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Adaptation of glycoalkaloids detection method for evaluation of Latvian potato genetic resources

I. Skrabule[1], D. Grauda[2], A. Mikelsone[2] and A. Vasariete[2]

[1]State Priekuli Plant Breeding Institute, Zinatnes 1A, Priekuli, LV-4126, Latvia;
e-mail: skrabuleilze@navigator.lv
[2]Institute of Biology, University of Latvia, Miera 3, Salaspils, LV-2169, Latvia;
e-mail: dace@email.lubi.edu.lv

Abstract. The glycoalkaloid content in potato tubers can be influenced by several factors: variety, weather, storage environment, maturity, damage, temperature and exposure to light. Potato varieties vary with regard to their inherited total glycoalkaloid (TGA) content. The problem in the practical use of most TGA detection methods is that they are money and time consuming. The aims of the investigation were adaptation of a rapid and rather cheap method and evaluation of TGA content of varieties included in Latvia potato genetic resources. The used method was based on three earlier elaborated protocols of different authors. 31 varieties of Latvian potato genetic resources were examined for TGA content for two years, 10 of them for three years. TGA content depending on the variety ranged from 2 till 27 mg 100 g^{-1} fresh weight, the variance between genotypes was high. The significant genotype influence on TGA content was established. The influence of growing conditions on TGA content was not significant.

Key words: potato, glycoalkaloids, TGA, colorimetric determination

INTRODUCTION

Potato tubers contain a small amount of naturally occurring steroidal glycoalkaloids – potentially toxic compounds, which are present throughout the family *Solanaceae.* Approximately 95% of the total glykoalkaloids present in potatoes are accounted for α-solanine and α-chaconine, both of which are structurally similar, being different glycosylated forms of aglycone, solanidine. The level of glycoalkoloids in potato tubers can be influenced by several factors: genotype or variety (cultivar), weather, storage environment, maturity, damage, temperature and exposure to light (Jadhav, 1981; Storey & Davies, 1992; Dale & Mackay, 2007). Potato varieties vary with regard to their inherited glycoalkaloid content; at low level it may enhance potato flavour, so it is acceptable. Higher concentration of glycoalkaloids (above 15 mg 100 g^{-1} fresh weight – FW) can develop bitterness. Content above 20 mg 100 g^{-1} fresh weight is considered unsuitable for human consumption because of toxicity (Kerlan & Ellisseche, 2000; Dale & Mackay, 2007). Glycoalkoloids content in tubers of potato varieties vary quite widely. The varieties with genetically high levels are apparently more susceptible to excessive glycoalkaloids production in extreme conditions (Storey & Davies, 1992). The research of German potato varieties showed glycoalkaloids

content in range from 2 to 22 mg 100 g^{-1} FW, American varieties – 2 to 13 mg 100 g^{-1} FW, United Kingdom varieties – 3.6 to 14.2 mg 100 g^{-1}FW (Dale & Mackay, 2007). This trait demonstrated quite high broad sense heritability (0.50–0.89 %) in different investigations (Kerlan & Ellisseche, 2000). Estimation of the glycoalkaloid levels of varieties used as parental genotypes and in the progenies should become an important criterion to take into consideration with aim to provide tuber glycoalkaloids content within accepted safety levels.

During more than 80 years different methods for determination of separate glycoalkaloids and total glycoalkaloid (TGA) content were developed. The first methods were only aware of solanine as the glycoalkaloid in tubers and the method for determination involved extraction, purification and weighing (Coxon, 1984). The developed quantitative assay of potato glycoalkaloids depends on the method of primary extraction, the work-up procedure and the ultimate quantitative assay. The ways for primary extraction are: hot alcoholic extraction, extraction with bi-solvent mixture and extraction with aqueous acidic alcohol. The work-up procedures vary from the classical precipitation in alkaline solution to absorption and hydrolysis, and extraction of the aglycones. Several techniques were used for quantitative assay: gravimetric, colorimetric, titrimetric, polarographic and chromatographic (Bergers, 1980). For determination of glycoalkaloids with chromatographic analysis several ways can be used, for example, gas chromatography can be applied for analyses of glycoalkaloids. Some authors suggested as a better choice for routine determination high-performance liquid chromatography (Houben & Brunt, 1994). In practical use of most of these methods the problem is the need for special equipment, and many steps involved are money and time consuming. In this case the aims of the investigation were adaptation of a rapid and rather cheap TGA detection method and TGA content evaluation of varieties included in Latvia potato genetic resources.

