level in 21 table potato cultivars. Bars represent wound × 2 d (W), heat × 7 d (H), and light × 8 d (L) treatments of tubers. Glycoalkaloid levels in treated tubers are expressed relative to that in untreated control samples (=100%). Mean values from data in Table 1.

cultivars (Table 1). This effect was significant at $p < 0.01$ (wounding) or $p < 0.05$ (light) (Student's *t*-test). Thus, for the group of 21 cultivars, the α-solanine/α-chaconine ratio was 0.57 ± 0.12 (mean value ± SD) in controls but 0.91 ± 0.24 in wounded tubers, and 0.88 ± 0.27 in light-exposed ones (Table 1). Examples of cultivars with a stronger α-solanine than α-

Table 2. Calystegine (CA) and Total Glycoalkaloid (SGA) Levels in Potato Tubers Subjected to Mechanical Wounding, Heat, or Light Exposure[a]

cultivar[b]	treatment	n	CA_{A3} (mg/kg)	CA_{B2} (mg/kg)	CA_{B4} (mg/kg)	sum CA (mg/kg)	CA alteration (mg/kg)	SGA alteration (mg/kg)[c]
1. Juliette	control	1	56	56	17	129	0	0
	wound	1	75	57	19	150	21	213
	light	1	38	32	16	86	−43	665
2. Maris Bard	control	1	14	18	3	35	0	0
	wound	1	26	20	3	49	14	149
	light	1	24	23	4	51	16	163
3. Princess	control	2	9 ± 3	13 ± 4	2 ± 2	23 ± 8	0	0
	wound	2	9 ± 4	14 ± 8	2 ± 2	24 ± 13	1	250
	light	2	10 ± 2	16 ± 5	2 ± 2	27 ± 8	4	249
4. King Edward	control	3	27 ± 13	28 ± 15	1 ± 1	55 ± 29	0	0
	heat	3	38 ± 8	40 ± 4	3 ± 0	80 ± 12	25	4
	wound	3	44 ± 5	44 ± 3	2 ± 1	89 ± 2	34	244
	light	3	35 ± 4	37 ± 3	3 ± 0	74 ± 7	19	380
8. Bintje	control	3	60 ± 3	52 ± 3	30 ± 5	141 ± 8	0	0
	heat	3	63 ± 18	55 ± 9	22 ± 9	140 ± 30	−1	24
	wound	3	45 ± 6	45 ± 9	35 ± 18	125 ± 33	−17	191
	light		41 ± 7	34 ± 5	17 ± 3	93 ± 13	−49	120
9. Marine	control	1	49	38	3	89	0	0
	wound	1	52	39	4	94	5	115
	light	1	69	54	3	127	38	524
10. Asterix	control	2	33 ± 6	46 ± 7	4 ± 1	83 ± 13	0	0
	wound	2	30 ± 1	33 ± 1	4 ± 0	66 ± 0	−17	116
	light	2	24 ± 4	30 ± 7	5 ± 2	58 ± 13	−26	168
11. Folva	control	2	13 ± 1	10 ± 1	2 ± 2	25 ± 4	0	0
	wound	2	18 ± 6	13 ± 5	n.d.	31 ± 11	6	59
	light	2	9 ± 2	7 ± 2	n.d.	16 ± 4	−9	108
13. Sava	control	2	22 ± 4	30 ± 5	9 ± 1	61 ± 9	0	0
	wound	2	23 ± 6	23 ± 7	7 ± 1	52 ± 12	−10	245
	light	2	19 ± 3	19 ± 2	8 ± 1	46 ± 6	−16	206
14. Terra	control	1	84	44	n.d.	128	0	0
Gold	wound	1	58	32	n.d.	91	−37	126
	light	1	72	34	n.d.	106	−22	−8
17. Melody	control	1	24	26	n.d.	49	0	0
	wound	1	22	20	n.d.	41	−8	75
	light	1	50	49	n.d.	99	50	−3
19. Fontane	control	1	27	28	23	79	0	0
	wound	1	18	21	16	54	−25	188
	light	1	23	28	12	62	−17	20
21. Desiree	control	1	15	21	n.d.	36	0	0
	wound	1	44	59	3	106	70	109
	light	1	39	50	3	92	56	22
mean ± SD (n = 13 cvs)	control	13	33 ± 22	32 ± 14	7 ± 9	72 ± 39	0	0
	wound	13	36 ± 18	32 ± 15	7 ± 10	75 ± 37	3	160
	light	13	35 ± 19	32 ± 13	6 ± 6	72 ± 31	0	201

