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INTERSPECIFIC HYBRIDS OF POTATO: DETERMINATION OF GLYCOALKALOID AGLYCONES AND INFLUENCE OF BACTERIAL INFECTION

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ABSTRACT

Wild *Solanum* species contain various desirable characters, including disease resistance traits, which may not be present in cultivated potato (*S. tuberosum* L. ssp. *tuberosum*). Many of these traits of interest have already been successfully transferred into interspecific hybrids between potato and wild *Solanum* spp., and subsequently through backcrosses into cultivated potato varieties. However, quite often such wild *Solanum* species also contain glycoalkaloids additional to the solanine and chaconine of *S. tuberosum*. High levels of glycoalkaloids are considered undesirable for human consumption as they can be toxic. Glycoalkaloids may also act synergistically, mixtures of them being of higher toxicity than expected. In the present study, interspecific somatic hybrids between cultivated potato and wild *Solanum* spp. (*S. brevidens* Phil., *S. etuberosum* Lindl. and *S. acaule* Bitt.) in various genome constitutions were analysed in terms of their glycoalkaloid aglycone contents. In addition, progenies derived from sexual backcrosses and somatic fusions to *S. tuberosum* were also included in the study.

The somatic hybrids between A genome *S. tuberosum* and E genome *Solanum* species (*S. brevidens* and *S. etuberosum*) contained a novel glycoalkaloid aglycone in their foliage, demissidine, in addition to the parental type aglycones solanidine, which originated from *S. tuberosum*, and tomatidine derived from the E genome species. In the present study, a hypothetical pathway for demissidine production was proposed. This was based on the expression of the hydrogenase enzyme encoded by the E genome species to form tomatidine from a teinemine precursor, but in the interspecific hybrids it instead caused hydrogenation of the double bond of solanidine and production of demissidine. Using the improved method of analysis developed in this study, tomatidenol and assumed soladulcidine aglycones were also detected in the E genome *Solanum* species and in interspecific A + E genome hybrids. This technique was based on the combined derivatisation method and gas chromatographic-mass spectrometric (GC-MS) analyses. As a result of the combined derivatisation method, specific fragments (*m/z* 417 and 419) of spirosolanes were formed in abundance. This method is well suited for screening purposes, especially when minor or novel glycoalkaloid aglycones are detected.

In the A + E genome hybrids, the total foliage glycoalkaloid aglycone concentrations were either similar or lower than the mid-parent level. However, the genome constitutions of the hybrids had an effect on the proportions of different aglycones. In the *S. brevidens* + *S. tuberosum* somatic hybrids, the relative proportion of tomatidine correlated positively with the genome portion of *S. brevidens*. In contrast, when the second generation somatic hybrids were analysed, the higher genome portion of *S. tuberosum* enhanced the production of solanidine. A similar trend was observed in the plant material derived from *S. etuberosum* + *S. tuberosum* hybrids. This confirmed that it was possible to reduce the formation of alien types of glycoalkaloid aglycones in a few generations from the plant material produced by interspecific somatic hybridisation.

Solanidine, demissidine and tomatidine were the main aglycones in the somatic hybrids between two closely related A genome *Solanum* species (*S. tuberosum* and *S. acaule*) as for the A + E genome hybrids. Before the present study was started, *S. acaule* was known to carry immunity to bacterial ring rot caused by *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*) (Spieck. & Kotth.) Davis et al., which was a desired trait to be incorporated into cultivated potato in this study. However, in this study the interspecific A + A genome somatic hybrids

were susceptible to infections, and the *S. acaule* species did not to carry complete immunity, but instead temperature-dependent immunity to *Cms*. In the hybrids however, the genome portions influenced *Cms* replications. The effect of the genome ratios was also noted in the glycoalkaloid aglycone contents of the *S. acaule* + *S. tuberosum* hybrids. Furthermore, *Cms* infection affected the aglycone concentrations in the plant material. The aglycone levels significantly decreased in the somatic hybrids and in *S. acaule* as a result of *Cms* infection. However, in *S. tuberosum* there was either a significant increase or no change in the aglycone concentrations.

LIST OF ORIGINAL PUBLICATIONS

The thesis consists of the following papers, which are referred to by their Roman numerals in the text.

I **Laurila, J., Laakso, I., Valkonen, J.P.T., Hiltunen, R. & Pehu, E. 1996.** Formation of parental-type and novel glycoalkaloids in somatic hybrids between *Solanum brevidens* and *S. tuberosum*. Plant Science 118: 145-155.

II **Laurila, J., Laakso, I., Väänänen, T., Kuronen, P., Huopalahti, R. & Pehu, E. 1999**. Determination of solanidine- and tomatidine-type glycoalkaloid aglycons by gas chromatography/mass spectrometry. Journal of Agricultural and Food Chemistry 47, 7: 2738-2742.

III **Laurila, J., Laakso, I., Larkka, J., Gavrilenko, T., Rokka, V.-M. & Pehu, E. 2001**. The proportions of glycoalkaloid aglycones are dependent on the genome constitutions of interspecific hybrids between two *Solanum* species (*S. brevidens* and *S. tuberosum*). Plant Science 161: 677-683.

IV **Laurila, J., Metzler, M.C., Ishimaru, C.A. & Rokka, V.-M. 2003.** Infection of plant material derived from *Solanum acaule* with *Clavibacter michiganensis* ssp. *sepedonicus*: temperature as a determining factor in immunity of *S. acaule* to bacterial ring rot. Plant Pathology 52: 496-504.

V **Rokka, V.-M., Laurila, J., Tauriainen, A., Laakso, I., Larkka, J., Metzler, M., & Pietilä, L.** Somatic hybrids between two A-genome potato species (*Solanum acaule* and *S. tuberosum*) with different Endosperm Balance Numbers (EBNs) – Surveys of the glycoalkaloid aglycone accumulations. Manuscript (submitted).

In addition, some data concerning the glycoalkaloid aglycone analyses of the somatic hybrids between *S. etuberosum* and *S. tuberosum* and their backcross progenies (Thieme et al. 2003) have been included in this thesis.

AUTHOR'S CONTRIBUTION

The contribution of the author of this thesis to the publications I-V is presented. The following text has been adapted from the documents where each author's contribution to the above-mentioned publications was stated and signed.

In paper I, the author of this thesis and Docent Into Laakso contributed equally to the work. The author of this thesis participated in the development of the analysis method and carried out the experimental part of the work. In addition, the author of this thesis contributed to writing the manuscript. Docent Into Laakso and Professor Jari Valkonen were responsible for planning the work and writing the manuscript. In addition, Docent Into Laakso was the supervisor of the experimental part of the work. Professor Raimo Hiltunen offered his guidance in suggesting the hypothetical pathway for demissidine formation. Professor Eija Pehu provided plant material for the experiments and participated in editing the manuscript.

In paper II, the author of this thesis carried out most of experimental work, analysed part of the results and participated in writing the manuscript. Docent Into Laakso offered the major contribution in planning of the combined derivatisation method and wrote the detailed analytical parts of the manuscript. The NMR analyses were carried out Dr. Pirjo Kuronen and Ms. Tiina Väänänen. Professor Rainer Huopalahti arranged the high-resolution MS analyses in his laboratory. Professor Eija Pehu supervised the work and participated in editing the manuscript.

In paper III, the author of this thesis participated in planning the work, carried out the glycoalkaloid aglycone and statistical analyses and wrote the manuscript. Docent Into Laakso and Dr. V.-M. Rokka participated in experimental design of the work and contributed in writing the manuscript. In addition, Dr. V.-M. Rokka provided plant material for the experiments. Mr. Juha Larkka carried out GISH analyses of some hybrids under the supervision of Dr. Tatjana Gavrilenko and assisted in digital image capture. Dr. Tatjana Gavrilenko carried out the GISH analysis for most hybrid genotypes. Professor Eija Pehu participated in editing the manuscript.

In paper IV, the author of this thesis was responsible for planning the experiments, performing the experimental part of the work, analysis of the results and writing the manuscript under the supervision of Dr. Mary Metzler and Dr. V.-M. Rokka. In addition, Dr. Mary Metzler provided the bacterial strains and supervised the inoculation of plant material. Professor Carol Ishimaru provided the plant material (*S. acaule* 7-8) and participated in editing the manuscript.

In paper V, Dr. V.-M. Rokka designed the experiments, participated in identification and characterisation of the hybrids and wrote most of the manuscript. The author of this thesis participated in planning the glycoalkaloid aglycone work, made the glycoalkaloid aglycone and statistical analyses and participated in writing the manuscript. Docent Into Laakso supervised the glycoalkaloid aglycone analyses and contributed to editing the manuscript. Ms. Airi Tauriainen participated in somatic hybridisation, anther culture experiments and flow cytometric analyses. Mr. Juha Larkka carried out chromosome counting. Dr. Mary Metzler participated in editing the manuscript. Mrs. Leena Pietilä, potato breeder, carried out the sexual backcrosses and contributed to editing the manuscript.

LIST OF ABBREVIATIONS

acl	*Solanum acaule*
adg	*Solanum andigena*
a.m.u.	atom mass unit
BC	backcross
ber	*Solanum berthaultii*
brd	*Solanum brevidens*
BSTFA	N,O-bis(trimethylsilyl)-trifluoroacetamide
bw	body weight
chc	*Solanum chacoense*
Cms	*Clavibacter michiganensis* ssp. *sepedonicus*
CV	coefficient of variation
cv.	cultivar
dw	dry weight
EBN	endosperm balance number
etb	*Solanum etuberosum*
FITC	fluorescein-conjugated anti-mouse IgM
fw	fresh weight
GC-MS	gas chromatography-mass spectrometry
GISH	genomic *in situ* hybridisation
HCl	hydrochloric acid
HPLC	high-performance liquid chromatography
IFAS	indirect immunofluorescent antibody staining
IFU/g	immunofluorescing unit per gram
I.S.	internal standard
mcd	*Solanum microdontum*
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
m/z	mass-to-charge ratio
NMR	nuclear magnetic resonance
PFAA	pentafluoropropionic acid anhydride
phu	*Solanum phureja*
R10	strain of *C. michiganensis* ssp. *sepedonicus*
3RC	strain of *C. michiganensis* ssp. *sepedonicus*
SIM	selected ion monitoring
ssp.	subspecies
tbr	*Solanum tuberosum*
TIC	total ion chromatogram
TLC	thin layer chromatography
TMCS	trimethylchlorosilane
TMS	trimethylsilylation
YGM	yeast extract glucose medium

1 INTRODUCTION

1.1 Potato

The tetraploid (2n=4x=48) cultivated potato (*Solanum tuberosum* L. ssp. *tuberosum*) is the fourth most important crop species in the world. The global production of potato was 311 million tons in 2003, of which the European share was approximately 40% (FAO 2004a). However, China is the world's largest potato producer. Potato is not only useful as a low fat energy crop, but also as a source of protein and vitamin C. Potato consumption in developing countries has increased during recent years but it is still at a low level in comparison with consumption in Europe (94.2 kg/capita/year) in 2001 (FAO 2004b).

There are six cultivated potato species other than *S. tuberosum* (Hawkes 1990). The cultivated potatoes and the remaining 228 wild potato species are classified into 21 series of the section Petota in the genus *Solanum*, family Solanaceae (Hawkes 1990, 1994). Furthermore, the section Petota consists of two subsections: Estolonifera and Potatoe. The subsection Potatoe with tuber-bearing species consists of 19 series, but the Estolonifera subsection consists of only two series (Hawkes 1990, 1994). Species in the subsection Estolonifera do not form either tubers or stolons.

Most of the species in the subsection Potatoe are diploid (2n=2x=24) but species with other ploidy levels also occur (3x, 4x, 5x, 6x) (Hawkes 1990; Matsubayashi 1991). Potato species are proposed to consist of five different basic genome formulae: A, B, C, D, and E. Cultivated potato is an A genome species like most of the potato species (Matsubayashi 1991), but the genome symbols for the three *Solanum* species of the Etuberosa series – *S. etuberosum* Lindl., *S. brevidens* Phil., and *S. fernandezianum* Phil. – are E^1, E^2, and E^3 (Ramanna & Hermsen 1981) or E^e, E^b, and E^f (Matsubayashi 1991).

1.2 Interspecific somatic hybrids of potato

Potato breeding using conventional methods has limitations because the genetic base of cultivated potato is narrow and the combination of specific traits through crosses of the tetraploid cultivars is difficult. These limitations include heterozygosity of the cultivated tetraploid potato, complex segregation of the traits at the tetraploid level, pollen sterility, germplasm storage and transport, and the accumulation of viruses through vegetative propagation cycles (Howard 1978). As a result of these, breeding at the tetraploid (4x-4x) level is slow, because large populations of seedlings have to be screened (Ross 1986). Breeding at the diploid level (2x-2x) is a potentially useful method to shorten the time of needed to release new cultivars. To increase the genetic base of the cultivated potato, new germplasm by utilisation of wild *Solanum* species can be introduced. This is beneficial, because many wild species contain desirable characters, such as several disease and pest resistance traits, which are not present in cultivated potato (Hawkes 1990). However, due to the differences in the genome compositions and endosperm balance numbers (EBNs) (Johnston et al. 1980), many *Solanum* species are sexually incompatible with *S. tuberosum*. Interspecific crosses are successful when both parents have the same EBN, thus resulting in a 2:1 female:male EBN ratio in the hybrid endosperm (Johnston et al. 1980).

