https://doi.org/10.17221/166/2018-PSE

Effect of genotype, flesh colour and environment on the glycoalkaloid content in potato tubers from integrated agriculture

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ABSTRACT

Urban J., Hamouz K., Lachman J., Pulkrábek J., Pazderů K. (2018): Effect of genotype, flesh colour and environment on the glycoalkaloid content in potato tubers from integrated agriculture. Plant Soil Environ., 64: 186–191.

The main aim of the study was evaluation of the content of α-solanine, α-chaconine and total glycoalkaloids (TGA) in fourteen new potato cultivars with purple and red flesh in comparison with yellow- and white-fleshed control potatoes cultivated in a friendly way in integrated agriculture. The results were obtained from three-year trials on two locations. TGA levels in tubers' flesh ranged from 33.69 to 167.77 mg/kg fresh matter (FM), and the ratio of α-chaconine to α-solanine from 1.18 to 3.78. No TGA safety limit was exceeded for any cultivar. The glycoalkaloids content was not significantly influenced by flesh colour, whereas the cultivar genotype had a decisive influence on their content. Eight cultivars with coloured flesh yielded a more favourable lower TGA content in comparison with the yellow-fleshed control cv. Agria (86.3 mg/kg FM); on the contrary six cultivars showed higher TGA values. The highest average TGA content was found in the purple-fleshed Bora Valley cultivar (165 mg/kg FM), the lowest was found in the red-fleshed Red Emmalie cultivar (43.6 mg/kg FM), whereas the white-fleshed cv. Russet Burbank reached 67.0 mg/kg FM. The glycoalkaloid content was significantly affected by location and year weather conditions.

Keywords: *Solanum tuberosum* L.; tuberous crop; toxic compounds; drought stress; year of cultivation

Potatoes and other Solanaceae species produce biologically active secondary metabolites called glycoalkaloids (GA) (Kaminski et al. 2016). They are toxic compounds involved in plant protection against pests and diseases and can be potentially harmful for humans if consumed in high quantities (Valcarcel et al. 2014, Kondamudi et al. 2017). GA levels above 200 mg/kg fresh matter (FM) are considered to pose a risk to human health (Tajner-Czopek et al. 2012, Petersson et al. 2013). Potato GA include the more toxic α-chaconine and the less toxic α-solanine, which together form approximately 95% of total glycoalkaloid content (TGA) (Amer et al. 2014). A number of factors can influence the formation of GA, such as growing, storage and transportation conditions, genotype, temperature, cutting, sprouting and exposure to phytopathogens and light (Friedman 2006, Valcarcel et al. 2014), peeling and cooking (Tajner-Czopek et al. 2012), herbicides and biostimulants (Gugała et al. 2016) and soil conditioners (Gugała et al. 2017). Over the last few years, red- and blue-fleshed potatoes have appeared in the retail trade in some European countries and on the U.S. market. They are quite popular in the countries of South America where they originate from, while in Europe the cultivars with coloured flesh are an attractive novelty and an interesting alternative to traditional potatoes with white or cream-coloured flesh (Rytel et al. 2013).

Cultivation of these special cultivars with coloured flesh beneficial to health (high in anti-

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Supported by the Ministry of Agriculture of the Czech Republic, Project No. QI101A184.

oxidants) is mainly domain of growers who use environmentally friendly cultivation methods that include the integrated way of agriculture. The aim of this work was to evaluate the content of TGA, α-chaconine and α-solanine in new purple- and red-fleshed cultivars, as well as in traditional yellow- and white-fleshed potatoes cultivated in integrated agriculture. Another goal was to find to what extent the GA content of cultivars with different flesh colours is influenced by location conditions, year of cultivation and flesh colour.