[a]Mean value ± SD or range; n, number of biological replicates; n.d., not detected. [b]Cultivar entry numbers refer to those in Table 1. [c]SGA levels and alterations were determined in the same tuber materials as CA.

chaconine production after wounding were Juliette (1), Eloge (6), and Maritema (16), and after light exposure Juliette (1), Marine (9), and Sava (13). As the conversion of solanidine into either α-solanine (**2**) or α-chaconine (**1**) is initiated by the enzymes solanidine galactosyltransferase (SGT1) or solanidine glucosyltransferase (SGT2), respectively, this observation might indicate a generally stronger effect by wounding and light on SGT1 activity than on SGT2 activity. Differences in availability of the respective sugar substrates may also be a contributing factor. Mechanical wounding of tubers by slicing stimulated glycoalkaloid production in all potato cultivars investigated (Table 1). The relative increase in glycoalkaloid content in wounded tubers as compared to undamaged control ones varied between 50% and 320% among the different cultivars (Figure 3). Light exposure increased glycoalkaloid levels up to 480%, although five cultivars showed no or only marginal response to light exposure. A comparison of the relative response of the tuber glycoalkaloid levels to mechanical wounding vs light exposure among the various potato cultivars identified some cultivars as responsive to both wounding and light exposure, whereas other cultivars were either predominantly wound-inducible or light-inducible, or responsive

neither to wounding nor to light exposure (Figure 3). On the basis of the upper limit for glycoalkaloids in potatoes for human consumption (200 mg/kg), 67% (n = 14) of the cultivars were classified as responsive to both wounding and light, 27% (n = 6) of the cultivars as predominantly wound-inducible, and 5% (n = 1) as predominantly light-inducible (Table 1). The degree of variation in glycoalkaloid responses to stress in the various potato cultivars indicates that glycoalkaloid-induction after mechanical wounding and light exposure are not traits that have been selected for or against during breeding of most modern table potato cultivars. The results also suggest that the glycoalkaloid-inducing effects by mechanical wounding and light exposure are transduced by different regulatory processes. These processes might act on the glycoalkaloid biosynthesis either directly, as part of the signal transduction pathway, or indirectly, e.g. through physiological or epigenetic mechanisms. In line with the latter suggestion are the observations that glycoalkaloid production after light exposure is influenced by the temperature and duration of postharvest storage[31] and that storage affects the DNA methylation pattern during the process of tuber dormancy release.[32] Thus, epigenetic effects due to alterations in DNA methylation during tuber storage might differentially affect glycoalkaloid production as a response to wounding and light exposure. It should be noted that special care was taken in our study to plant, grow, harvest, store, and handle/treat tubers in parallel, allowing the conclusion that the relative differences in glycoalkaloid stress responses observed most likely are cultivar-dependent traits. Whatever the regulatory mechanism, our results show that for a given period of tuber storage the potato cultivars respond very differently to wounding or light exposure with regard to glycoalkaloid production. This has implications for postharvest storage recommendations, since the need to avoid light exposure is clearly urgent for certain potato cultivars, but might be much less critical for others. For the breeding industry, it should be of interest to further characterize tuber responses to various types of stress and environmental conditions, since potato cultivars with low basal glycoalkaloid levels but a strong potential for postharvest increase may request special treatments not to accumulate unhealthy levels of these natural toxins for the consumer.