Somatic hybridisation. Protoplast fusion is an alternative technique to sexual crossing in potato breeding. Therefore, symmetric interspecific somatic hybrids produced by protoplast

fusion, in which the complete genomes (nuclear and cytoplasmic) are combined, provide increased genetic diversity for crop improvement (Helgeson 1989). In this way, it is possible to overcome breeding barriers (sexual and taxonomic incompatibility) between species and desirable characters of the wild species can more effectively be incorporated into cultivated potato germplasm. Another advantage of somatic hybridisation is that the dominant traits of both parental species are expressed in the hybrids (Wenzel et al. 1979). In addition, polygenic traits, such as high vigour, yield, fertility, and traits of unknown genetic basis can be transferred (Millam et al. 1995; Waara & Glimelius 1995; Pehu 1996; Waara 1996; Orczyk et al. 2003). Fish et al. (1987) wrote "Somatic hybridisation can potentially provide a greater diversity of combination than sexual hybridisation. Somatic hybrids may possess either parental chloroplast genome, as well as new mitochondrial genomes produced by recombination of the parental types. To this may be added a background of somaclonal variation, thus providing breeders with a range of novel hybrids for selection." Somaclonal variations are genetic changes that arise following cell culture and plant regeneration (rev. in Rokka 1998). There are several reasons for somaclonal variation, such as point mutations, doubling of the ploidy level, changes in chromosome number, transposon insertion, and translocations, but most of them may be negative for application of the methods. In addition, incompatibility barriers between *Solanum* species can also be overcome and successful crosses produced by 2n gametes coupled with a knowledge of the EBNs of the species used (Ehlenfeldt & Hanneman 1984; Camadro & Espinillo 1990), combining compatible second pollination and embryo rescue techniques (Iwanaga et al. 1991; Watanabe et al. 1995), or treatmenting sterile hybrids with colchicine (Ross 1986).

Somatic hybrids. Various interspecific symmetric somatic hybrids have been produced between *S. tuberosum* and several partly or completely sexually incompatible wild *Solanum* species (see Table 1). In addition, asymmetric interspecific somatic hybrids between various *Solanum* species have also been produced (Sidorof et al. 1987; Fehér et al. 1992; Xu et al. 1993c; Oberwalder et al. 1997, 2000) in order to eliminate certain parts of the donor species genome by irradiation and to produce hybrids with variant genetic backgrounds. The asymmetric hybrids formed contained a complete genome of the recipient and only a limited part of the donor genome.

General requirements for the somatic hybrids are fertility and ability to cross sexually (Ehlenfeldt & Helgeson 1987; Waara & Glimelius 1995). The general objective is to incorporate through backcrosses the desirable traits of the hybrids into cultivated potato but to remove the undesirable wild traits. However, potato breeding using somatic hybridisation may cause genetic instability and low fertility in the hybrids (Barsby et al. 1984; Mattheij et al. 1992; Austin et al. 1993; Cardi et al. 1993a; Novy & Helgeson 1994b). Difficulties in the segregation of the fusion partner genomes may result in elimination of chromosome(s) especially when taxonomically distant species are hybridised. Thus, aneuploid interspecific somatic hybrids are common (Puite et al. 1986; Pijnacker et al. 1987, 1989; Fish et al. 1988b; Pehu et al. 1989; Ramulu et al. 1989; Preiszner et al. 1991; Kim et al. 1993; Ward et al. 1994; Masuelli et al. 1995; Yamada et al. 1997, 1998b; Dong et al. 1999). Also other cytogenetic alterations, including structural rearrangements of chromosomes (deletions, duplications, translocations) are possible (Pijnacker et al. 1987, 1989; Williams et al. 1990; Yamada et al. 1998b). Genetic stability was higher in the tetraploid somatic hybrids between *S. brevidens* and *S. tuberosum* than in the hexaploid hybrids, but hexaploid somatic hybrids were, however, easier to backcross with *S. tuberosum* (Ehlenfeldt & Helgeson 1987).

Table 1. *Examples of various somatic hybrids of* Solanum *expressing traits of interest (according to Orczyk et al. 2003, modified).*

Somatic hybrids	**Traits transferred to somatic hybrids**	**Ref.**
*tbr** + *S. acaule*	Transfer of resistance to *Potato virus X* (PVX)	Yamada et al. (1997)
tbr + *S. brevidens*	Transfer of resistance to *Potato leaf roll virus* (PLRV)	Austin et al. (1985)
	Increased resistance to late blight *Phytophthora infestans*	Austin et al. (1986)
	Increased tuber resistance to bacterial soft rot *Erwinia* sp.	Austin et al. (1988)
	Transfer of resistance to PLRV and *Potato virus Y* (PVY)	Gibson et al. (1988)
	Increased resistance to *Erwinia carotovora* ssp. *carotovora*	Polgar et al. (1999) Rokka et al. (2000)
	Transfer of resistance against *Erwinia* and *P. infestans* to sexual progeny of somatic hybrids	Helgeson et al. (1993)
	Somatic hybrids combined two, different mechanisms of virus (PVY, PVA, PVX) resistance: hypersensitive response (from *S. tuberosum*) and functional through slow virus cell-to-cell movement (from *S. brevidens*)	Valkonen & Rokka (1998)
	Introgression and stabilisation of resistance to *Erwinia*	McGrath et al. (2002)
tbr x *S. berthaultii* + *S. etuberosum*	Higher resistance to PVY	Novy & Helgeson (1994a)
tbr + *S. bulbocastanum*	Transfer of resistance to nematode *Meloidogyne chitwoodi*	Austin et al. (1993)
tbr + *S. commersonii*	Somatic hybrids with cold-hardy species *S. commersonii* and *S. brevidens*. Transfer of freezing tolerance from *S. commersonii* to somatic hybrids	Cardi et al. (1993a) Chen et al. (1999) Bastia et al. (2000) Seppänen et al. (2000)
tbr + *S. commersonii*	Increased frost tolerance and higher capacity to cold acclimation	Nyman & Waara (1997)
tbr + *S. phureja*	Transfer of resistance to bacterial wilt *Ralstonia solanacearum*	Fock et al. (2000)
tbr + *S. commersonii*	Transfer of resistance to *R. solanacearum*	Laferriere et al. (1999)
tbr + *S. stenotomum*	Transfer of resistance to *R. solanacearum*	Fock et al. (2001)
tbr + *S. pinnatisectum* or *S. pinnatisectum* x *S. bulbocastanum*	Transfer of resistance to *P. infestans*	Thieme et al. (1997)
tbr + *S. bulbocastanum*	Transfer of resistance to *P. infestans*	Helgeson et al. (1998)
tbr + *S. verrucosum*	Transfer of resistance to PLRV	Carrasco et al. (2000)
two dihaploid clones of *tbr*	Transfer of resistance for potato cyst nematode *Globodera pallida*	Cooper-Bland et al. (1994)
	Combined resistances to different pathotypes of *G. pallida*	Rasmussen et al. (1996)
	Combined foliage and tuber resistance to *P. infestans*	Rasmussen et al. (1998)
	Higher production of tubers and higher tuber yield	Mattheij & Puite (1992) Gavrilenko et al. (1999)
	Combined from both lines tuber yield and starch content	Möllers et al. (1994)
tbr + *tbr* (*stoloniferum*)	Cytoplasmic male sterility of cybrids between *S. tuberosum* and alloplasmic *S. tuberosum* (*stoloniferum*)	Perl et al. (1990)

**tbr = S. tuberosum*

If fertility of the somatic hybrids generated is low, there are alternative ways to overcome it. Rokka (1998) presented an alternative scheme for sexual backcrosses based on an analytic-synthetic breeding scheme of Wenzel et al. (1979), who had proposed the utilisation of dihaploids in potato breeding through somatic hybridisation. After the first somatic hybridisation between *S. tuberosum* and *S. brevidens* (Rokka et al. 1994), a selected anther-derived (somato)haploid plant (Rokka et al. 1995) was re-fused with *S. tuberosum* to produce "second generation" somatic hybrids (Rokka et al. 2000). The purpose of the production of these second generation somatic hybrids was to reduce ploidy and the proportion of the wild species genome, while the desired traits were effectively incorporated into cultivated potato.

1.2.1 Genetic analyses of genome compositions of the somatic hybrids

There are several molecular and cytological methods for analysing genome compositions of somatic hybrids (meaning hybrid nature, chromosome elimination and rearrangement, intergenomic introgression). Identification and characterisation of chromosome complements in interspecific and intergeneric somatic hybrids of potato and their progenies can be carried out using species-specific repetitive DNA sequences (Schweizer et al. 1988; Pehu et al. 1990b; Polgár et al. 1993; Stadler et al. 1995; Zanke & Hemleben 1997), RFLP (restriction fragment length polymorphism) markers (Williams et al. 1990, 1993; McGrath et al. 1994; Novy & Helgeson 1994b; Menke et al. 1996; Garriga-Calderé et al. 1997; Oberwalder et al. 1997, 2000; Yamada et al. 1998b), RAPD (randomly amplified polymorphic DNA) markers (Baird et al. 1992; Kim et al. 1993; Xu et al. 1993a; Rokka et al. 1994; Masuelli et al. 1995; Yamada et al. 1997; Polgar et al. 1999), slot-blot hybridisation (Matthews et al. 1997), inter-SSR PCR (inter-simple-sequence repeat polymerase chain reaction) markers (Matthews et al. 1999), GISH (genomic *in situ* hybridisation) (Wolters et al. 1994; Jacobsen et al. 1995; Ramulu et al. 1996; Garriga-Calderé et al. 1997, 1999; Dong et al. 1999; Gavrilenko et al. 2001; Horsman et al. 2001), FISH (fluorescence *in situ* hybridisation) (Rokka et al. 1998a), and with sequential GISH and FISH analyses (Dong et al. 2001).

RAPD markers are commonly used in verification of the putative somatic hybrids. RAPDs are PCR-based markers, mostly dominant, produced by short oligonucleotide primers of arbitrary nucleotide sequence. The method is simple because it demands only a small amount of DNA and no radioactivity is used. Reproducibility, however, is a weakness of the RAPD markers but more reproducible assays have been achieved by converting RAPD markers to SCAR (sequence characterised amplified region) markers (Paran & Michelmore 1993; Masuelli et al. 1995). Inter-SSR PCR has been reported to be a more reliable PCR-based method than RAPD analysis due to the co-dominant nature of the markers and higher level of polymorphisms (Zietkiewicz et al. 1994; Matthews et al. 1999). With RFLP markers even fine chromosomal regions of the hybrids can be detected, but many RFLP markers are needed when an entire chromosome (with different chromosomal regions) is analysed completely. In addition, RFLP requires large amounts of DNA and is relatively labour intensive. With the cytological GISH technique, total genomic DNA is used in chromosome analyses to differentiate and distinguish between chromosomes from different species. It can identify alien chromatins in translocated chromosomes (Jiang & Gill 1994), but it may not detect small deletions of chromosomes. FISH is a chromosome-labelling technique based on the use of fluorochromes for signal detection of repetitive DNA sequences (Jiang & Gill 1994). A combination of the RFLP technique with GISH analysis has been shown to be an excellent characterisation method for interspecific and intergeneric hybrids and their progenies (Jacobsen et al. 1995; Garriga-Calderé et al. 1997; Dong et al. 1999).