MATERIAL AND METHODS

Plant material. Potato tubers for chemical analyses were grown in the 2012 and 2014 in the Czech Republic in field trials with four replicates on two locations at different altitudes. In the location Prague – Uhříněves (298 m a.s.l., soil type Luvisol) the experiment was carried out at the Research Station of the University of Life Sciences in Prague and in the Valečov location (460 m a.s.l., soil type acid Cambisol) at the Experimental Station of the Potato Research Institute Havlíčkův Brod. Weather conditions in both locations in experimental years are given in Table 1. In the trials, a total of 16 cultivars were assessed – control yellow-fleshed cv. Agria, 9 cultivars with purple- and 5 cultivars with red-fleshed tubers and white-fleshed cv. Russet Burbank. Potatoes at both locations were cultivated with practically identical integrated technologies, environmentally friendly (without the use of herbicides and mineral nitrogen fertilizers). At both locations, 30 t/ha manure was incorporated into the soil in the autumn, and in the Valečov location according to the results of soil analyses 400 kg/ha Patenkali (i.e. 96.3 kg K/ha and 24 kg Mg/ha) were added. Weed control at both sites was ensured by mechanical cultivation from planting to standing plants in rows. To control the Colorado potato beetle, two sprays were performed on the Uhříněves location with Spintor (active compound: spinosad – a mix of spinosyn A and spinosyn D in the 17:3 ratio, 0.15 L/ha). Protection against potato late blight consisted here of three sprays of preventive preparation Flowbrix (copper oxychloride, 2.3 L/ha). Valečov was the cooler region and in experimental years, beetle did not extend and protection against potato late blight was ensured only with three fungicides sprays (two times with Infinito 1.6 L/ha, one time with Revus (active ingredients: mandipropamid and difenoconazole, 0.6 L/ha). The experimental crops were not damaged with potato late blight at either of the two sites. After harvest, fresh tubers were analysed for the GA content.

Determination of glycoalkaloids by high performance liquid chromatography with electro-

Table 1. Basic characteristics of weather on the vegetation period in experimental years

Location	Month	Average temperature (°C)			Σ precipitation (mm)		
		2012	2013	2014	2012	2013	2014
Uhříněves	April	9.7	13.4	9.6	39.8	17.2	32.4
	May	15.9	12.9	14.0	59.3	82.4	117.8
	June	18.5	17.7	17.5	60.3	157.9	32.6
	July	19.5	21.9	20.6	87.1	61.8	178.6
	August	19.8	19.8	17.6	83.6	89.3	58.6
	September	14.7	14.0	15.5	33.3	49.0	87.6
	average IV–IX	16.4	16.6	15.8			
	Σ IV–IX				363.4	457.6	507.6
Valečov	April	8.1	8.2	9.9	23.8	27.2	29.8
	May	14.6	12.3	12.2	68.2	119.2	129.1
	June	17.2	15.7	16.4	56.0	154.9	36.0
	July	18.5	19.7	19.6	118.6	45.8	56.4
	August	18.4	17.9	16.1	76.0	95.0	85.4
	September	13.4	11.8	14.0	50.0	72.0	106.1
	average IV–IX	15.03	14.27	14.70			
	IV–IX				371.6	514.1	442.8

https://doi.org/10.17221/166/2018-PSE

spray tandem mass spectrometry (HPLC-ESI/MS/MS). The analysis was carried out using a high performance liquid chromatograph Ultimate 3000 RS (Dionex, Sunnyvale, USA) with a binary pump, refrigerated autosampler and column heater. An analytical column Pinnacle DB C18 (50 × 2.1 mm; 1.9 µm) (Restek, Bellefonte, USA) was used at a flow rate of 0.3 mL/min with isocratic elution of a mixture of acetonitrile: methanol (MeOH): Milli-Q water: 0.1 mol/L ammonium acetate (200:100:550:50) (v/v/v/v), pH 3.5 (formic acid). Injection volume was 1 µL and the column temperature was 40°C. The HPLC instrument was coupled to a 3200 Qtrap hybrid triple quadrupole-linear ion trap mass spectrometer (AB Sciex, Foster City, USA) with an electro-spray ionisation source. Mass spectrometric detection of positively charged ions was performed by selected multiple reaction monitoring mode using ion transitions as follows: quantification: α-chaconine 852.6 > 98.2; α-solanine 868.6 > 98.1; confirmation: α-chaconine 852.6 > 380.4; α-solanine 868.6 > 398.5. The applied experimental conditions were as follows: temperature 650°C; ion spray voltage 5500 V; curtain gas 30 psi; collision gas medium; nebulizer gas 60 psi; turbogas 60 psi.

