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Glycoalkaloid content in potato tubers with different levels of resistance to *Phytophthora infestans*

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SUMMARY
In this paper the results of investigation of relationship between potato plant tuber resistance and glycoalkaloid content are reported. The plant material was represented by *Tuberosum* type breeding lines and potato cultivars as well as by accessions of four *Solanum* species and two interspecific hybrids. Within *Tuberosum* accessions tuber resistant, moderately resistant and susceptible clones were registered. Wild species accessions and interspecific hybrid populations were segregating for resistance. Glycoalkaloid content data was also characterized by a wide range of values. The α-solanine and α-chaconine content varied between different types of potatoes (*Tuberosum* contra wild species) or between groups with different resistance levels. Spearman analyses showed that in potato species accessions as well in interspecific hybrids there were no significant correlations between resistance and α-solanine as well as α-chaconine contents. A significant correlation (Spearman′s rho=0.224, p=0.03) was however observed between lesion size and α-chaconine in the *Tuberosum* material.

KEYWORDS
α-solanin, α-chaconine, TGA, late blight

INTRODUCTION

Many Solanaceae family members synthesize steroidal glycoalkaloids; to date there are over 90 described in the literature with diverse chemical structures. α-chaconine and α-solanine are the most abundant (95%) in most commercial potatoes and are often referred to as Total GlycoAlkaloids (TGA). The average ratio of α-solanine to α-chaconine is 40:60, but deviations have been reported. Glycolakaloid content varies depending on tissue as well as between species and cultivars and expression is determined by both genetic and environmental factors (Friedman 2006; Friedman and McDonald 1997; Maga 1994). Many of the *Solanum* L. species of interest to potato breeders may contain levels in excess of 200 mg/kg (fresh weight) which is the standard maximum level allowed by international health regulations.

It has been assumed that glycoalkaloids are a part of the natural defence against some pests as well as diseases. The situations in Colorado Potato Beetle (CPB) (Tingey, 1984; Deahl *et al.*, 1991), potato cyst nematode (Grassert and Lellbach, 1987), leafhopper (Sanford *et al.*, 1992) as well as in the diseases caused by *Erwinia carotovora* subsp. *atroseptica* (Andrivon *et al.*, 2003), *Rhizoctonia solani*(Morrow and Caruso, 1983) and *Phytophthora infestans* (Deahl *et al.*, 1973; Sarquis *et al.*, 2000; Andrivon *et al.*, 2003) have been investigated. Results showed a significant influence of glycoalkaloid content on resistance to CPB (Deahl *et al.*, 1991). The steroidal aglycone leptine (a form of solanidine) found in *S. chacoense* was identified as responsible for resistance to CPB. To achieve resistance against CPB in potato cultivars, high-leptine genotypes of *S. chacoense* were integrated in a breeding program (Yencho *et al.*, 2000). No influence of glycoalkaloid content on tuber resistance to late blight was found in the study performed by Deahl *et al.* (1973). Total glycoalkaloid contents from blight-infected plants were not significantly different than TGA contents from healthy plants (Deahl *et al.*, 1973). Data obtained by Sarquis *et al.* (2000) showed that the correlation between tuber and foliage alkaloids is poor. In view of the observed field resistance to late blight, it was also concluded that tuber glycoalkaloid content might not be responsible for such resistance. The results of research performed by Andrivon *et al.* (2003) indicated that neither race-specific nor partial resistance to late blight and soft rot in the accessions used as progenitors of resistance depend on high α-solanine or α-chaconine concentrations. With the exception of low, but statistically significant, correlations between the concentration of α-solanine and late blight resistance in progenies derived from *S. vernei*, no consistent relationship between resistance to disease and concentrations of α-solanine and/or α-chaconine was observed (Andrivon *et al.* 2003).
Sources of resistance to *P. infestans* found in potato species are intensively used in potato breeding. The glycoalkaloid content in such plant material exceeds these in cultivated potatoes (Sarquis *et al.*, 2000). The composition of glycoalkaloids in potato hybrids derived from some wild species show, beside α-chaconine and α-solanine, the specific glycoalkaloid demissidine (Mattheij *et al.*, 1992). Tuber resistance to *P. infestans* has been found in a number of wild potato species (Zoteyeva, 2006). For breeding purposes it is therefore important to find and select breeding lines derived from wild potato species that possess traits for both resistance and low glycoalkaloid content.
The aims of our study were

1) to investigate if a relationship exists between the *P. infestans* tuber resistance components mycelium growth and lesion size on one hand and TGA, α-chaconin and α-solanin concentrations in tubers on the other hand
2) to study if the proportion of α-solanine to α-chaconine varies between different types of potatoes (*Tuberosum* contra wild species) or between groups with different resistance levels.