1.2.2 Genetic enhancement of potato using wild germplasm

Wild *Solanum* species contain several desirable traits – such as resistance to diseases, insects, and tolerance to abiotic stresses – which may not be present in cultivated potato (Hawkes 1990). One of the most intensively studied wild *Solanum* species, *S. brevidens* (*S. palustre* Poepp.), is extremely resistant to *Potato leaf roll virus* (PLRV) (Jones 1979; Valkonen et al. 1992), *Potato virus Y* (PVY) and *A* (PVA) (Gibson et al. 1988; Valkonen et al. 1992), moderately resistant to *Potato viruses X* (PVX), *M* (PVM) and *S* (PVS) (Gibson et al. 1990; Valkonen et al. 1992) as well as resistant to *Andean potato mottle virus* (APMV) (Valkonen et al. 1992). Freezing tolerance of *S. brevidens* has also been reported (Ross & Rowe 1969). *Solanum etuberosum* is another species resistant to the viruses mentioned above except *M* and *S* viruses (Valkonen et al. 1992; Thieme & Thieme 1998). Furthermore, resistance to *Aphis frangulae* Kaltenbach, *Aulacorthum solani* Kaltenbach, *Myzus nicotianae* Blackman, and *M. persicae* Sulzer has also been found in *S. etuberosum* (Thieme & Thieme 1998; Novy et al. 2002). *Solanum acaule* Bitt., an A genome wild potato species, is resistant to PLRV, PVX, PVY, and *Spindle tuber viroid* (Bamberg et al. 1994; Hawkes 1994). It is also partially resistant to silver scurf (*Helminthosporium solani* Durieu & Mont.) (Rodriquez et al. 1995), powdery scab (*Spongospora subterranean* Wallr. Lagerh.) (Mäkäräinen et al. 1994), wart (*Synchytrium endobioticum* Schilb.) and nematodes (*Globodera* spp.) (Hawkes 1990), and has shown immunity to bacterial ring rot caused by *Clavibacter michiganensis* ssp. *sepedonicus* (Spieck. & Kotth.) Davis et al. (Ishimaru et al. 1994; Kriel et al. 1995a,b). In addition it is freezing tolerant (Chen & Li 1980) and tolerates various other abiotic stresses well, including heat and drought (Hawkes 1990). Several other species containing desirable resistance characters, but not mentioned here, were reviewed by Hawkes (1990, 1994) and Flanders et al. (1992).

Bacterial ring rot of potato. Bacterial ring rot of potato, which was studied in the present work, is caused by *C. michiganensis* ssp. s*epedonicus* (*Cms*). It is a quarantine disease, causing symptoms of rotting vascular tissue in tubers. Typical foliage symptoms consist of wilting, chlorosis, and necrosis (De Boer & Slack 1984). Some cultivars also exhibit stunting. Infected tubers and contaminated potato equipment spread bacterial ring rot. Elimination of this disease is particularly difficult due to latent infections (De Boer & McNaughton 1986; Franc 1999). In addition, different growth conditions affect *Cms* infection and variable expression of disease symptoms complicate eradication of the disease. As bacterial ring rot is a quarantine disease, legislative control requires absence of the bacterium, not just of the disease symptoms. Resistant or tolerant cultivars will not fulfill the demand of zero-tolerance but may increase the problem of spread through symptomless or latent infections. Only immune potato cultivars would both prevent *Cms* infections and spread of the disease. A range of wild *Solanum* species has been screened for potential immunity to infection by *Cms* (Kurowski & Manzer 1992; Kriel et al. 1995a). One wild potato species, *Solanum acaule* ssp. *acaule*, was found to be immune to the bacterium (Ishimaru et al. 1994; Kriel et al. 1995a,b).

Expression of resistance characters in hybrids. Many traits of interest have been successfully transferred from wild *Solanum* species to cultivated potato through somatic hybridisation (Table 1), e.g. the virus resistance traits of *S. brevidens* (PLRV, PVY, and PVX) (Austin et al. 1985; Helgeson et al. 1986; Gibson et al. 1988; Pehu et al. 1990a, Rokka et al. 1994). Interestingly, resistance to *Erwinia* bacterial soft rot was also examined in tubers of the *S. tuberosum* + *S. brevidens* somatic hybrids, but the trait subsequently segregated in the backcross progeny produced (Austin et al. 1988; Helgeson et al. 1993; McGrath et al. 2002). As *S. brevidens* is a non-tuber bearing species, the origin of the soft rot resistance was

difficult to trace. Austin et al. (1988) concluded that there was a genetic factor with an effect on the segregation of the trait rather than a physiological or a nutritional explanation. Based on the backcross population analysed, McGrath et al. (2002) strongly assumed that *S. brevidens* was the source of *Erwinia* resistance. The resistance, when derived from *S. brevidens*, seemed, however, to be oligogenic. This example suggested that even novel traits not previously expressed in the parental species might show up in the resulting hybrids produced.

1.3 Glycoalkaloids

Steroidal glycoalkaloids are naturally occurring secondary metabolites of plants. The term "secondary metabolites" indicates compounds that are not required for plant growth and development but presumed to function in communication or defence (Luckner 1990). Steroidal glycoalkaloids derived from a cholestane skeleton are mainly found in the Solanaceae family and therefore termed *Solanum* glycoalkaloids. In addition to the Solanaceae, glycoalkaloids are also found in the Liliaceae family but then they are called *Veratrum* alkaloids. Besides potato, there are also other plants in the Solanaceae that contain glycoalkaloids: e.g. tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melongena* L.). Much effort has been invested in glycoalkaloid research since 1820 when the alkaloid solanine was discovered. Another milestone was reached in 1954 when solanine was shown to be a mixture of two glycoalkaloids, α-solanine and α-chaconine (Kuhn et al. 1955a,b; see also Jadhav et al. 1981).

1.3.1 Structure

Alkaloids are a large group of secondary metabolites produced by plants. A common feature of the structure of various alkaloids is that they contain nitrogen in a heterocyclic ring and that the entire skeleton of an amino acid may be incorporated into an alkaloid. Steroidal glycoalkaloids (cholestane C_{27}-derivatives) belong to a subgroup of pseudoalkaloids (or isoprenoid alkaloids) because nitrogen is inserted into a non-amino acid residue (Goodwin & Mercer 1988). According to various classifications, steroidal glycoalkaloids can also be grouped as basic steroid saponins or saponins (Mahato et al. 1982; Hostettmann & Marston 1995) or as steroids (Heftmann 1983). The structure of steroid/steroidal saponins corresponds with glycoalkaloids. The only difference between them is an oxygen atom in an F-ring of sapogenin. However, steroidal saponins do not contain nitrogen and are thus not alkaloids. Steroidal sapogenins have been isolated from many *Solanum* species (Schreiber 1968; Heftmann 1983).

Potato glycoalkaloids are glycosides, which consist of two structural components – a hydrophobic C_{27}-carbon skeleton of cholestane (aglycone) and a hydrophilic carbohydrate side chain. Six-ring steroid aglycones are also referred to as alkamines and are structurally divided into five different groups: the solanidanes, e.g. solanidine; the spirosolanes, e.g. tomatidine; the epiminocholestanes, e.g. solacongestidine; the alkaloids with a solanocapsine skeleton, e.g. solanocapsine; and the 3-aminospirostanes, e.g. jurubidine (Schreiber 1968; Ripperger & Schreiber 1981). At least 90 structurally different steroidal alkaloids have been found in over 350 *Solanum* species (Friedman & McDonald 1997), but the major solanidane and spirosolane aglycones found in potatoes are solanidine, demissidine, solasodine, tomatidenol, and tomatidine (Figure 1).

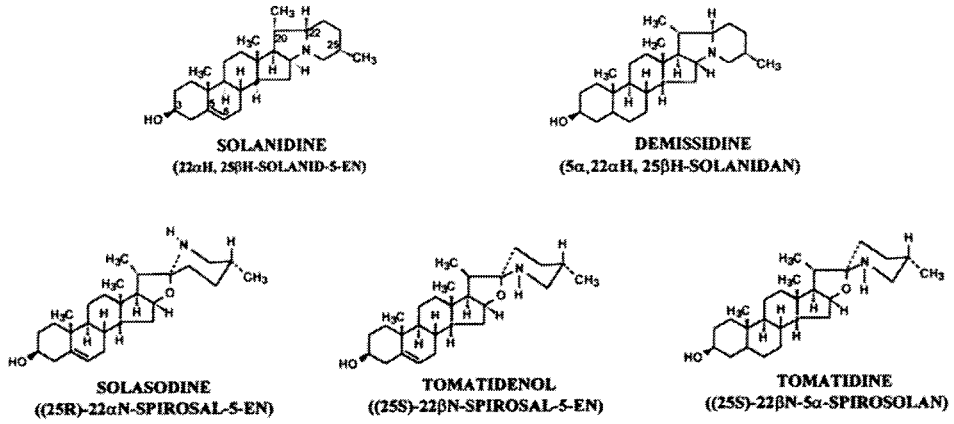

Figure 1. *Structures of the most common glycoalkaloid aglycones found in* Solanum *species (according to Friedman & McDonald 1997).*

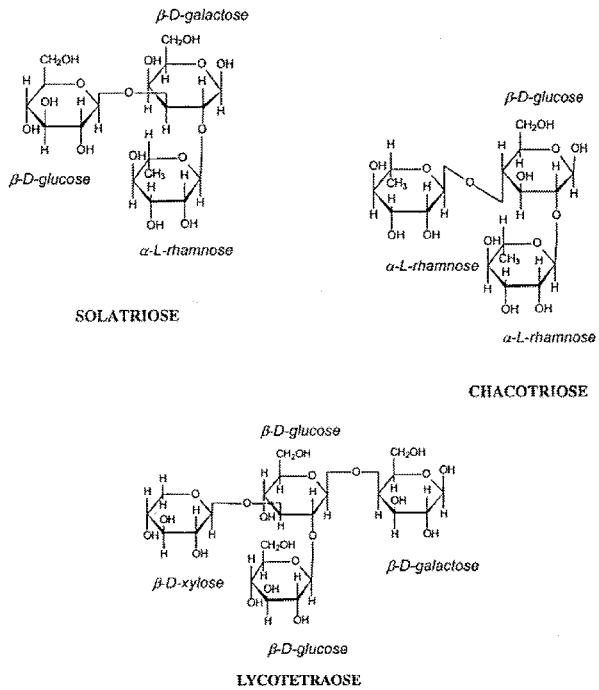

Figure 2. *Structures of three common sugar side-chains found in* Solanum *species (according to Friedman & McDonald 1999). Sugar side-chains of solatriose and lycotetraose are attached to aglycone through the OH-group of C-1 of β-D-galactose and chacotriose through OH-group of C-1 of β-D-glucose.*

The steroid alkaloids generally exist as glycosides. The carbohydrate moiety of the glycoalkaloid is attached to the 3-hydroxy group of the first ring of aglycone. Tri- and tetrasaccharides are the most common carbohydrate parts of the glycoalkaloids (Figure 2). Mono-, di-, and trisaccharides (β, γ, and δ glycosides) have also been found but they are mostly hydrolysis products of α-glycosides.

The most common individual sugars of carbohydrates are D-glucose, D-galactose, D-xylose, and L-rhamnose (Schreiber 1968). β-Solatriose consisted of D-glucose, D-galactose, and L-rhamnose is a carbohydrate moiety of α-solanine. Correspondingly, α-chaconine is formed from β-chacotriose, while the sugar moiety of tomatidine and demissidine is β-lycotetraose. The most common glycoalkaloids are presented in Table 2. Their chemistry and structure have been extensively reviewed during recent decades (Schreiber 1968; Ripperger & Schreiber 1981; van Gelder 1991; Maga 1994; Friedman & McDonald 1997, 1999).

Table 2. *The most common glycoalkaloids found in* Solanum *species (according to van Gelder 1991). The systematic names of glycoalkaloids are presented in the appendix on page 81.*

SGA[a]	Sugar Moiety	Glycoside Structure[b]	
Solanidine glycosides			
α-Solanine	Solatriose	A: R-Gal <	Rham Glu
β-Solanine[c]	Solabiose	B: R-Gal-Glu	
γ-Solanine[c]	Galactose	C: R-Gal	
α-Chaconine	Chacotriose	D: R-Glu <	Rham Rham
β_1-Chaconine[c]	Chacobiose	E: R-Glu-Rham	
β_2-Chaconine[c]	Chacobiose	F: R-Glu-Rham	
γ-Chaconine[c]	Glucose	G: R-Glu	
Dehydrocommersonine	Commertetraose	H: R-Gal-Glu <	Glu Glu
Demissidine glycosides			
Demissine	Lycotetraose	I: R-Gal-Glu <	Glu Xyl
Commersonine	Commertetraose	As H	
Leptinidine glycosides			
Leptinine I	Chacotriose	As D	
Leptinine II	Solatriose	As A	
Acetylleptinide glycosides			
Leptine I	Chacotriose	As D	
Leptine II	Solatriose	As A	
Tomatidenol glycosides			
α-Solamarine	Solatriose	As A	
β-Solamarine	Chacotriose	As D	
Solasodine glycosides			
Solasonine	Solatriose	As A	
Solamargine	Chacotriose	As D	
Tomatidine glycosides			
α-Tomatine	Lycotetraose	As I	
Sisunine (neotomatine)	Commertetraose	As H	

[a] Aglycone
[b] R=aglycone; Gal=β-D-galactose; Rham=α-L-rhamnose; Glu=β-D-glucose; Xyl=β-D-xylose
[c] Minor SGAs may be artefacts or metabolites

1.3.2 Biosynthesis and degradation

Biosynthesis. The biosynthesis of the glycoalkaloids has been extensively studied, but the pathway from cholesterol to glycoalkaloids has yet to be completely elucidated. Glycoalkaloid synthesis commences during germination (Heftmann 1983). Based on the studies in tomato, microsomal organelles of cells are the probable biosynthetic sites of glycoalkaloids (Roddick 1976). Other sites (such as plastids) have also been suggested (rev. in Bergenstråle et al. 1992). After synthesis, accumulation of glycoalkaloids occurs in the soluble phase of the cytoplasm and/or in the vacuoles (Roddick 1976, 1977). Glycoalkaloids are not transported from one part of the plant into another (Roddick 1982; Heftmann 1983), and thus tend to remain at the site of synthesis.