Influence of Wounding and Light Exposure on Calystegine Levels. To investigate whether also calystegine levels are influenced by wounding and light exposure, 13 potato cultivars, selected to represent the range of glycoalkaloid responses, were analyzed for the tuber content of calystegine A_3 (**3**), B_2 (**4**), and B_4 (**5**), in the same tuber samples that were used for glycoalkaloid analyses. These calystegines were chosen for analysis, since Keiner and Dräger[33] identified them as the most prominent calystegines in potato, and they could be quantitated in all tissues analyzed. Other parts of the potato plant may contain low levels of calystegine A_5, B_1, and B_3, but these have not been identified in tubers of commercial potato varieties. Likewise, calystegine C_1 and N-methyl-calystegine B_2 have not been identified in potatoes. As shown in Table 2, the total amount of the three major calystegines in potatoes varied between 23 and 141 mg/kg f.w. The difference in analytical results between batches of a particular cultivar was very small, indicating that differences between cultivars are likely to have a genetic basis. The total calystegine level observed in the present study was higher than that reported previously, e.g. 3.4−7.0 mg/kg f.w. in two undefined potato samples,[15] or 5.4−-68.1 mg/kg f.w. in eight potato varieties on the North American market.[20] Some of the differences between these studies may be explained by different genotypes, biological materials, and analytical methods. For instance, it is well established that the peel contains substantially higher levels than the flesh,[20,33,34] and a difference in the proportion of peel to flesh between studies may lead to variation in total calystegine levels. An arbitrary classification would define three cultivars in Table 2 [Juliette (1), Bintje (8), and Terra Gold (14)] as having a high content (>120 mg/kg) of total calystegine; three other cultivars [Marine (9), Asterix (10), and Fontane (19)] as containing intermediate levels (61−120 mg/kg); and the remaining seven cultivars [Maris Bard (2), Princess (3), King Edward (4), Folva (11), Sava (13), Melody (17), and Desiree (21)] as displaying low calystegine contents (<60 mg/kg).

The potato cultivars differed in the proportion of the various calystegines in tubers. In most cultivars, calystegine A_3 (**3**) and B_2 (**4**) dominated and were found at fairly similar levels, whereas calystegine B_4 (**5**) was present only in trace amounts or at very low levels. However, in some cultivars, notably Bintje (8) and Fontane (19), the proportion of calystegine B_4 (**5**) was above 20%. Intermediate levels (13%−14%) of this calystegine were found in Juliette (1) and Sava (13). No correlation was found between the total content of calystegines and the proportion of calystegine B_4 (Table 2). Our observations differ to some extent from those of an earlier investigation,[20] where it was shown that calystegine B_2 (**4**) sometimes occurs at significantly higher levels than calystegine A_3 (**3**). We do not know the reason(s) for the differences between these two investigations, but it is conceivable that genetical, physiological, as well as analytical factors may be of importance.

Mechanical wounding and light exposure did not have any significant effect on the total calystegine level (Student's t-test). However, some exceptions to this general trend were noted; for example, Desiree (21) showed increased calystegine levels after both wounding and light exposure, and Melody (17) displayed increased levels after light exposure. Moreover, there was no significant correlation between altered total glycoalkaloid and calystegine levels in tubers after wounding or light exposure (F-test; α 0.05) (Figure 4). These observations agree with the lack of a wounding effect on calystegine levels in wounded potato sprouts[33] and with findings in root cultures of *Atropa belladonna*, where chitosan or the wound stress-related hormones abscisic acid and jasmonic acid did not elicit an increase in calystegine levels.[35]