Characterization of weather conditions. The characteristics of weather conditions in years of experimental cultivation (2012–2014) are given in Table 1. The year 2012 was characterized by warm and exceptionally dry weather in the vegetation period, while the years 2013 and 2014 were characterized by lower temperatures and higher sums of total precipitation in a period April–September.

Statistical analysis. The obtained results were statistically evaluated by the analysis of variance (ANOVA). The differences between average values were evaluated by the Tukey's *HSD* (honestly significant difference) test in the SAS computer program (SAS Institute, version 9.4., Carry, USA) at the level of significance $P = 0.05$.

RESULTS AND DISCUSSION

Glycoalkaloids content

Influence of cultivar and flesh colour. The content of glycoalkaloids in the flesh of tubers varied for α-solanine from 10.39 to 77.01 mg/kg FM, α-chaconine from 19.14 to 90.76 mg/kg FM and TGA 33.69 to 167.77 mg/kg FM (Table 2). Importantly, the TGA content did not exceed 200 mg/kg FM for any cultivar (equivalent to roughly 1000 mg/kg dry matter, assuming a water content of 80%), which is considered to be hazardous to human health (Tajner-Czopek et al. 2012, Petersson et al. 2013). The TGA contents found by us are in line with other literature data, which also indicates a significant variability in TGA ranges among cultivars. Valcarcel et al. (2014) indicated a range from 4 to 957 mg/kg dry matter (DM), Friedman et al. (2003) from 5 to 592 mg/kg DM, Deusser et al. (2012) from 7 to 466 mg/kg DM and Petersson et al. (2013) indicated normal ranges between 5 and 100 mg/kg FM.

The ratio of α-chaconine to α-solanine content varied in the present experiments from 1.18 to 3.78 (Table 2) and in all samples a higher α-chaconine content was found in comparison with α-solanine content in the flesh of tubers. Friedman et al. (2003) in their experiment with eight cultivars found the ratio of α-chaconine to α-solanine greater than 1 in all cases, Deusser et al. (2012) found the range of ratios 0.83–2.38. On the other hand, Valcarcel et al. (2014) reported α-chaconine to α-solanine ratio range between 0.3–3.1, with higher levels of α-chaconine found in 4 to 30 cultivars out of the total of 60 cultivars, depending on location and the year of cultivation.

In our experiments, the cultivar proved to be the factor that has the greatest impact on the TGA content. Among 16 evaluated cultivars, on average of three years on two locations, a number of evidence of TGA differences was determined, regardless of the colour of the flesh (Table 2). The highest α-solanine content was found in the cv. Bora Valley (77.01 mg/kg FM, purple-fleshed) in the Valečov location, which reached 1.56 to 7.41 times higher values than the other cultivars. In the case of α-chaconine content, the highest content (90.76 mg/kg FW) achieved the same cultivar on the same location (1.23 to 4.74 times higher than in the other cultivars). A significant effect of genotype on α-solanine and α-chaconine levels was reported by Valcarcel et al. (2014) and by the other authors cited above in relation to the knowledge of the ratios of α-chaconine to α-solanine content.

Results in the present study also show a decisive influence of the genotype of cultivars with different flesh colour on the TGA content (Figure 1). The average values of three years and both loca-

https://doi.org/10.17221/166/2018-PSE

Table 2. Glycoalkaloids content (mg/kg fresh matter) in the flesh of sixteen potato cultivars in two locations