Biosynthesis of glycoalkaloids was reviewed by Heftmann (1983), Petersen et al. (1993), Bergenstråle (1995), and Valkonen et al. (1996). A starting point of glycoalkaloid synthesis is considered to be the general pathway of steroid biosynthesis. The pathway begins from a reaction of acetate with acetyl-coenzyme A and then follows through the intermediates of mevalonic acid, squalene, lanosterol, and cycloartenol to cholesterol. Very recently it was suggested that cholesterol acts as a precursor in glycoalkaloid biosynthesis (Arnqvist et al. 2003). Two possible pathways for glycoalkaloid synthesis from cholesterol have been proposed: Petersen et al. (1993) postulated that solasodine and soladulcidine are formed from cholesterol, while tomatidenol, tomatidine, solanidine, and demissidine are formed from saturated cholesteranol (Figure 3). In addition, tomatidenol and solanidine are more alike than could be expected from their chemical structures. In contrast, solasodine and tomatidine, which are alike in their chemical structure, are biosynthetically different. Friedman and McDonald (1997) further concluded, according to the presumptions of Petersen et al. (1993), that aglycones with double bonds were formed first and subsequently saturated aglycones. Another pathway for glycoalkaloid formation was proposed by Kaneko et al. (1972, 1976, 1977a,b, 1978) after studies carried out with *Veratrum grandiflorum* O. Loes.. They presented, for example, the biosynthetic steps for solanidine formation (Figure 4) and suggested that L-arginine was most likely to be the nitrogen source for solanidine (Kaneko et al. 1976). Also glycine and alanine were proposed to be the donor molecules for nitrogen synthesis (Jadhav et al. 1973).

The next step in biosynthesis after aglycone formation is glycosylation. Many studies have shown that, after their formation, aglycones are rapidly enzymatically glycosylated by sugar to the α-form of glycoalkaloids (Liljegren 1971; Jadhav & Salunke 1973; Jadhav et al. 1973; Lavintman et al. 1977; Osman et al. 1980). Therefore, free solanidine does not occur in healthy potato tuber tissue (Osman et al. 1980). The enzymes involved in the glycosylation processes have been described lately (e.g. Stapleton et al. 1991; Bergenstråle et al. 1992).

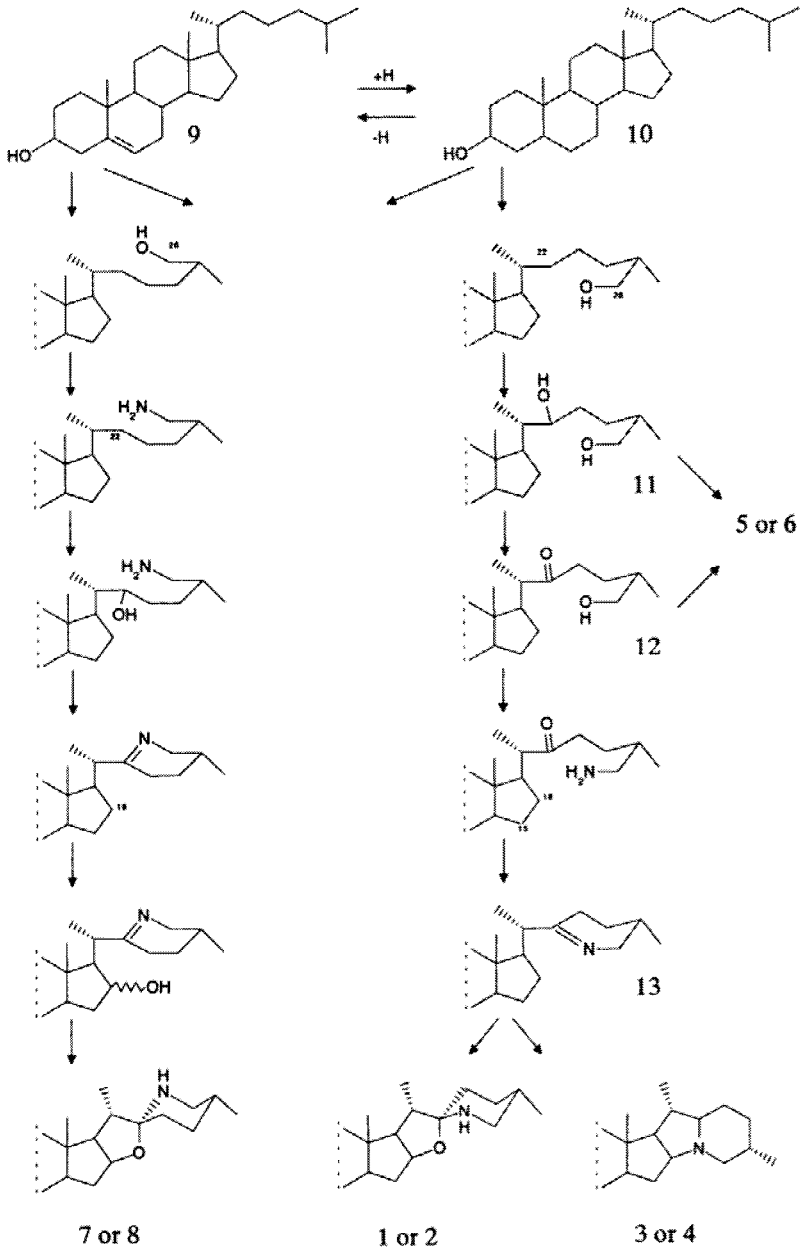

Figure 3. *Proposed biosynthetic pathways from cholesterol (9) and from saturated cholesteranol (10) for glycoalkaloid aglycones; 1 tomatidenol, 2 tomatidine, 3 solanidine, 4 demissidine, 5 diosgenin, 6 tigogenin, 7 solasodine, 8 soladulcidine (according to Petersen et al. 1993).*

Plant transformation offers possibilities for the manipulation of glycoalkaloid metabolism in transgenic potato plants. Moehns et al. (1997) demonstrated that by cloning of solanidine glucosyltransferase (SGT), an enzyme that catalyses the glucosylation of solanidine to α-chaconine, glycoalkaloid metabolism can be manipulated. Later on it was shown that the antisense SGT gene inhibited the biosynthesis of tuber glycoalkaloids (Joyce et al. 1999; McCue et al. 2003). Overexpression of a *SMT1* cDNA (a soybean type 1 sterol methyltransferase) can be used to down-regulate glycoalkaloid levels in potato (Arnqvist et al. 2003). In addition, DNA-based studies concerning the identification of molecular markers associated with glycoalkaloid production (Yencho et al. 1998, 2000; Ronning et al. 1999) are valuable prerequisites to the cloning of genes associated with glycoalkaloid biosynthesis. Modification of glycoalkaloid levels may generate new ways to use potatoes, such as using potato pulp wastes from the starch industry as animal fodder, and may also increase the genetic pool available for breeding programmes, such as insect and disease resistance (Yencho et al. 2000; Arnqvist et al. 2003). However, detailed testing of the genetically modified potatoes is needed to evaluate changes in other characters.

Degradation. Enzymatic degradation of glycoalkaloids occurs by stepwise cleaving of sugar(s) from carbohydrate moieties (Schreiber 1968; Swain et al. 1978; Heftmann 1983; Bushway et al. 1988, 1990). Degradative enzymes and substrates are located in different compartments within cells, thus, enzymes are activated after tissues are disrupted. In tomato, enzymatic degradation of glycoalkaloids occurs when tomato ripens (rev. in Friedman 2002). The aglycone metabolism of glycoalkaloids was described by Heftmann and Schwimmer (1972).

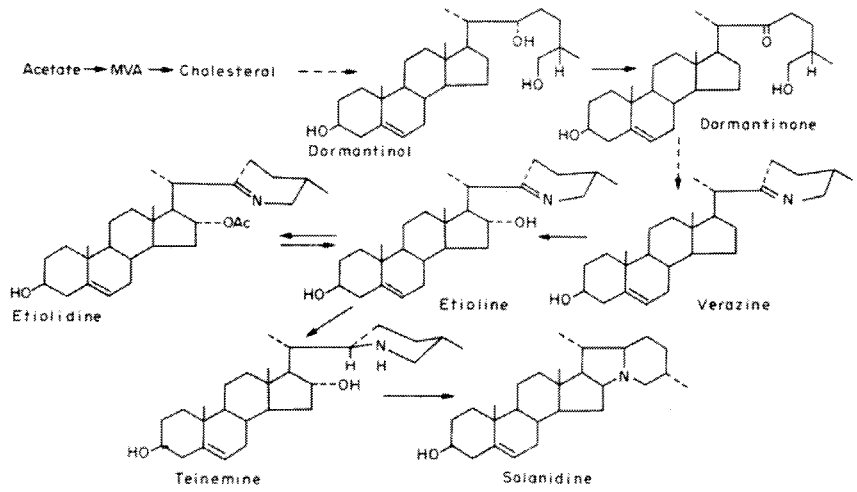

Figure 4. *Proposed biosynthetic pathway for solanidine (according to Kaneko et al. 1976).*

1.3.3 Toxicity

Glycoalkaloids are toxic compounds. An official safety or acceptable limit of total glycoalkaloid content for human consumption in tubers is 200 mg/kg fresh weight (fw) (1000 mg/kg dry weight, dw) or 1 mg/kg body weight (bw). Glycoalkaloid contents below this guideline are not thought to represent health risks for humans. However, lower acceptable levels (60–70 or 100 mg/kg fw) (Ross et al. 1978; Vorne & Hallikainen 2003) for potato varieties have been recommended because of the variation in glycoalkaloid concentrations between years and growth locations. According to estimates based on poisoning cases reported, a toxic dose of glycoalkaloids in human consumption can vary between 2–5 mg/kg bw and a lethal dose about 3–6 mg/kg bw (Morris & Lee 1984) or it can be even as low as from 1 to 2 mg/kg bw (rev. in Friedman & McDonald 1997). Symptoms of poisoning caused by glycoalkaloids have been reported to be gastrointestinal, causing vomiting, diarrhoea, abdominal pain, neurological resulting in restlessness, confusion, delirium, stuporose, drowsiness, hallucination, and others such as nausea, malaise and skin lesions (rev. in van Gelder 1991). However, it is unlikely that humans would eat potatoes containing high, fatal glycoalkaloid doses because glycoalkaloid concentrations above 140 mg/kg are associated with a bitter taste and other unpleasant flavour characteristics (Sinden et al. 1976). However, not all the glycoalkaloids cause bitterness (Ross et al. 1978) and thus flavour is not a reliable indicator (van Gelder 1991) and there is variation in human perception of bitterness. However, low glycoalkaloid levels in tubers improve the flavour of potatoes (Morris & Lee 1984). It is worth noting that glycoalkaloids are stable and are not destroyed by cooking, except during frying where a minor reduction in glycoalkaloid levels has been reported (Bushway & Ponnampalam 1981). Glycoalkaloids are toxic compounds for all mammals. However, animals are generally less susceptible to glycoalkaloids than humans. The effects of glycoalkaloids on animals have been reviewed by Morris and Lee (1984) and Friedman and McDonald (1997).

The major toxic properties of glycoalkaloids are due to i) the ability of glycoalkaloids to bind with membrane 3β-hydroxy sterols and to disrupt membrane function and ii) the ability to inhibit acetylcholinesterase. The modes of toxicity and toxicity studies of glycoalkaloids in humans and animals have recently been reviewed by van Gelder (1991), Roddick (1996), and Friedman and McDonald (1997, 1999). However, it has even been suggested that “the toxicity of glycoalkaloids has largely been overestimated and their dangers are probably not as high as history would imply” (Hostettmann & Marston 1995). Briefly, chacotriose-based glycoalkaloids are highly active whereas solatriose-based glycoalkaloids have exhibited lower or no activity with regard to membrane-disruptive activity. Orally consumed tomatine (in future in the present study, tomatine indicates α-tomatine, solanine and chaconine indicate α-solanine and α-chaconine) was shown to be non-toxic in studies with hamsters and it was even suggested to have some health promoting properties for humans (Friedman et al. 2000a,b; Friedman 2002; Kozukue & Friedman 2003) because of the ability of tomatine to form an insoluble complex with cholesterol in the digestive tract which is then eliminated in the faeces. This type of insoluble complex, for example with cholesterol, was reported earlier (rev. in Jadhav et al. 1981). The features of the carbohydrate side-chain of the glycoalkaloid, as well as the type of sterol, are thought to be important for the mechanism of cell disruption.