MATERIALS AND METHODS

Potato growing conditions. Potato variety samples were grown in the State Priekuli Plant Breeding Institute field of potato collection during 2007–2009; plot size for each variety was 5.04 m^2. Plots were arranged in a random design with three replications. The soil type was sod-podzolic (PVv), loamy sand in 2007 and loam in 2008, 2009. The organic matter content in soil was 12–23 g kg^{-1}, pH_{KCl} was 5.3–6.1, available P 44–80 mg kg^{-1} and K 105–160 mg kg^{-1}. Fertilizer N 55–65, P 33–95, K 46–110 kg ha^{-1} was used. Potatoes were planted in the second decade of May and harvested in the last decade of August. The furrowing three times was used and herbicide Zenkor was applied for weeding in 2007–2008, but furrowing only twice and herbicides Mistral and Pantera were used in 2009 because of bluegrass (*Agropyron repens* L.*)* presence in field. Spraying two times with fungicides Ridomil or Penkoceb and Gloria was applied after flowering to limit late blight (*Phythophthora infestans* (Mont.) de Bary) damage. The application with insecticides Kalipso and Karate was used in 2007 for Colorado

beetle (*Leptinotarsa decemlineata* (Say) control and with Fastac in 2009 for aphid control. Winter cereals were pre crop each trial year.

Concerning weather conditions, average air temperature was slightly higher than long-term average in all three trial years. However, precipitation was different during growing periods 2007–2009. The rainfall exceeded a little long-term average data in the whole growing season in 2007. Next year period from May to July was quite dry with low rainfall. In August heavy rain followed (173% of long-term average data). Comparatively, the beginning of the growing period was rainy (precipitation 174% of long term average data) and the second part fairly dray in 2009. The total rainfall in trial years was respectively: 418 mm in 2007, 287 mm in 2008 and 343 mm in 2009.

The tubers of each variety for TGA content detection were taken as average sample of total potato tuber yield.

Tested potato varieties. The Latvian potatoes genetic resources consist of varieties developed in Latvia and are so called local varieties – potato genotypes adapted to local growing conditions without information about the origin and been grown in fields and gardens for a long time. TGA content was detected for 31 varieties of Latvia potato genetic resources during 2007–2008 and for 10 of the tested varieties in 2009 as well. Seven of the examined 31 potato varieties were developed in period from 1926 to 1950, nine – from 1951 to 1990, twelve from 1991 to 2008, three of the used varieties were local varieties with unknown time of creation. The tested potato varieties belonged to different maturity types: 7–early maturity, 5–medium early, 13–medium late, 2–late and 5 unknown maturities.

Method for detection of TGA content in potato tubers. The adaptation of method for detection of TGA content in potato tubers and TGA evaluation was carried out in laboratory of the Institute of Biology. The extraction and precipitation of used protocol was based on quantitative assay for solanidine glycoalkaloids (Bergers, 1980; Frydecka-Mazurczyk, 2001).

Washed unpeeled potato tubers were cut into small pieces and ground. 50 g of ground potato mass was weighed in the flask and 200 ml 96% ethanol was added. The flask with the potato-alcohol pulp was placed in a water bath with temperature of 90ºC for 10 minutes and was heated. The obtained extract was filtered through Whatman No. 1 filter paper by suction over a Bühner's funnel. The filtered extract was evaporated at a temperature of 60ºC till 20 ml using the rotary evaporator. After evaporating 50 ml of 10% acetic acid was added to the obtained concentrate, it was mixed and transferred to 50 ml tubes of the same volume. The tubes were put in the centrifuge (Eppendorf 5810 R), at 10ºC and centrifuged for 30 minutes at 133.36 Hz. The centrifuged solution was poured into the narrow-necked Erlenmeyer's flask with ground glass stopper and ammonia was added to provide final solution's pH = 10. The flask was immediately closed with a stopper to avoid ammonia evaporating. The flask with the solution was heated in a 70ºC water bath for 20 min. After heating the solution was poured into 15 ml tubes to be equal in the volume, and cooled for at least 3 h in temperature +4ºC. Solution was centrifuged for 30 min at 10ºC at 133.36 Hz. After centrifugation, the liquid part was carefully poured off and precipitation of the glycoalkaloids remained at the bottom of the tube. Oorthophosphoric acid 500 µl, 7%

was added to the precipitation and the obtained glycoalkaloids sample was transferred to Eppendorf tubes.

The samples were stored in a freezer at –20ºC (Grunenfelder et al., 2006) until performing of quantitative colorimetric determination of TGA content (Bergers, 1980; Frydecka-Mazurczyk, 2001; Grunenfelder et al., 2006). Stored glycoalkaloids samples were taken from the freezer, defrosted and stirred with a mixer in a homogeneous mass (Vortex-2 Genie kombi-spin). A specific solution was prepared from 40 ml of phosphoric acid and 12 mg of paraformaldehyde. The solution was thoroughly stirred to dissolve the paraformaldehyde. The 100 µl of the extracted glycoalkaloids sample and 1 ml of the previously prepared solution of phosphoric acid and paraformaldehyde were mixed together in the Eppendorf's tube with a mixer. Prepared samples were kept for 30 min until color changes. After coloring the prepared samples were transferred to disposable 1 ml cells and the absorption A600 was measured with a spectrophotometer (Eppendorf Bio Photometer). The TGA concentration was determined based on a γ-solanine (ROTH Rotichrom® CMR) standard curve (Bergers, 1980; Frydecka-Mazurcyk, 2001; Grunenfelder et al., 2006). The results were calculated as mg in 100 g of fresh weight (FW) of potato tubers.