MATERIALS AND METHODS

The study was performed at Alnarp (southern Sweden), Swedish University of Agricultural Sciences University during two years (2008 and 2010).

Tubers of 12 *Tuberosum* breeding lines, 4 cultivars, 15 accessions from different *Solanum* species (*S. andigenum*, *S. neoantipoviczii*, *S. papita* and *S. ruiz-ceballosii*) and 12 interspecific hybrids (derived from crosses using *S. microdontum*, *S. neoantipoviczii*, *S. tarijense* and *S. phureja*) were evaluated for tuber resistance to *P. infestans* as well as for TGA, α-solanine and α-chaconine contents. The tubers originated from field grown plants.

Evaluation of tuber resistance

Tuber resistance to *P. infestans* was evaluated by inoculating tubers with an aggressive isolate (SE 03058, mating type A1, virulence genes 1.3.4.7.10.11). The inoculum comprised 20,000 sporangia/

ml. The incubation period lasted 6 days and the disease rating was performed on the 7th day after inoculation. Mycelium growth was scored using a scale 0–3 (0=lack of mycelium and 3=very abundant growth) and afterwards lesion sizes were scored on longitudinally cut tubers using a scale with grades 1 – 9 (1=up to 90% of diseased area and 9=highest resistance, no lesion).

Quantification of glycoalkaloid content
For TGA analysis, five tubers from each genotype were selected and rinsed in tap water. Each sample was finely diced (skin and cortex) and mixed. Then 20 g was homogenized with an Ultra Turrax homogenizer TP 18/10 with shaft 18-N (Janke & Kunkel KG, IKA-Werk, D-7813, Staufen, Germany) for 2 min with 100 ml water:acetic acid:ascorbic acid, 100:5:1 (vol/vol/wt). The volume was adjusted to 200 ml with the same solvent, clarified by centrifugation at 4°C at 10,000 rpm (Sorvall Evolution RC) for 10 min and filtered through 1F (Munktells). Ten ml of the supernatant was put onto a Sep-Pak C18 cartridge previously activated by acetonitrile in accordance with the method of Hellenäs and Branzell (1997), which was also used for the subsequent analytical procedure that was performed on HPLC. The α-solanine and α-chaconine (Sigma Chemical Co.) were used as standards. The concentrations were given in mg/kg fresh weight (FW).

Statistical analysis
Spearman 1-tailed analyses of correlation were performed with the computer program SPSS.

RESULTS AND DISCUSSION

Average TGA content found in tubers from accessions of wild species and interspecific hybrids from wild parents (838 mg/kg FW) highly exceeded the one in the *Tuberosum* group (135 mg/kg FW). None of the cultivars and only one of the breeding lines showed TGA values exceeding the maximum level allowed by the international health regulations whereas only four of the wild species and interspecific hybrids had acceptable TGA values. This shows the importance of regular analyses of TGA contents of wild accessions and their hybrids during the breeding process.

No significant correlations were found neither between TGA or α-solanine and the two evaluated resistant factors nor between α-chaconine and mycelium growth in the *Tuberosum* material. A significant correlation (Spearman´s rho=0.224, p=0.03) was however observed between lesion size and α-chaconine in this material (Table 1). In the wild potato species as well as in the interspecific hybrids no significant correlation were found between the resistance components (lesion size and mycelium growth) and α-chaconine or α-solanine.

The material was sorted in 3 resistance groups (HR=high resistance (grades 7.0-9.0), MR=moderate resistance (5.0 to 6.9) and S=susceptible (1.0 to 4.9)) depending on results in the tuber inoculation test (lesion size). The HR–group consisted of 5 breeding lines and 12 wild species accessions/ interspecific hybrids. Five breeding lines and cv. Bintje were found susceptible together with 4 wild accessions/interspecific hybrids. The rest of the material, including cv. Asterix, Matilda and Superb, had moderate resistance levels.

All *Solanum* species accessions and interspecific hybrids strongly segregated for tuber resistance to *P. infestans*. Resistance in the accession of *S. andigenum* was scored with average grade 6.8 and ranged from 4.5 to 7.3. Average mycelium growth was 0.4 grades. Tubers of all tested plants of *S. neoantipoviczii* expressed resistance scored 7.7 on average and were lacking mycelium growth. Plants of *S. papita* segregated in the proportion 1S:1MR:2R. Their resistance was scored on average 5.7. The accession of *S. ruiz-ceballosii* segregated for resistance in equal proportions (1R:1MR:1S). The hybrid

Table 1. Correlations between late blight resistance components (lesion size and mycelium growth intensity) and α-chaconin, α- solanine and total glycoalkaloid content in tubers of potato accessions.