Both solanine and chaconine are strong inhibitors of acetylcholinesterase activity. Also demissine and commersonine are more inhibitory than tomatine (Bushway et al. 1987). The aglycone part of glycoalkaloid may be more important during the inhibition of acetylcholinesterase, but the importance of the carbohydrate moiety cannot be excluded. In

producing toxic effects, glycoalkaloids may act synergistically, i.e. a mixture of glycoalkaloids has greater/different toxicity than could be expected from their individual effects. Friedman and McDonald (1997) have concluded that teratogenic effects of glycoalkaloids, i.e. non-heriditable birth defects in offspring, are still an open question. However, factors modifying the teratogenic effects have been recently reported: the nitrogen of the steroid is required for teratogenicity (Friedman et al. 1992), and differences in embryotoxity between two glycoalkaloids with the same aglycone arise from the different structures of the carbohydrate side-chains (Rayburn et al. 1994; Blankemeyer et al. 1998). As Friedman et al. (1992) discussed, "an unanswered question is whether the glycoalkaloids would induce teratogenicity in pregnant mammals when, as part of a normal diet, they are subject to an interaction with dietary nutrients and non-nutrients, digestion, absorption, transport, metabolism, and elimination." To be concluded, different glycoalkaloids and aglycones possess various, specific biological activities and toxicity depends on their structural characteristics.

1.3.4 Distribution of glycoalkaloids in potato parts

The major glycoalkaloids of cultivated potato are solanine and chaconine (aglycone solanidine), but α- and β-solamarine (tomatidenol) have also been found in some potato cultivars (Shih & Kuć 1974; Sinden & Sanford 1981). The ratio of solanine to chaconine is on average 40:60 in potato but this is not consistent, thus other ratios have also been determined (Sinden & Webb 1974; Morris & Petermann 1985; Friedman & Dao 1992; Percival et al. 1993, 1994; Percival 1999a). Continuous illumination was assumed to be one of the reasons for the varying ratios. *Solanum* species other than *S. tuberosum* also contain glycoalkaloids additional to solanine and chaconine (see paragraph 1.5.).

Glycoalkaloids occur in all parts of potato, the concentrations varying widely, as seen in Table 3. The highest glycoalkaloid levels were found in plant parts with high metabolic activity, e.g. young leaves, flowers, fruits, and sprouts. Kozukue et al. (1987) reported that the upper leaves of potato plants contained higher total glycoalkaloid concentrations (as a fresh weight) than the lower, older leaves. Glycoalkaloid content in brown, senescent leaves of tomato was higher than in the fresh leaves. However, when the amounts were expressed in the dry weights the concentrations were similar (Friedman & Levin 1998). Since most of the glycoalkaloids exist in the peel of tubers (Bushway et al. 1983; Uppal 1987; Kozukue et al. 1987; Mondy & Gosselin 1988; Wünsch 1989; Peksa et al. 2002) they can be removed to a large extent by peeling. However, if tubers normally or as a result of a stress contain high amounts of glycoalkaloids, they can only be partly removed (Petersen 1993; Hellenäs et al. 1995b). Tubers of early potato varieties may contain high levels of glycoalkaloids (Hellenäs et al. 1995a). The glycoalkaloid concentrations in tubers and foliage were reported to correlate positively (Deahl et al. 1973; Uppal 1987) but contrary results were also reported (Sarquís et al. 2000).

Table 3. Total glycoalkaloid content found in different parts of cultivated potato (mg/kg fresh weight) (according to Friedman & McDonald 1997).

Plant part	Lampitt et al., 1943	Wood and Young, 1974	Kozukue et al., 1987	Friedman and Dao, 1992	Coxon, 1981
Roots	180–400	—	—	860[a]	
Stems	23–33	30	30–71	320–450[a]	
Leaves	550–610	400–1,000	230–1,000	1450[a]	
Flowers	2,150–4,160	3,000–5,000	3,000–5,000		
Berries	420	—	—	380	255–1,355
Sprouts	1,950	2,000–4,000	—	2,750–10,000[a]	
Skin	300–640	300–600	—	—	
Peel	150–155	150–300	13–400		
				850[b]	
Flesh	12–100	12–50	—	16–60, 110[b]	
Peel + eye	300	300–500	—	—	

[a] NDA1725-1, a cultivar known to be high in glycoalkaloids.
[b] Lenape, a cultivar known to be high in glycoalkaloids.

1.3.5 Factors influencing glycoalkaloid formation

1.3.5.1 Genetic factors

Glycoalkaloid content is a genetically controlled trait (Sinden & Webb 1972; Sanford & Sinden 1972; van Dam et al. 1999). Highly heritable, polygenic inheritance for glycoalkaloid content demonstrated by Sanford and Sinden (1972) and Sanford et al. (1995, 1996b) was subsequently confirmed by van Dam et al. (1999). The number of genes involved is considered to be quite low (3–7 genes) (van Dam et al. 1999). In contrast, Ross (1966) concluded that the suppression of glycoalkaloid synthesis is a dominant trait and that low glycoalkaloid synthesis is controlled by a few dominant alleles (i.e. multiple recessive alleles are required for the expression of elevated glycoalkaloid levels). According to Ross (1966), solanine content is low in the interspecific hybrid between a wild species and *S. tuberosum*. Lower total glycoalkaloid content than the mid-parent mean content was reported in the F_2 population derived from a cross between *S. tuberosum* (4x) and *S. chacoense* Bitt. (2x) (Sanford et al. 1994, 1995), but the content was supposed to be too high to indicate complete dominance. It was also speculated that apparent dominance was related to differences in tuber size. Yencho et al. (2000) reported that the frequency distributions of leptines in the F_2 progenies of *S. tuberosum* (4x) and *S. chacoense* (4x) were skewed toward reduced glycoalkaloid production.

In general, varieties with a high mean glycoalkaloid content can produce excessive amounts, in comparison with varieties having a low mean glycoalkaloid content, when subjected to non-ideal environmental conditions (Sinden & Webb 1972; Papathanasiou et al. 1999a). On the other hand, the varieties that produced the highest amounts of glycoalkaloids also consistently produced the highest glycoalkaloid concentrations regardless of the growing conditions or the tuber ages (Sinden & Webb 1972, 1974; Dimenstein et al. 1997). Thus, the varieties with low mean glycoalkaloid content should be selected for breeding. However, selection of new cultivars based on the tuber glycoalkaloid content at harvest is not necessarily a good criterion because no relationship has been established between glycoalkaloid amount at harvest and the rate of glycoalkaloid accumulation in response to

storage temperature and time (Griffiths et al. 1997). An important selection criterion for a cultivar is also a slow rate of accumulation of glycoalkaloids during light exposure (Percival 1999a).

Additionally other detailed studies concerning the inheritance and genetic control of glycoalkaloids have been published. Single major genes with co-dominant alleles were shown to affect the synthesis of solanine and commersonine in the progenies derived from crosses between accessions of *S. chacoense* (McCollum & Sinden 1979). The major gene for chaconine segregated independently from the above mentioned genes. It has been proposed that there is a single gene or a few genes controlling leptine production in *S. chacoense* (Sinden et al. 1986b). In the hybrids between *S. tuberosum* and *S. chacoense*, the ability to synthesise leptines was suggested to be controlled by a few dominant genes (Sanford et al. 1996b). The theory that homozygous dominant genes are responsible for leptine production was also supported (Veilleux & Miller 1998). Results of a study of leptine biosynthesis in intraspecific *S. chacoense* hybrids indicated a single recessive gene model for leptine synthesis (Ronning et al. 1998), but it was considered that additional factors were also involved. According to the authors, this was not contradictory to the polygenic control by dominant gene(s) based on the study of Sanford et al. (1996b) because of the more complicated nature of the *S. chacoense* x *S. tuberosum* cross. Molecular markers showed that the chromosome 1 of *Solanum* species may contain gene(s) for glycoalkaloid accumulation (Ronning et al. 1999). Also, Yencho et al. (1998) after screening low to non-detectable levels of solanidine and solasodine in the F_1 hybrids between *S. tuberosum* and *S. berthaultii* Hawkes postulated that recessive genes controlled the expression of the aglycones. In the backcross populations, quantitative trait loci (QTLs) for the accumulation of solasodine were identified on chromosomes 4, 6, and 12 and for solanidine on chromosomes 4, 8, and 11 and for both solasodine and solanidine on chromosomes 1 and 4 (Yencho et al. 1998). After segregation studies in the hybrids between *S. chacoense* genotypes, molecular markers linked to leptinine production were located on chromosome 1 (Hutvágner et al. 2001) supporting the earlier studies that the short arm of chromosome 1 was involved in glycoalkaloid synthesis. In addition, four molecular markers associated with leptine production in reciprocal backcross families of diploid potato [*S. phureja* Juz. & Buk x (*S. phureja* x *S. chacoense*) and (*S. phureja* x *S. chacoense*) x *S. phureja*] (Bouarte-Medina et al. 2002) and one marker associated with total glycoalkaloid content in potato tubers (van Dam et al. 2003) were published. Van Dam et al. (2003) concluded that the expression of total glycoalkaloid content in tubers was modulated by two interacting loci. Genes associated with aglycone synthesis were suggested to segregate independently from genes associated with carbohydrate synthesis of the glycoalkaloids (McCollum & Sinden 1979). As it can be seen, the qualitative and quantitative inheritance of glycoalkaloid content is complex and not totally understood. The situation is even more complex when wild *Solanum* species are utilised in potato breeding. However, the molecular-based research strategies are now expected to produce more information in this area.

1.3.5.2 Environmental conditions

In addition to the genetic factors, environmental conditions during the growing season greatly influence glycoalkaloid levels. It was proposed that any environmental factor that stresses a plant can change glycoalkaloid content (Sinden et al. 1984). For instance, extended daylength increased glycoalkaloid content of tubers of cultivated potato (Nitithamyong et al. 1999) and it also had a significant influence on the composition and proportion of aglycones in *S. vernei*

Bitt. & Wittm. (van Gelder & Scheffer 1991). Higher light intensity/irradiance significantly stimulated glycoalkaloid synthesis in foliage of *S. chacoense*, *S. tuberosum* (Deahl et al. 1991) and in two leptine-producing genotypes (Lafta & Lorenzen 2000) as well as in the tubers of *S. tuberosum* (Nitithamyong et al. 1999). Furthermore, glycoalkaloid concentrations were significantly increased by temperatures above and below 16 °C (Nitithamyong et al. 1999). Low temperatures favoured glycoalkaloid formation (Chungcharoen et al. 1987; van Swaaij 1992). A temperature of 32/27 °C (day/night) induced significantly higher levels of glycoalkaloids in plants than one of 22/17 °C (Lafta & Lorenzen 2000).

Due to the variability of several factors, evaluation of the effects of differing climatic conditions has been complex. Potato tubers grown in a hot and dry climate generally accumulate more glycoalkaloids than those grown in cooler climates (Morris & Petermann 1985; Dimenstein et al. 1997). Moreover, unusually short and cool growing seasons (Sinden & Webb 1972), as well as cold and rainy growth conditions (Hellenäs et al. 1995b) supposedly promote elevated glycoalkaloid levels. Tests in the growth chamber showed that drought stress at 24/18 °C and waterlogging at 12/9 °C increased the total glycoalkaloid concentration of cv. British Queen compared with the favourable conditions of 18/14 °C. Other varieties tested changed little if at all (Papathanasiou et al. 1999b). The differences in tuber glycoalkaloid concentrations between two testing years were statistically significant (Love et al. 1994; Papathanasiou et al. 1999a), possibly as a result of the differences in weather conditions. The weather conditions probably affected the production of the immature tubers and, thus, subsequently caused the abnormally high glycoalkaloid levels. Generally, glycoalkaloid levels decrease during the tuber maturation process (Papathanasiou et al. 1998; Nitithamyong et al. 1999; Peksa et al. 2002). For example, the potato cv. Magnum Bonum was grown for more than 50 years in Sweden until one growing season, when its tuber glycoalkaloid levels exceeded the acceptable level and as a result, a sale restriction was imposed (Hellenäs et al. 1995b). It was suspected that the environmental conditions caused the high glycoalkaloid levels. Elevated carbon dioxide concentrations have both increased (Nitithamyong et al. 1999) and decreased (Donnelly et al. 2001; Vorne et al. 2002) tuber glycoalkaloid levels by decreasing the chaconine levels. On the other hand, increase in relative humidity from 50 to 80% had no effect (Nitithamyong et al. 1999), but ozone increased tuber glycoalkaloid content (Donnelly et al. 2001).