The results were evaluated using the statistical package (MS EXCEL) and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The method for TGA detection was established on base of three earlier elaborated protocols of different authors: W. Bergers (1980), L. Grunenfelder et all. (2006) and A. Frydecka-Mazurcyk (2001). The combined protocol was simple to use and relatively cheap, it needed only basic equipment of biochemistry laboratory and some specific and comparatively expensive (γ-solanine) chemicals. The included sample freezing in protocol gave possibility to collect bulk of samples and to detect TGA content for all samples together at the same time. It saved time for detection of one sample (in two replications) and simplified laboratory procedure.

The TGA content of 31 potato varieties varied from 2.09 to 26.2 mg 100 g^{-1} FW in 2007 and from 3.9 to 27.2 mg 100 g^{-1} FW in 2008 (Table 1). Acceptable for human consumption TGA content (< 20 mg 100 g FW) in tubers was detected for most of the examined potato varieties. TGA content more than 20 mg 100 g^{-1} FW was considered unsuitable for human consumption, so detected during trial years TGA content of some potato genotypes was higher than acceptable. TGA content in tubers of four potato varieties in 2007 and two potato varieties in 2008 was higher than 20 mg 100 g^{-1} FW. The significant influence of genotype on TGA content was detected using two factors analysis of variance (p = 0.025 <0.05), but the impact of growing conditions was not significant. The coefficient of variation in both years was high (Table 1) it means that genotypes or varieties were greatly different. No influence of maturity type on TGA content was found. As well as diversity of TGA content was similar for variety groups developed in different times.

Table 1. TGA content in tubers of potato varieties included in Latvian potato genetic resources.

Characteristics		Unit	2007	2008
TGA content	mean	mg 100 g^{-1} FW	14.26	12.70
	min		2.09	3.90
	max		26.20	27.20
Standard deviation			6.4	6.1
Coefficient of variation		%	45.1	48.1
Number of varieties		Piece	31	31

Table 2. TGA content (mg 100 g of FW) of potato varieties during 2007–2009.

Variety	Maturity	2007	2008	2009	Mean
Agrie Dzeltenie	Early	8.47	4.15	6.05	6.22
Vita	Medium late	14.78	3.95	5.50	8.07
Magdalena	Medium late	2.09	13.49	9.63	8.40
Lenora	Medium early	6.59	12.23	6.98	8.60
Bete	Medium late	14.16	8.08	4.98	9.07
Vale	Medium late	7.44	5.79	14.40	9.21
Imanta	Medium late	12.92	12.73	5.00	10.21
Brasla	Medium late	12.08	13.45	11.43	12.32
Monta	Early	19.75	11.18	10.88	13.93
Vietejie Biesu		23.44	25.40	25.65	24.83
Mean		12.17	11.04	10.05	
Probability (0.05)			p = 0.61		p = 0.006

The TGA content for ten potato varieties was detected in the three years (Table 2). The results varied from 2.09 (‘Magdalena’, 2007) to 26.65 (‘Vietejie Biesu’, 2009) mg 100 g of FW. No correlation between TGA content and maturity type of potato varieties was found. TGA content of two early varieties was different, and of medium late varieties varied quite widely – in an average from 8.07 (‘Vita’) to 12.32 (‘Basal’) mg 100 g of FW. Even the difference between TGA content of some varieties in separate years was important, for instance – variety ‘Magdalena’ 2.09 mg 100 g FW in 2007 and 13.49 100 g FW in 2008. For varieties ‘Agrie Dzeltenie’ and ‘Vietejie Biesu’ TGA content was more stable in all three years. However, the established impact of genotype (variety) on TGA content was significant ($p = 0.006 < 0.05$). The average TGA content in 2007 was a little higher than in other years. Concerning meteorological or growing conditions the total rainfall was 418 mm in 2007, slightly exceeding total rainfall in both following years. Rainfall could induce TGA accumulation in tubers. However, no significant influence of growing conditions or trial year on TGA content ($p = 0.61 > 0.05$) was found.

CONCLUSIONS

The combined method for TGA content detection in potato tubers was developed. Using this method TGA content in tubers of potato varieties included in Latvian potato genetic resources was evaluated.

The TGA content in tubers of potato varieties was different. TGA content of most the genotypes were below 20 mg 100 g FW, which is considered acceptable for human consumption. Some genotypes contained higher amount of TGA in tubers than accepted as suitable for human consumption.

The influence of potato genotype on TGA content in tubers was significant, but the impact of growing conditions or year was insignificant.

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