Taken together, our results show that the calystegine metabolism is generally not influenced by postharvest exposure of potato tubers to wounding or light. Furthermore, there were no indications that the basal levels of glycoalkaloids and calystegines, or the altered levels after wounding and light exposure, are interrelated. This contrasts to the cultivar-dependent induction of glycoalkaloid levels in tubers as a response to mechanical wounding and light exposure and suggests that the biosynthetic pathways of these two types of alkaloids are uncoupled in potato tubers. Thus, the incorporation of carbon and nitrogen into glycoalkaloids during light and wounding conditions does not influence calystegine metabolism, either positively or negatively. Clearly, the cultivar-dependent aspects of postharvest glycoalkaloid increases in potato tubers need further studies, as do the combined effects of glycoalkaloids and calystegines with regard to food quality and safety.

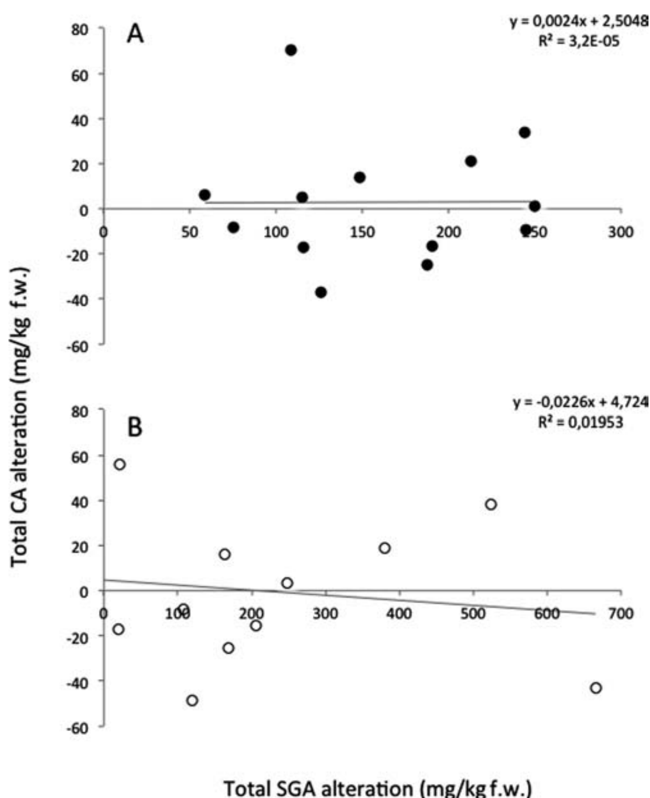

Figure 4. Alteration of total glycoalkaloid and calystegine levels in tuber sections from 13 potato cultivars subjected to mechanical wounding (A) or light exposure (B).

ASSOCIATED CONTENT

Supporting Information

Figure showing the spectral distribution of the fluorescent light source, and table showing the calystegine analytes and parameters of LC-MS/MS detection. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +4618673243. Fax: +4618673389. E-mail: Folke.Sitbon@slu.se.

Funding

This work was financed by a grant from The Swedish Farmers' Foundation for Agricultural Research, and it was also supported by The National Food Agency. The method for determination of calystegines was developed within project NAZVII OH82149 supported by the Ministry of Agriculture of the Czech Republic and project MSM No. 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

Notes

The authors declare no competing financial interest.

[†]E.V.P. and U.A. should be regarded as joint first authors.

ACKNOWLEDGMENTS

We thank Urban Pettersson and Joel Sitbon for plant cultivation, and Per Lindén for light source characterization. Anders Magnusson, Svegro AB, Sweden, is thanked for advice concerning the choice of cultivars.

ABBREVIATIONS USED

f.w., fresh weight; LOD, limit of detection; UV, ultraviolet

REFERENCES

(1) Valkonen, J. P. T.; Keskitalo, M.; Vasara, T.; Pietila, L. Potato glycoalkaloids: a burden or a blessing? *Crit. Rev. Plant Sci.* **1996**, *15*, 1−20.

(2) Friedman, M.; McDonald, G. M. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. *Crit. Rev. Plant Sci.* **1997**, *16*, 55−132.