It was shown that irrigation methods affect glycoalkaloid levels (Gosselin et al. 1988). Also, the effects of fertilisation practices on glycoalkaloid levels were studied: elevated levels of nitrogen increased (Mondy & Munshi 1990a; Love et al. 1994) or decreased (van Swaaij 1992) the tuber glycoalkaloid levels. Reduced glycoalkaloid levels in foliage were reported after elevated supply of nitrogen (Fragoyiannis et al. 2001). Hoffland et al. (1999) speculated that in tomato, tomatine could be a carbon-based rather than nitrogen-based compound and, therefore the carbohydrate rather than the nitrogen concentration limits the rate of tomatine production. The addition of molybdenum (Munshi & Mondy 1988) and selenium (Mondy & Munshi 1990b) also decreased glycoalkaloid levels, but magnesium (Evans & Mondy 1984) promoted glycoalkaloid formation. Foliar application of the plant growth regulator indoleacetic acid significantly decreased glycoalkaloid levels in two potato cultivars (Ponnampalam & Mondy 1986). Friedman and McDonald (1997) reviewed the effect of pesticides and haulm killing on the glycoalkaloid content. In conclusion, the influences of different environmental factors and agronomic procedures on glycoalkaloid content can be variable.

1.3.5.3 Pathogen infections

Pathogen infections are known to affect glycoalkaloid content. Suppression of glycoalkaloid accumulation in tuber slices or potato leaves after fungal inoculations or treatments with *Phytophthora infestans* (Mont.) de Bary, *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian, or *H. carbonum* Ullstrup. (Shih et al. 1973; Zacharius et al. 1975; Zook & Kuć 1987; Mucharromah et al. 1995; Dimenstein et al. 1997; Andreu et al. 2001) was reported. In addition, eicosapentaenoic and arachidonic acid extracts or elicitors of *P. infestans* caused inhibition in glycoalkaloid accumulation (Tjamos & Kuć 1982; Choi et al. 1994). In contrast, zoospores of *P. infestans* induced glycoalkaloid accumulation in potato leaves (rev. in Friedman & McDonald 1997). However, no significant differences in tomatine content in the stem vascular tissue, root tissue or petiolar tissue between the control plants and *Verticillium albo-atrum* Reinke & Berthold infected tomato plants were recorded (Pegg & Woodward 1986). Bacterial soft rot infection caused by *Erwinia carotovora* increased glycoalkaloid production in potato tubers (rev. in Friedman & McDonald 1997). The suppression of glycoalkaloid production after exposing plant tissues to elicitors or fungal pathogens that induce a hypersensitive response was also demonstrated at the biosynthetic level (Stermer & Bostock 1987; Vögeli & Chappell 1988; Zook & Kuć 1991).

An additional aspect in plant-pathogen interactions is that fungal pathogens of tomato and potato have a metabolite effect on glycoalkaloids by the removing sugar(s) from the glycoalkaloid sugar chain (Pegg & Woodward 1986; Lairini & Ruiz-Rubio 1997, 1998; Weltring et al. 1997; Becker & Weltring 1998; Quidde et al. 1998; Sandrock & VanEtten 1998; Morrissey & Osbourn 1999; Oda et al. 2002). Pathogens thereby have the ability to resist glycoalkaloids and pathogen growth is not arrested during the infection process in the plant.

Pest-related stress following foliar damage by Colorado potato beetles (*Leptinotarsa decemlineata* Say) significantly increased the glycoalkaloid concentrations in potato tubers, while leafhoppers caused no change (Hlywka et al. 1994). In contrast, infestation by peach potato aphids (*M. persicae*) reduced total foliage glycoalkaloid contents (Fragoyiannis et al. 2001).

1.3.5.4 Post harvest conditions

Light. Post harvest conditions also affect glycoalkaloid formation in storage. Tubers exposed to light increase glycoalkaloid synthesis (Sinden 1972; Uppal 1987; De Maine et al. 1988; Dale et al. 1993; Kaaber 1993; Percival et al. 1993, 1994, 1996; Griffiths et al. 1994, 2000; Edwards & Cobb 1999) or there is formation of previously undetected glycoalkaloids (Griffiths et al. 2000). However, in two commercial potato varieties there was no increase in glycoalkaloid concentrations as a result of light treatments (De Maine et al. 1988; Percival et al. 1993). Light has also been shown to alter the ratio of solanine and chaconine (Dale et al. 1993). Enhanced solanine synthesis was increased over that for chaconine after particular illumination regimes (Percival et al. 1993, 1994, 1996; Percival 1999a). The bias toward solanine synthesis is thought to result from the conversion of starch to sugar. Solanine production could be linked to the higher proportion of reducing sugars, such as galactose, which are more freely available than rhamnose. This event results in sequential addition of monosaccharide units to the aglycone and, thus, favours solanine production. However, Percival (1999b) reported that the synthesis of chaconine exceeded that of solanine after

exposure to high-pressure mercury light. Aerial tubers exhibited a significantly different ratio compared with subterranean tubers (Percival & Dixon 1996). In a study of the effect of the type of illumination, mercury light was found to elicit reduced glycoalkaloid accumulation in comparison with fluorescent and sodium light (Percival et al. 1994; Percival 1999b). The study demonstrated the importance of the light wavelength (Percival et al. 1994). Furthermore, freshly harvested tubers were more responsive to intense illumination than physiologically older tubers (Percival et al. 1994; Edwards & Cobb 1999). The responsiveness of tubers to photoinduced glycoalkaloid synthesis was reduced by curing tubers at 25 °C for 10 days before storage at 5 °C (Zitnak 1981). However, from the practical point of view, tubers can be exposed to light for at least 24 hours before any major increase in glycoalkaloids occurs (Percival et al. 1996).

It has been widely speculated that there is a relationship between the light induced greening of tubers and accumulation of glycoalkaloids, but in two recently published studies directly opposing results were presented. Edwards et al. (1998) found no direct metabolic link between chlorophyll and glycoalkaloid biosynthesis, when tubers were treated with chlorophyll biosynthesis inhibitors and subsequently exposed to daylight for up to 10 days. In contrast, Percival (1999b) established a strong correlation between glycoalkaloid and chlorophyll accumulation in response to light, when tubers were analysed following 15 days of continuous illumination.

Temperature. The effects of storage temperature and storage time are difficult to separate. There is no evidence of an optimal storage temperature that promotes minimal glycoalkaloid accumulation. Higher storage temperatures resulted in raised glycoalkaloid concentrations (Wu & Salunkhe 1976; Kaaber 1993; Percival et al. 1993; Love et al. 1994). However, raising the temperature from 5 to 25 °C stimulated no significant changes in glycoalkaloid concentrations (Edwards & Cobb 1997). Griffiths et al. (1997) reported that the highest mean glycoalkaloid content was found in tubers stored at 7 °C, but the largest increase was recorded after short-term storage at 4 °C. When tubers stored at 10 °C for nine weeks were transferred to a lower temperature, no increase in glycoalkaloid concentrations was detected. Therefore, it was concluded that once the tubers were fully dormant they were insensitive to variation in storage temperatures. Studies conducted by Coria et al. (1998), using a heat-susceptible cultivar, showed that there is 74% increase in total glycoalkaloid concentration when the plants are treated at 35 °C for 4 h over when they maintained at 22 °C. Conversely, a heat-resistant cultivar showed a 50% reduction in total glycoalkaloid content after similar treatments.

Time. Increased storage times raise glycoalkaloid levels (Percival et al. 1993; Griffiths et al. 1997). During the first three months (Love et al. 1994) or the first nine weeks (Griffiths et al. 1997) of storage the increase in glycoalkaloid content is rapid, but subsequently remains constant. After 34 or 40 weeks of storage little change was recorded (Fitzpatrick et al. 1977; Edwards & Cobb 1999), but some fluctuation in total levels was measured. Even a slight, non-significant reduction in glycoalkaloid content during storage was reported (Wünsch & Munzert 1994).

Mechanical damage. Mechanical damage to tubers also causes increased synthesis of glycoalkaloids (Sinden 1972; Mondy et al. 1987). The type of damage determines the level of increase: severe impact and cutting were associated with the highest glycoalkaloid levels (Wu & Salunke 1976; Olsson 1986). In the response to injury, similarly to the effect of light, temperature, and storage time, the rate of glycoalkaloid accumulation is genotype-dependent

(Olsson 1986; Dale et al. 1998). Furthermore, the rates of glycoalkaloid synthesis in response to damage were in good agreement with cultivar response to light and cold temperature stress (Dale et al. 1998).

Accumulation of glycoalkaloids in potato tubers continues after harvesting. Sub-optimal storage conditions, represented by light exposure, temperature extremes, mechanical damage, and duration of storage, affect glycoalkaloid levels. In addition, relationships between these factors increase the difficult of determining their effects and also those of genetic factors on glycoalkaloid formation.

1.4 Methods of analysis for glycoalkaloids

1.4.1 Sample preparation for glycoalkaloid analyses

Glycoalkaloid analysis methodology has been studied intensively (rev. in Coxon 1984; Friedman & McDonald 1997; Edwards & Cobb 1998). Analytical methods include three stages: extraction, purification, and analysis. Various extraction systems based on earlier published studies were evaluated (Friedman & McDonald 1995b, rev. in Friedman & McDonald 1997). It was concluded that acetic acid (2%, v/v) was the most efficient solvent for dried samples, nonaqueous solvents not being a good choice. For fresh samples, a solvent mixture of methanol and chloroform (Wang et al. 1972) or of tetrahydrofuran-water-acetonitrile-glacial acetic acid (Bushway et al. 1985) was recommended. Bushway et al. (1985) used their own original extraction method for dehydrated samples and concluded that it was more suitable for dehydrated samples than the method of Mondy and Ponnampalam (1983), in which water was included. Furthermore, other solvents used for extraction have included e.g. 5% (v/v) acetic acid (Speroni & Pell 1980; Gregory et al. 1981; Bacigalupo et al. 2000), methanol (Saito et al. 1990; Plhak & Sporns 1992; Keukens et al. 1994; Driedger et al. 2000a), methanol with acetic acid (Osman & Sinden 1977; Jonker et al. 1992), heptane sulfonic acid with acetic acid (Carman et al. 1986; Houben & Brunt 1994; Simonovska & Vovk 2000) and a mixture of tetrahydrofuran, water, acetonitrile and acetic acid (Bushway et al. 1985; Friedman & Levin 1992; Zhao et al. 1994). After extraction, various clean-up methods for glycoalkaloid purification can be used. Traditional precipitation with ammonium hydroxide is not reliable because many glycoalkaloids can remain in the liquid phase (Gregory et al. 1981). The most commonly used purification method is solid-phase extraction (SPE). Different cartridges of C_{18} (Bushway et al. 1986; Carman et al. 1986; Saito et al. 1990; Houben & Brunt 1994; Keukens et al. 1994; Edwards & Cobb 1996), NH_2 (Saito et al. 1990), and CN (Jonker et al. 1992) have been used and tested (Väänänen et al. 2000). In addition to extraction and purification, glycoalkaloid samples can also be hydrolysed.

Since there is no single method that can satisfy the needs of all users (breeders, scientists, authorities in the National Food Agency), numerous reports dealing with analysis of glycoalkaloids using different separation and detection methods have been published. High-performance liquid chromatography and immunoassays are the most common and practical methods (Edwards & Cobb 1998), but standard chromatographic methods (gas chromatography and thin layer chromatography) are also applied. In addition, colorimetric methods (Wang et al. 1972; Coxon et al. 1979; Bushway et al. 1980), for example, were also used. New methods for glycoalkaloid detection are still being developed, and include indirect assay of time-resolved fluorescence using a europium chelator entrapped in liposomes

(Bacigalupo et al. 2000) and an enzyme biosensor based on pH-sensitive field effect transistors (Korpan et al. 2002).

1.4.2 Gas chromatography

The history and main principle of gas chromatography (GC) was recently reviewed (Bartle & Myers 2002). Separation of volatile samples in GC is based on a series of partitions/events between the carrier gas phase and the stationary liquid phase in the column. Two different column types have been used. A capillary column, used currently, has certain advantages over a packed column: increased separation efficiency, lower column/working temperature, better separation and shorter analysis time. A wide range of capillary columns is available and therefore the choice of the column for an analysis depends on the mixture of compounds to be analysed and the stationary phase of the column. The other main instruments of the basic gas chromatograph apparatus are carrier gas supplier, injector, detector, and data recorder. The carrier gas in the column is helium or hydrogen; previously nitrogen was most commonly used. The detector is used to monitor compounds eluted from the column. A number of types of detectors have been developed, but the most widely used detectors are the flame ionisation detector (FID), electron-capture detector (ECD), photo-ionisation detector (PID), thermionic detector (TID), and flame photometric detector (FPD). It is important to note that temperature, which varies depending on the compounds to be analysed, is decisive for the separation process of the GC system.