Cultivar	Flesh colour	Uhříněves				Valečov			
		α-solanine (S)	α-chaconine (C)	TGA (S + C)	ratio (C/S)	α-solanine	α-chaconine	TGA (S + C)	ratio (C/S)
Agria	y	41.22^{de}	69.29^{b}	110.51^{d}	1.68	23.87^{ef}	38.23^{de}	62.10^{e}	1.60
Blaue Anneliese	p	12.42^{j}	47.00^{e}	59.42^{hi}	3.78	10.39^{i}	36.22^{ef}	46.61^{g}	3.49
Blaue Elise	p	11.05^{j}	35.93^{fg}	46.97^{j}	3.25	14.50^{hi}	38.68^{de}	53.18^{fg}	2.67
Blaue St. Galler	p	21.0^{1i}	29.78^{h}	50.79^{ij}	1.42	15.98^{gh}	20.76^{h}	36.74^{h}	1.30
Blue Congo	p	31.17^{g}	47.49^{e}	78.66^{f}	1.52	23.53^{ef}	36.32^{ef}	59.85^{ef}	1.54
Bora Valley	p	73.89^{a}	88.25^{a}	162.14^{a}	1.19	77.01^{a}	90.76^{a}	167.77^{a}	1.18
Königsblau	p	36.61^{ef}	55.62^{d}	92.23^{e}	1.52	39.62^{c}	53.73^{c}	93.35^{c}	1.36
Salad Blue	p	25.44^{hi}	40.68^{f}	66.12^{gh}	1.60	17.85^{gh}	28.14^{g}	45.99^{g}	1.58
Valfi	p	20.94^{i}	37.07^{f}	58.00^{hi}	1.77	27.18^{de}	39.35^{de}	66.53^{e}	1.45
Vitelotte	p	43.05^{d}	67.89^{bc}	110.94^{d}	1.58	26.12^{e}	32.66^{fg}	58.78^{ef}	1.25
Herbie 26	r	32.96^{fg}	62.13^{c}	95.09^{e}	1.89	31.70^{d}	52.87^{c}	84.57^{d}	1.67
Highland B. Red	r	43.79^{d}	82.67^{a}	126.46^{c}	1.89	45.45^{b}	73.98^{b}	119.43^{b}	1.63
Königspurpur	r	48.91^{c}	65.74^{bc}	114.65^{d}	1.34	24.24^{ef}	28.54^{g}	52.78^{fg}	1.18
Rosemarie	r	56.28^{b}	86.63^{a}	142.91^{b}	1.54	49.26^{b}	70.62^{b}	119.88^{b}	1.43
Red Emmalie	r	22.83^{i}	30.76^{gh}	53.58^{ij}	1.35	14.55^{hi}	19.14^{h}	33.69^{h}	1.32
Russet Burbank	w	28.31^{gh}	40.80^{f}	69.11^{g}	1.44	20.46^{fg}	42.34^{d}	62.80^{e}	2.07
Average		33.75^{A}	55.18^{A}	88.92^{A}	1.80	29.10^{B}	44.09^{B}	73.19^{B}	1.67
$HSD_{cultivars}$		4.814	5.889	9.229		4.648	5.012	7.938	

Differences between means with the same letter (for cultivars lowercase letters, for locations uppercase letters) are statistically non-significant; locations: $HSD_{solanine}$ = 0.630; $HSD_{chaconine}$ = 0.725; HSD_{TGA} = 1.142; flesh colour: y – yellow; p – purple; r – red; w – white; HSD – honestly significant difference; TGA – total glycoalkaloid

tions show that the highest TGA content was found in cv. Bora Valley (165.0 mg/kg FM), followed by cvs. Rosalinde and Highland B. Red-fleshed cultivars ranked second and third (131.4 and 123.0 mg/kg FW, respectively), while the fourth place was occupied by the purple-fleshed cv. Königsblau (92.8 mg/kg FM). The control yellow-fleshed cv. Agria (86.3 mg/kg FM) occupied the sixth place of 16 cultivars and the total of 10 cultivars with coloured flesh achieved more favourable TGA values (1.02 to 1.98 times less) and only 5 cultivars obtained higher values (1.04 to 1.91 times). Globally significant white-