(3) Andersson, C. Calystegine alkaloids in Solanaceous food plants. *TemaNord* **2002**, *513*, 1−54.

(4) Biastoff, S.; Dräger, B. Calystegines. In *The Alkaloids: Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: New York, 2007; pp 49−102.

(5) Friedman, M. J. Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. *J. Agric. Food Chem.* **2006**, *54*, 8655−8681.

(6) Ginzberg, I.; Tokuhisa, J. G.; Veilleux, R. E. Potato steroidal glycoalkaloids: biosynthesis and genetic manipulation. *Potato Res.* **2009**, *52*, 1−15.

(7) Milner, S. E.; Brunton, N. P.; Jones, P. W.; O'Brien, N. M.; Collins, S. G.; Maguire, A. M. Bioactivities of glycoalkaloids and their aglycones from *Solanum* species. *J. Agric. Food. Chem.* **2011**, *59*, 3454−3484.

(8) Jadhav, S. J.; Sharma, R. P.; Salunkhe, D. K. Naturally occurring toxic alkaloids in foods. *CRC Crit. Rev. Toxicol.* **1981**, *9*, 21−104.

(9) The National Food Administration's Regulations on Certain Foreign Substances in Food. SLVFS 1993.36. http://www.slv.se/upload/nfa/documents/food_regulations/0702_foreign_substances_1993_36.pdf (accessed May 5, 2013).

(10) Sinden, S. L.; Webb, R. E. Effect of environment on glycoalkaloid content of six potato varieties at 39 locations. *USDA Tech. Bull.* **1974**, *1472*, 1−30.

(11) Hellenäs, K. E.; Branzell, C.; Johnsson, H.; Slanina, P. High levels of glycoalkaloids in the established Swedish potato variety Magnum bonum. *J. Sci. Food Agric.* **1995**, *68*, 249−255.

(12) Sinden, S. L.; Sanford, L. L.; Webb, R. E. Genetic and environmental control of potato glycoalkaloids. *Am. Potato J.* **1984**, *61*, 141−156.

(13) Friedman, M.; McDonald, G. Postharvest changes in glycoalkaloid content of potatoes. In *Impact of Food Processing on Food Safety*; Jackson, L., Knize, M., Eds.; Plenum Press: New York, 1999; pp 121−143.

(14) Haase, N. U. Glycoalkaloid concentration in potato tubers related to storage and consumer offering. *Potato Res.* **2010**, *53*, 297−307.

(15) Asano, N.; Kato, A.; Matsui, K.; Watson, A. A.; Nash, R. J.; Molyneux, R. J.; Hackett, L.; Topping, J.; Winchester, B. The effects of calystegines isolated from edible fruits and vegetables on mammalian liver glycosidases. *Glycobiology* **1997**, *7*, 1085−1088.

(16) Winchester, B.; Vellodi, A.; Young, E. The molecular basis of lysosomal storage diseases and their treatment. *Biochem. Soc. Trans.* **2000**, *28*, 150−154.

(17) Heftmann, E. Biogenesis of steroids in the Solanaceae. *Phytochemistry* **1983**, *22*, 1843−1860.

(18) Arnqvist, L.; Dutta, P. C.; Jonsson, L.; Sitbon, F. Reduction of cholesterol and glycoalkaloid levels in transgenic potato plants by overexpression of a type-1 sterol methyltransferase cDNA. *Plant Physiol.* **2003**, *131*, 1792−1799.

(19) Biastoff, S.; Brandt, W.; Dräger, B.; Putrescine. *N*-methyltransferase—The start for alkaloids. *Phytochemistry* **2009**, *70*, 1708−1718.

(20) Friedman, M.; Roitman, J. N.; Kozuke, N. Glycoalkaloid and calystegine contents of eight potato cultivars. *J. Agric. Food Chem.* **2003**, *51*, 2964−2973.