Glycoalkaloids are usually hydrolysed to corresponding aglycones before GC analyses because of their high molecular weight and low volatility. However, entire glycoalkaloids have also been analysed after derivatisation. The first report of a GC analysis of glycoalkaloids was made by Herb et al. (1975), who permethylated the samples and then successfully determined entire glycosides. Osman et al. (1978) and Gregory et al. (1981) also repeated permethylation of entire glycosides. Later on, GC methods for aglycone analyses with good separation were developed (Osman & Sinden 1977; Coxon et al. 1979; King 1980). Marked progress in the separation and quantification of different aglycone mixtures was achieved using a capillary GC method (van Gelder 1985; van Gelder et al. 1988a) incorporating simultaneous flame ionisation (FID) and nitrogen-specific (NPD) detection (van Gelder et al. 1988a). Separation of solanidine, solasodine, leptinidine, tomatidine, and acetylleptinidine was also achieved using the capillary GC method after combined extraction and hydrolysis, but not between solanidine and demissidine (Lawson et al. 1992). The most important advantage of GC analysis is good separation of aglycone mixtures. Hydrolysis of glycoalkaloids, cleaving the sugar moiety from aglycone, is considered to be a disadvantage in GC analysis because information on glycoside composition is lost. Various types of hydrolysis methods and times [e.g. aqueous acid hydrolysis (Osman & Sinden 1977; Coxon et al. 1979), alcohol acid hydrolysis (King 1980; van Gelder 1984; Lawson et al. 1992; Friedman & McDonald 1995a), and a two-phase system (van Gelder 1984)] have been used. The optimum hydrolysis time for solanine and tomatine was 2.5-3 h in a two-phase system (van Gelder 1984). It has been stated that “hydrolyses rates in methanol increased with HCl concentration and temperature and decreased with amount of water in methanol-water solutions” (Friedman et al. 1993). The nature of the alcohol solvent influenced the rate and specificity of the hydrolysis (Friedman & McDonald 1995a). According to Friedman et al. (1998), a 70 min hydrolysis time in 1 N HCl at 100 °C caused complete hydrolysis of tomatine to tomatidine. Furthermore, acid hydrolysis with ethanol or methanol solution (King 1980; van Gelder 1984; Lawson et al. 1992) was reliable in preventing the degradation of

solanidine and tomatidine. Finally in sample preparation, derivatisation of aglycones by conversion to more volatile and thermally stable derivatives has not become a common procedure, but acetylation (King 1980) and trimethylsilylation (Juvik et al. 1982) have been used.

1.4.3 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is the most commonly used method for glycoalkaloid detection. Separation of components in HPLC is based on mass-transfer between stationary and liquid mobile phases in a column. Choosing suitable solvent(s) for the sample and mobile and stationary phases may have an effect on the separation process. An important advantage in HPLC analysis of glycoalkaloids is that entire glycosides and aglycones can be analysed without any derivatisation. However, a problem can arise because of the weak absorption and low sensitivity of the glycoalkaloids in the short UV wavelengths (200–215 nm) because glycoalkaloids do not have strong chromophores. Therefore, the choice of eluents and clean-up methods to remove contaminating compounds, which would hinder glycoalkaloid detection, is important. Since the introduction of the first HPLC methods for glycoalkaloid separation and quantification (Bushway et al. 1979; Morris & Lee 1981), continuous developments and improvements of solvent systems, sample preparations, and column chemistry have been made (e.g. Bushway et al. 1986; Carman et al. 1986; Saito et al. 1990; Jonker et al. 1992; Friedman & Levin 1992; Bushway et al. 1994; Friedman et al. 1994; Houben & Brunt 1994; Edwards & Cobb 1996; Friedman et al. 2003). Also, simultaneous detection of steroidal glycoalkaloids and their aglycones with solanidane and spirosolane structures has been developed (Kuronen et al. 1999).

1.4.4 Thin layer chromatography

Thin layer chromatography (TLC) is based on variable partitioning behaviour of the components between the mobile liquid phase and stationary phase. TLC consists of a stationary phase immobilised on a glass or plastic plate and a solvent. For separation, the sample is deposited as a spot on the stationary phase. After running, separated spots are detected with ultraviolet light or by placing the plate in iodine vapour. TLC, which is a simple, qualitative detection method for both the glycoalkaloids and their aglycones, was widely used before the development and improvement of GC and HPLC methods. The advantage of TCL is the quick screening of a large number of samples. Solvent systems for the separation and visualising methods for the detection of glycoalkaloids using various reagents were recently reviewed by Friedman and McDonald (1997). It is also possible to quantify glycoalkaloids with TLC using, for example, densitometry (Ferreira et al. 1993; Simonovska & Vovk 2000).

1.4.5 Mass spectrometry

Mass spectrometry (MS) provides information about the molecular weight and chemical structure of the compound analysed. The mass spectrometer creates charged ions from molecules. These ions are extracted into the analyser region of the mass spectrometer and are separated according to their mass-to-charge ratios (*m/z*). The separated ions are detected and the signals are sent to the data system where the *m/z* ratios are stored together with their

relative abundance for presentation in the format of a *m/z* spectrum. Because of its specific "finger-print" property, MS is very useful in qualitative analyses of unknown compounds. MS is generally used as a detector in GC or HPLC apparatuses. Structural and quantitative information from GC-MS (van Gelder et al. 1989), LC-MS (Bushway et al. 1994), liquid secondary ion MS (Friedman et al. 1994), tandem MS (Evans et al. 1993; Chen et al. 1994), high resolution MS (Lawson et al. 1997), capillary electrophoresis coupled with electrospray ionization-ion trap MS (CE-ESI-MS) (Bianco et al. 2002), and electrospray LC-MS (LC-ESI-MS) (Stobiecki et al. 2003) analyses of glycoalkaloids and their aglycones has been published. Also quantification using a new matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) method was successful (Abell & Sporns 1996). The advantages of MALDI-TOF MS were rapid analysis, short sample preparation time and suitability for routine glycoalkaloid analyses. According to Wolfender et al. (1994), LC-MS has become a successful analytical tool in phytochemical analyses and it can now be used in routine practical applications, and the situation is also the same with glycoalkaloid analyses.

1.4.6 Immunoassay

Immunoassay is based on the use of antibodies that have been designed to bind to a target compound or class of compounds. Quantification of compounds is made by comparing the colour developed from a sample of unknown concentration with the colour formed by the standard of known concentration. The immunoassay methods used for glycoalkaloid determination have been radioimmunoassay (RIA) (Weiler et al. 1980), enzyme linked immunosorbent assay (ELISA) (Morgan et al. 1983; Ward et al. 1988; Plhak & Sporns 1992, 1994; Stanker et al. 1994; Friedman et al. 1998), and fluorescence polarisation immunoassay (FPIA) (Thomson & Sporns 1995). Recently an alternative method to ELISA, a solution-phase immunoassay with capillary electrophoresis (CE) laser-induced fluorescence (LIF) detection was developed (Driedger et al. 2000a,b). Immunoassays are specific, relatively quick and simple to use. However, immunoassays are used for quantification, and monoclonal antibodies should be suitable for large-scale and international screening of total glycoalkaloid content (Edwards & Cobb 1998).

Summary of different analysis methods. The development of new glycoalkaloid analysis techniques is still continuing although hundreds of research articles, based on analytical methods, have already been published. HPLC is the most commonly used application and its advantage is that both entire glycosides and also aglycones can be analysed. However, if the glycoalkaloid profile of material is unknown or novel compounds are expected, the techniques coupled with MS are relevant, such as LC-MS or GC-MS. The most important advantage of GC analysis is its sensitivity and good separation of aglycone mixtures. The high cost and complexity are disadvantages of LC-MS and GC-MS. Immunoassays may be alternative methods for analyses of total glycoalkaloid content. Immunoassays are used for quantification and their advantages are that large sample amounts can be analysed quickly and without high costs. Furthermore, methodologies based on modern techniques, such as MS and capillary electrophoresis (CE), deserve further exploration (Edwards & Cobb 1998). The choice of the glycoalkaloid analysis method is dependent on the task – quantification or/and identification.

1.5 Glycoalkaloids in *Solanum* species and their hybrids

Glycoalkaloid composition and content in wild *Solanum* species, their interspecific sexual hybrids and somatic hybrids have been the focus of numerous studies, because wild species are used in potato improvement. Foliar or tuber glycoalkaloid content of at least 70 *Solanum* species has been analysed (Osman et al. 1978; Gregory et al. 1981; van Gelder et al. 1988b; Deahl et al. 1993). The concentrations of a wide range of glycoalkaloids among various species have been reported and also different glycoalkaloids from those present in *S. tuberosum* have been identified. The most common glycoalkaloids in wild *Solanum* species are e.g. tomatine in *S. acaule, S. brevidens, S. commersonii* Dun.; demissine in *S. chacoense, S. commersonii, S. demissum* Lindl.; solasonine in *S. berthaultii, S. dulcamara* L., *S. vernei*; commersonine in *S. commersonii*; leptines and leptinines in *S. chacoense*. Several *Solanum* species contain more than one glycoalkaloid. For instance, *S. chacoense* contains, dependent on the accession, solanine, chaconine, leptine (I, II), leptinine (I, II) (Kuhn & Löw 1961a,b; Sinden et al. 1986b), demissine and commersonine (Osman et al. 1976). Other types of glycoalkaloid are also present in *S. chacoense*, which was reported to synthesise at least 10 different glycoalkaloids (Sinden et al. 1991). *Solanum commersonii* synthesises commersonine, demissine, tomatine, dehydrocommersonine, and Δ^5-demissine (Vázquez et al. 1997). *Solanum dulcamara* can contain solasonine, α-solamarine, and soladulcidinetetraoside (Sander 1963), whereas *S. vernei* synthesises several solanidane-based and spirosolane-based glycoalkaloids (van Gelder & Scheffer 1991). New glycoalkaloids, such as minor amounts of dehydrotomatine from tomato and Δ^5-demissine from *S. commersonii*, were recently identified from previously analysed species as a result of improved analysis techniques (Bushway et al. 1994; Friedman et al. 1994; Vázquez et al. 1997).

Leptines. Foreign steroidal glycoalkaloids can be transferred from wild *Solanum* species to their interspecific hybrids. In order to transfer leptine synthesis into cultivated potato, foliage and tuber glycoalkaloid levels were very intensively studied in hybrids between cultivated potato and wild potato *S. chacoense*, the source of the leptine glycoalkaloids. The studies also included self-pollination and backcross progenies derived from the interspecific hybrids (Sanford et al. 1994, 1995, 1996b, 1998). When the F_2 and F_4 populations were analysed, their foliage (Sanford et al. 1994) and tuber (Sanford et al. 1995) glycoalkaloid levels were significantly lower than in the parental species *S. chacoense*, but higher than in the *S. tuberosum* parent. As the result of the first backcross to *S. tuberosum*, the total glycoalkaloid levels were close to the mean level of the F_2 population, but the second backcross to *S. tuberosum* resulted in decreased total glycoalkaloid levels, which were close to the levels of the *S. tuberosum* parent. Furthermore, the ratio between chaconine and solanine changed as a result of self-pollination and backcrossing (Sanford et al. 1994). The interspecific F_1 hybrids between *S. tuberosum* and *S. chacoense* synthesised not only solanine and chaconine, but also leptine and leptinine glycoalkaloids. The highest levels of leptines were only one quarter of those originally synthesised in the *S. chacoense* parents. Although the *S. tuberosum* lines did not express leptines, they did have a marked influence on the leptine levels and the relative proportions of different glycoalkaloids. The choice of the *S. tuberosum* genotype was thus essential for leptine production (Sanford et al. 1996b). According to Sanford et al. (1998), all the F_2 plants synthesised leptines and leptinines, but Yencho et al. (2000), however, later reported that leptines were not detected in all of the F_2 plants. In another combination (*S. chacoense* x *S. phureja*), all the resulting hybrids contained the glycoalkaloid aglycones (solanidine, leptinidine, and acetylleptinidine) of *S. chacoense* (Veilleux & Miller 1998). Similarly to the influence of *S. tuberosum*, the leptidine levels of the hybrids were also dependent on the *S. phureja* parental line, which did not synthesise acetylleptinidine (Veilleux

& Miller 1998). Overall, it was concluded that leptine production both in *S. tuberosum* x *S. chacoense* hybrids and in *S. phureja* x *S. chacoense* hybrids and their progenies was dependent on the genome constitutions of the hybrids. As a result of backcrossing, the genome proportion of *S. chacoense* was decreased and therefore the pool of a substrate precursor for glycoalkaloid biosynthesis was directed instead to the production of *S. tuberosum* glycoalkaloids (Sanford et al. 1998; Veilleux & Miller 1998).