Spearman's rho		α-solanine	α-chaconine	TGA	Mycelia	Lesion
α-solanine	Correlation Coefficient	1,000	,848(**)	,920(**)	-,120	,029
	Sig. (1-tailed)	.	,000	,000	,153	,404
α-chaconine	Correlation Coefficient		1,000	,983(**)	-,181	,224(*)
	Sig. (1-tailed)		.	,000	,060	,027
TGA	Correlation Coefficient			1,000	-,181	,181
	Sig. (1-tailed)			.	,060	,061
Mycelia	Correlation Coefficient				1,000	-,465(**)
	Sig. (1-tailed)				.	,000
Lesion	Correlation Coefficient					1,000
	Sig. (1-tailed)					.

between *S. microdontum* and *S. tarijense* showed a distribution for resistance 1R:1S with mycelium growth scored 0.9 on average. Lack of mycelium growth was noted on all tubers of another hybrid (*S. neoantipoviczii* × *S.phureja*). Its tuber resistance was scored on average 7.1 and ranged from 6.0 to 8.4.

As noted in other studies,α-chaconine was the predominant glycoalkaloid in all cultivars and breeding lines in the *Tuberosum* group and in most of the accessions from the wild species/ interspecific hybrids when studied for the 2008 analyses. Only in six out of nine of the hybrids between *S. neoantipoviczii* and *S. phureja* as well as in one of the three accessions belonging to the species *S. neoantipoviczii* α-solanine was the predominant glycoalkaloid. For the rest, α-chaconine was predominant just as in the *Tuberosum* group. Also when average values were calculated for the three different resistance groups of the accessions from the wild species/interspecific hybrids α-chaconine was the predominant glycoalkaloid. The percentage of α-chaconine out of the total glycoalkaloid content were higher in the *Tuberosum* group compared to the group of wild species and interspecific hybrids (Fig. 1, 1–6). However, the different resistance groups showed slightly different proportions between the two glycoalkaloids and the domination of α-chaconine was most evident for the most sensitive genotypes in both groups.

The conclusions about the lack of relationship between glycoalkaloid content and tuber resistance to late blight have been made in investigations performed at different sites and time periods (Deahl *et al.*, 1973; Sarquis *et al.*, 2000). In the results of a study performed by Andrivon *et al.* (2003) the correlations between concentration of α-solanine and two late blight resistance components (incubation period and spore production per unit lesion area) was found. In opposite, in our study, the higher content of α-chaconine was found might be responsible for resistance. One explanation for the differences in results obtained might be genetically determined glycoalkaloid concentrations in potato (Kozukue *et al.*, 2008). Beside differences in genetic background of tested materials the

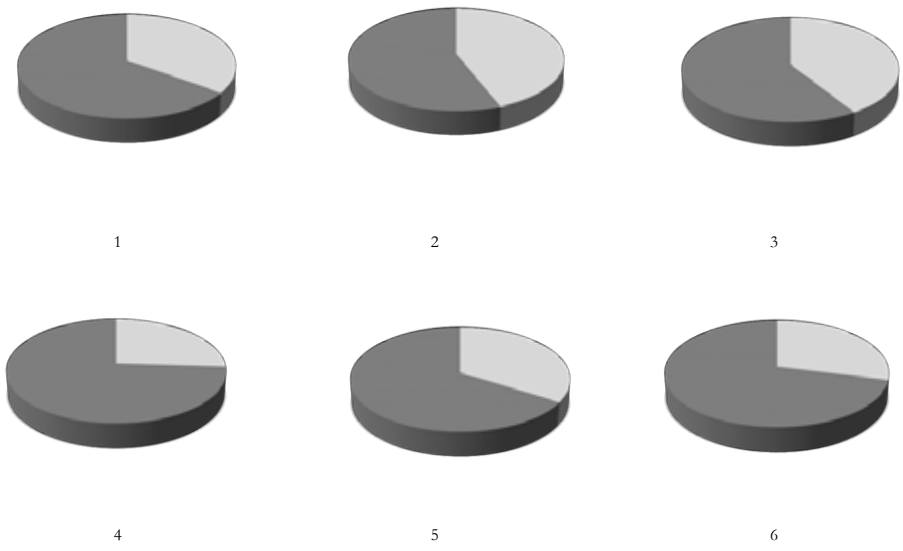

Figure 1. Ratio of α-solanine and α-chaconine in tubers from accessions originating from wild species and interspecific hybrids (1–3) and genotypes of *Tuberosum* typ (4–6). S=1 and 4, MR=2 and 5 HR=3 and 6. Dark grey = α-chaconine, light grey = α-solanine.

glycoalkaloid content may be strongly affected by environmental factors, particularly by light and limiting temperature regimes (Dao and Friedman, 1994; Bowles *et al.*, 2006).

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