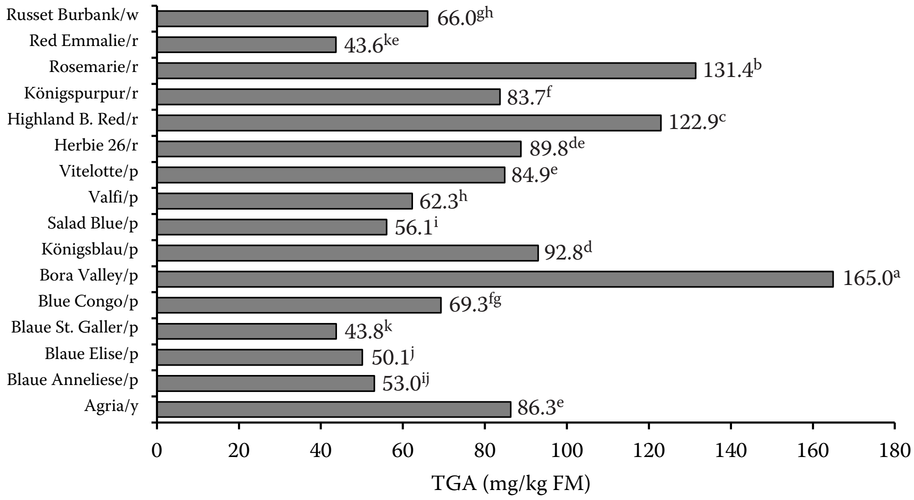

Figure 1. Influence of cultivar and flesh colour on the total glycoalkaloid (TGA) content; average of two locations and three years 2012–2014. Differences between averages with the same letter are statistically non-significant. Tukey's *HSD* = 5.88; flesh colour: w – white; r – red; p – purple; y – yellow; FM – fresh matter; HSD – honestly significant difference

fleshed cv. Russet Burbank (66.0 mg/kg FM) ranked 10th between the examined cultivars. The lowest TGA content was determined in red-fleshed cv. Red Emmalie (43.6 mg/kg FM). The decisive influence of the cultivar on the TGA content was found also by other authors (Lachman et al. 2013, Amer et al. 2014, Gugała et al. 2016, 2017).

The average TGA content for the period of three years and both locations was not significantly affected by the colour of tubers pulp (Table 3). However, a trend (the inconclusive difference) to the highest TGA content in red-fleshed cultivars was found, where it reached 1.09 times higher values in comparison with the control cv. Agria, 1.27 times higher in the group of cultivars with purple flesh and 1.41 times higher content in the white-fleshed cv. Russet Burbank. In a detailed analysis of the results, it may be concluded that within the group of coloured-flesh cultivars, the TGA content of each individual cultivar is decisive. Evidence for this statement is the fact that in a group of five red-fleshed cultivars the differences in TGA levels between individual cultivars were very significant and in all cases conclusive (Figure 1). A similar finding was also reported in a group of nine purple-fleshed cultivars, where only in one case the difference in TGA levels between cultivars did not exceed the limit of statistical significance. The TGA content in tubers of three red-fleshed cultivars and four blue-fleshed cultivars was evaluated by Tajner-Czopek et al. (2012), where they found a significantly higher TGA (on average by 8%) in red-coloured cultivars. In contrast, Rytel et al. (2013) found a higher TGA in blue-fleshed cultivars in a similar experiment. These two contradictory results support our understanding of the dominant influence of the genotype on TGA content compared to the flesh colour.

Table 3. Influence of flesh colour on the total glycoalkaloid (TGA) content (mg/kg fresh matter); 2012–2014

Flesh colour	$\bar{x}^1$		
	Uhříněves	Valečov	average of locations
Yellow	110.51^a	62.10^a	86.30^a
Purple	79.65^{ab}	68.96^a	74.20^a
Red	106.14^a	84.16^a	94.31^a
White	69.11^b	62.80^a	67.01^a

For flesh colour: $HSD_{\text{Uhříněves}}$ = 36.35; $HSD_{\text{Valečov}}$ = 45.18; $HSD_{\text{average of locations}}$ = 27.79. [1]Average of all cultivars with the above-mentioned flesh colour (four replicates); Differences between averages marked with the same letter are statistically non-significant. HSD – honestly significant difference