(21) Hellenäs, K. E.; Branzell, C. Liquid chromatographic determination of the glycoalkaloids α-solanine and α-chaconine in

potato tubers: NMKL interlaboratory study. *J. AOAC Int.* **1997**, *80*, 549−554.
(22) Knuthsen, P.; Jensen, U.; Schmidt, B.; Larsen, I. K. Glycoalkaloids in potatoes: Content of glycoalkaloids in potatoes for consumption. *J. Food Compos. Anal.* **2009**, *22*, 577−581.
(23) Fitzpatrick, T. J.; Herb, S. F.; Osman, S. F.; McDermott, J. A. Potato glycoalkaloids: increases and variations of ratios in aged slices over prolonged storage. *Am. J. Potato Res.* **1977**, *54*, 539−544.
(24) Olsson, K. The influence of genotype on the effects of impact damage on the accumulation of glycoalkaloids in potato tubers. *Potato Res.* **1986**, *29*, 1−12.
(25) Dale, M. F. B.; Griffith, D. W.; Bain, H.; Todd, D. Glycoalkaloid increase in *Solanum tuberosum* on exposure to light. *Ann. Appl. Biol.* **1993**, *123*, 411−418.
(26) Percival, G.; Dixon, G.; Sword, A. Glycoalkaloid concentration of potato tubers following continuous illumination. *J. Sci. Food Agric.* **1998**, *66*, 139−144.
(27) Morris, S. C.; Petermann, J. B. Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato (*Solanum tuberosum* L.). *Food Chem.* **1985**, *18*, 271−282.
(28) Coria, N. A.; Sarquís, J. I.; Peñalosa, I.; Urzúa, M. Heat-induced damage in potato (*Solanum tuberosum*) tubers: membrane stability, tissue viability, and accumulation of glycoalkaloids. *J. Agric. Food Chem.* **1998**, *46*, 4524−4528.
(29) Ginzberg, I.; Barel, G.; Ophir, R.; Tzin, E.; Tanami, Z.; Muddarangappa, T.; de Jong, W.; Fogelman, E. Transcriptomic profiling of heat-stress response in potato periderm. *J. Exp. Bot.* **2009**, *60*, 4411−4421.
(30) Rensink, W. A.; Lobst, S.; Hart, A.; Stegalkina, S.; Liu, J.; Buell, C. R. Gene expression profiling of potato responses to cold, heat, and salt stress. *Funct. Integr. Genet.* **2005**, *5*, 201−207.
(31) Griffiths, D. W.; Bain, H.; Dale, M. F. B. The effect of storage temperature on potato (*Solanum tuberosum* L.) tuber glycoalkaloid content and the subsequent accumulation of glycoalkaloids and chlorophyll in response to light exposure. *J. Agric. Food Chem.* **1998**, *46*, 5262−5268.
(32) Law, R. D.; Suttle, J. C. Transient decreases in methylation at 5′-CCGG-3′ sequences in potato (*Solanum tuberosum* L.) meristem DNA during progression of tubers through dormancy precede the resumption of sprout growth. *Plant Mol. Biol.* **2003**, *51*, 437−447.
(33) Keiner, R.; Dräger, B. Calystegine distribution in potato (*Solanum tubersosum*) tubers and plants. *Plant Sci.* **2000**, *150*, 171−179.
(34) Griffiths, D. W.; Shepherd, T.; Stewart, D. Comparison of the calystegine composition and content of potato sprouts and tubers from *Solanum tuberosum* group Phureja and *Solanum tuberosum* group Tuberosum. *J. Agric. Food Chem.* **2008**, *57*, 5197−5204.
(35) Rothe, G.; Hachiya, A.; Yamada, Y.; Hashimoto, T.; Dräger, B. Alkaloids in plants and root cultures of *Atropa belladonna* over-expressing putrescine *N*-methyltransferase. *J. Exp. Bot.* **2003**, *54*, 2065−2070.