Influence of the location. The location significantly affected TGA and individual glycoalkaloids (Table 2). In the average of three years and 16 experimental cultivars, significantly higher TGA, α-solanine and α-chaconine contents were found in tubers from the Uhříněves location (TGA 1.21 times, α-solanine 1.16 times, and α-chaconine 1.25 times higher) in comparison to the Valečov location. In a more detailed assessment of the difference of the TGA content among different cultivars, it was found that at the Uhříněves location, a higher TGA content in 13 cultivars, α-solanine in 11 cultivars and α-chaconine in 12 cultivars was found than in at the Valečov location. Thus, it is evident that the effect of location on the TGA content is cultivar-specific. Differences in TGA levels between locations are likely related to their climatic and soil conditions. At the Uhříněves location, higher TGA accumulation in the tubers was promoted by a warmer and drier climate in comparison to the Valečov location (Table 1) where more frequent drought stresses were observed, which corresponds to the findings of Bejarano et al. (2000). In terms of soil conditions, the higher content of TGA in Uhříněves can be supported by the heavier clay soil (in Valečov sandy loams prevail), as Haase (2010) attributed the differences between glycoalkaloids content from different locations to the soil characteristics, associating the loamy soil with higher levels of glycoalkaloids.

Influence of the year of cultivation. Year of cultivation influenced the TGA content in tubers in both locations, however each in a different way (Table 4). In Uhříněves (298 m a.s.l., significantly warmer location compared to Valečov), the highest content of TGA and also α-chaconine was found in 2012, which was characterized by warm and particularly dry weather in the vegetation period in comparison with other years; a very dry month was mainly September, but also May and June (Table 1). The highest TGA content in Uhříněves in 2012 was probably due to the drought stress during some vegetation periods. On the other hand, the highest TGA, α-solanine and α-chaconine contents were found in Valečov (460 m a.s.l.) in 2013; in this location, the year 2013 was the coldest and most rainy and the average temperature during the vegetation period was lower in comparison with Uhříněves by 2.3°C (Table 1). The highest TGA content in 2013 thus seems to be related to cold and water stress. In Uhříněves, cold (June and September) and rainy (June) weather was reported only in some phases

Table 4. Influence of year on the glycoalkaloids (GA) content; average of sixteen cultivars

	Year	α-solanine	α-chaconine	TGA
Uhříněves	2012	34.8^{b}	67.9^{a}	102.8^{a}
	2013	38.1^{a}	41.2^{c}	79.3^{c}
	2014	29.1^{c}	58.9^{b}	86.9^{b}
Valečov	2012	16.2^{c}	36.6^{b}	52.8^{c}
	2013	45.1^{a}	47.9^{a}	93.0^{a}
	2014	24.7^{b}	46.6^{a}	71.3^{b}

Differences between averages with the same letter are statistically non-significant; Uhříněves: $HSD_{\text{solanine}} = 1.329$; $HSD_{\text{chaconine}} = 1.625$; $HSD_{\text{TGA}} = 2.547$; Valečov: $HSD_{\text{solanine}} = 1.314$; $HSD_{\text{chaconine}} = 1.417$; $HSD_{\text{TGA}} = 2.244$; HSD – honestly significant difference; TGA – total glycoalkaloid

of vegetation in 2013. A significant influence of the year on the GA content is presented by a number of authors, e.g. by Valcarcel et al. (2014) and Bejarano et al. (2000). In contrast, in experiments provided by Gugała et al. (2017), weather conditions in experimental years had an insignificant influence on GA levels in potato tubers. It is difficult to determine the exact causes of increased GA accumulation in tubers, but many authors agree that the synthesis of GA is related to stress in general, during both growth and storage (Petersson et al. 2013, Valcarcel et al. 2014, Mekapogu et al. 2016). Papathanasiou et al. (1999) reported that heat stress increases the glycoalkaloid content, with various results reported for low temperatures. Drought stress seems to increase the GA content as well, whereas the excess of water has the same effect only at low temperatures during later stages of development (Papathanasiou et al. 1999, Bejarano et al. 2000). Cold and wet periods during summer were also associated with higher levels of glycoalkaloids (Haase 2010, Valcarcel et al. 2014).

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Received on March 13, 2018
Accepted on April 5, 2018