Additional glycoalkaloids. In the hybrids between *S. vernei* and *S. tuberosum*, reduced amounts of solanidine and solasodine aglycones were detected in comparison with the *S. vernei* parental species (van Gelder & Scheffer 1991). Hybrids did not contain the full range of aglycones found in *S. vernei*. Tuber glycoalkaloid levels in half of the analysed hybrids with three genome compositions (*tbr* x APB [*adg* x (*phu* x *ber*)], *tbr* x APC [*adg* x (*phu* x *chc*)], *tbr* x APM [*adg* x (*phu* x *mcd*)]) were higher than the acceptable safety value of 200 mg/kg fw (Veilleux et al. 1997). The F_1 hybrids between *S. tuberosum* and *S. berthaultii* contained low to very low levels of the parental glycoalkaloids solanidine and solasodine. In the BC populations, the total glycoalkaloid levels were similar or lower than in the parental lines, solasodine being the major aglycone in both BC populations (Yencho et al. 1998). Total glycoalkaloid concentrations in tubers and foliage of *S. demissum* and *S. iopetalum* Bitt. (Hawkes) were 70 and 76 (tubers), 88 and 92 mg/100 g fw (Sarquís et al. 2000), whereas in the BC_2, BC_3, and BC_4 hybrids backcrossed with *S. tuberosum* the average tuber glycoalkaloid content was 19 mg/100 g fw. Although not all the differences were statistically significant, backcrossing was again found to be an important tool for reduction of tuber glycoalkaloid level. On the other hand, foliage glycoalkaloid content in the BC hybrids was only 15% lower than in the parents (Sarquís et al. 2000). In the F_1 and BC_1 populations derived from hybrids between *S. commersonii* and *S. tuberosum*, the parental-type glycoalkaloids were present: dehydrodemissine, dehydrotomatine, dehydrocommersonine from *S. commersonii* and solanine, chaconine from *S. tuberosum* (Carputo et al. 2003). In some of their BC_2 hybrids, only solanine and chaconine were detected. The mean total glycoalkaloid concentrations in the BC_1 and BC_2 hybrids were 166 and 193 mg/kg fw, respectively.

In the tubers of the somatic hybrids between *S. circaeifolium* Bitt. and *S. tuberosum*, demissidine aglycone was produced in addition to the parental-type glycoalkaloid aglycones solanidine and tomatidine (Mattheij et al. 1992). The tetraploid hybrid also contained tomatidenol, which was an aglycone produced by *S. circaeifolium*. The total glycoalkaloid content in the hybrids (253–405 mg/kg fw) was at similar levels to those in the parental species. In the sexual F_1 hybrids between *S. circaeifolium* and *S. tuberosum* (Louwes et al. 1992), solasodine aglycone was found in one hybrid in addition to the aglycones mentioned above. Also, the glycoalkaloid level in the F_1 hybrids was the same as in the parental species. However, in both hybrid types (sexual and somatic), higher total glycoalkaloid levels were detected in comparison with those in *S. circaeifolium*. Not only were solanine and chaconine detected in the foliage of a somatic hybrid between *S. brevidens* and cultivated potato, but also two unknown glycoalkaloid compounds derived from *S. brevidens* (Vallin et al. 1996). The total glycoalkaloid contents in the somatic hybrids (724 mg/kg dw) were higher than in *S. brevidens* (233 mg/kg dw) but lower than in the cultivated potato (1718 mg/kg dw). Glycoalkaloids of parental origin i.e. solanine, chaconine, and tomatine, together with additional minor previously unidentified compounds, were recorded in the somatic hybrids between potato and tomato (Roddick & Melchers 1985). Ninety-eight per cent of the glycoalkaloids in the leaves of these hybrids originated from potato whereas in “tubers” (resembling thickened stolons) tomatine was the predominant glycoalkaloid (60–70%). Foliar

glycoalkaloid contents were at similar levels to those of the parental species. However, total glycoalkaloid contents in the tubers of the hybrids were 5–15 times higher than in tubers of cultivated potato.

1.5.1 Beneficial effects of glycoalkaloids

All effects of glycoalkaloids are not necessary deleterious, because they can induce resistance against several diseases and pests. In addition, the rise of new approaches such as metabolomics (Fiehn 2002) and the search for more benign insecticides/fungicides are important drivers for the investigation of secondary metabolites for previously unrealised properties.

1.5.1.1 Resistance to diseases

Fungal resistance. Anti-fungal activities of potato glycoalkaloids have been difficult to demonstrate. Laboratory tests using solanine and chaconine demonstrated their anti-fungal activity against the pathogens *Ascobolus crenulatus* Karst., *Alternaria brassicicola* (Schw.) Wiltshire, *A. solani* (Ellis & Mart.) Jones & Grout, *Phoma medicaginis* Mal. & Roum., and *Rhizoctonia solani* Kühn (Sinden et al. 1973; Fewell & Roddick 1993). There are various factors influencing activity against fungal pathogens, such as growth conditions, developmental stage, pH, pathogen type as well as the synergistic effects of glycoalkaloids (Fewell & Roddick 1993, 1997; Fewell et al. 1994). In addition, some glycoalkaloids show increased activity, for example chaconine is more fungitoxic than solanine (Fewell & Roddick 1993, 1997; Fewell et al. 1994). Anti-fungal activities of tomatine of tomato are more pronounced (Osbourn 1996a,b) than the corresponding effects of the glycoalkaloids of cultivated potato. For potato there are also numerous reports inferring there is no relationship between tuber/foliage glycoalkaloids and resistance to fungal diseases, including studies on *P. infestans* (Andrivon et al. 2003) and *R. solani* (Morrow & Caruso 1983). In addition, a direct relationship was not established between glycoalkaloid concentrations and resistance to *Phoma exigua* Desm. or *F. solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth (Olsson 1987).

Bacterial resistance. There are only a few published reports on anti-bacterial properties of glycoalkaloids. For example, tuber glycoalkaloid content was not linked to resistance to bacterial ring rot (Paquin 1966), common scab (Frank et al. 1975) or soft rot (Andrivon et al. 2003). However, many questions still remain unresolved with widely opposing views expressed, some of those supporting the linkage between glycoalkaloids and anti-bacterial activities (Bobeica et al. 1996; Lachman et al. 2001; Dr. Anne Osbourn, personal communication). For example, tomatine was reported to have some anti-bacterial effects on gram positive bacteria that infect humans (Jadhav et al. 1981).

1.5.1.2 Insect resistance

Colorado potato beetle. Leptine glycoalkaloids found in *S. chacoense* were shown to induce resistance (decreased feeding and increased mortality) to Colorado potato beetle (Stürckow & Löw 1961; Sinden et al. 1986a; Sinden et al. 1988; Deahl et al. 1991; Silhavy et al. 1996; Sanford et al. 1997). A special property of leptines is that they only occur in foliage, not in

tubers. The ability of *S. chacoense* to resist Colorado potato beetle was introgressed into somatic hybrids between *S. tuberosum* and *S. chacoense*, into diploid *S. phureja* x *S. chacoense* sexual hybrids, as well as into tetraploid *S. tuberosum* x *S. chacoense* hybrids (Cheng et al. 1995; Rangarajan et al. 2000; Yencho et al. 2000). Decreased feeding of Colorado potato beetles in hybrids correlated with increased foliar concentrations of leptines (Rangarajan et al. 2000; Yencho et al. 2000). Moderate resistance to Colorado potato beetle in the hybrids between *S. chacoense* and *S. tuberosum* was also reported (Sinden et al. 1991). In addition to the resistance conferred by leptines, solanocardenine in *S. neocardenasii* Hawkes & Hjert. and tomatine in *S. pinnatisectum* Dun. may also have a role in the resistance to Colorado potato beetle (Sinden et al. 1991). In addition, *S. chacoense* genotypes with commersonine and dehydrocommersonine were more resistant to the beetles than those genotypes containing only solanine and chaconine (Sinden et al. 1980). Flanders et al. (1992) reported tomatine to be associated with field resistance to Colorado potato beetles. Furthermore, in studies using synthetic diets it was shown that increased concentrations of tomatine caused retarded growth and delayed development of Colorado potato beetles (from eggs to adults) (Kowalski et al. 2000). Tomatidine had however no effect (Kowalski et al. 2000). It was concluded that the tetrasaccharide moiety of tomatine was crucial for insectical activity because of its membranolytic action.

Other insects. Glycoalkaloids can also have other kinds of anti-insect properties, not only to Colorado potato beetle. Positive correlations were reported between total glycoalkaloid content in leaves and resistance to potato leafhopper (*Empoasca fabae* Harris) in several *Solanum* species (Tingey et al. 1978; Raman et al. 1979; Sanford et al. 1990; Sanford & Ladd 1992). The type of glycoalkaloid was also an important factor in resistance to potato leafhopper. When separate glycoalkaloids were studied, tomatine caused the greatest mortality of potato leafhopper compared with other glycoalkaloids such as solanine, chaconine, leptine I and II, solasonine and solamargine (Sanford et al. 1996a). Solamargine and chaconine caused the second greatest mortality. Flanders et al. (1992) also suggested that tomatine was connected with field resistance to potato leafhopper.

Glycoalkaloids may be of minor importance in resistance to other pests. Flanders et al. (1992) found no correlation between glycoalkaloid-type and resistance to peach aphids (*M. persicae*), potato aphids (*Macrosiphum euphorbiae* Thomas), and potato flea beetles (*Epitrix cucumeris* Harris). Later it was reported that potato aphid was quite tolerant of glycoalkaloids, but chaconine and tomatine had some negative effects on the vital functions of the aphids (Güntner et al. 1997). The effects of glycoalkaloids on non-insect pests are minor or there are no effects at all (rev. in Friedman & McDonald 1997).

2 AIMS OF THE STUDY

Many resistance and tolerance traits associated with wild *Solanum* species would benefit cultivated potato. Although most wild *Solanum* species are sexually incompatible with *S. tuberosum*, incorporation of the desirable resistance characters can be achieved through somatic hybridisation followed by a series of backcrosses or re-fusions of somatic cells. However, when the complete genomes are somatically combined, many undesirable traits, such as high levels of glycoalkaloids, will also be transferred from the wild parental species to the progeny.

The present study included both glycoalkaloid aglycone analyses and bacterial disease resistance tests on the interspecific somatic hybrids of potato and their progenies. The aims of the analytical part of the study were to optimise the gas chromatographic-mass spectrometric (GC-MS) method for glycoalkaloid aglycone analyses, particularly for the identification and quantification of different glycoalkaloids in various potato structures, and to analyse aglycone contents in interspecific somatic hybrids and their progenies with several kinds of genome constitutions. The study was composed of both identification and also quantification of different glycoalkaloid aglycones in the potato material analysed.

The plant material included hybrids between A and E genome *Solanum* species possessing resistance to viral diseases and to *Erwinia* soft rot, and also of hybrids between two A genome species (*S. acaule* and *S. tuberosum*). According to this, additional aims of the present work were to study the effect of genome composition in various A + A hybrids in terms of immunity to the plant pathogen *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*), which causes bacterial ring rot. Following this, some observations of the relationships between *Cms* infection and glycoalkaloid aglycone contents were made in *S. tuberosum*, *S. acaule* and their interspecific hybrids.

3 MATERIALS AND METHODS

3.1 Plant material (I, II, III, IV, V)

3.1.1 Hybrids between distantly related species with A and E genomes (I, II, III)

The following interspecific hybrids between A and E genome species were selected for glycoalkaloid aglycone analyses: i) tetraploid (2n=4x, AAEE) and hexaploid (2n=6x, AAAAEE and AAEEEE) primary somatic hybrids between diploid (2n=2x=24, EE) *S. brevidens* acc. CPC 2451 (*brd*) and dihaploid (2n=2x=24, AA) *S. tuberosum* PDH40 (*tbr*) (Fish et al. 1987, 1988a; Xu et al. 1993b) (I) and ii) tetraploid and hexaploid primary somatic hybrids between *S. brevidens* acc. CPC 2451 and dihaploid *S. tuberosum* Pito 4 (anther culture derived line 45/4 of cv. Pito) (Rokka et al. 1994) (II, III), anther culture derived diploid (AE) and triploid (2n=3x, AEE) (somato)haploid lines (Rokka et al. 1995) (III), and pentaploid (2n=5x, AAAEE) second generation somatic hybrids (Rokka et al. 2000) (III). The scheme for the production of the plant material used in study III is presented in Figure 5.

Furthermore, an additional diploid E genome species *S. etuberosum* k-9141 (*etb*) and dihaploid *S. tuberosum* T67 and their hexaploid (2n=6x, AAAAEE and AAEEEE) primary somatic hybrids (Thieme et al. 1999, 2003) were selected for the present study. In addition, one BC_1 hybrid derived from a cross between the hexaploid primary somatic hybrid 27/2/14/1 and tetraploid *S. tuberosum* N90, and eight BC_2 hybrids derived from a cross between the pentaploid (2n=5x, AAEEE) BC_1 hybrid 64/10 and *S. tuberosum* N90 were analysed (see *Table 4* in paragraph 4.2.2).

3.1.2 Hybrids between closely related A genome species (*S. acaule* and *S. tuberosum*) (IV, V)

Tetraploid and hexaploid interspecific somatic hybrids between dihaploid (10, 86, 119) or tetraploid [acc. PI 472655.8 (7-8)] lines of *S. acaule* (*acl*) and dihaploid (Pito 179, Petra 63, White Lady 7, White Lady 15) or tetraploid (Pito, Petra, White Lady) lines of *S. tuberosum* (Rokka et al. 1998b,c) (IV) were selected. In addition, dihaploid lines of *S. acaule* (59, 77, 110, 135) were included in the study.

Parental lines were also analysed in each experiment. *In vitro* and greenhouse culture conditions of plantlets and plants were described in the papers.

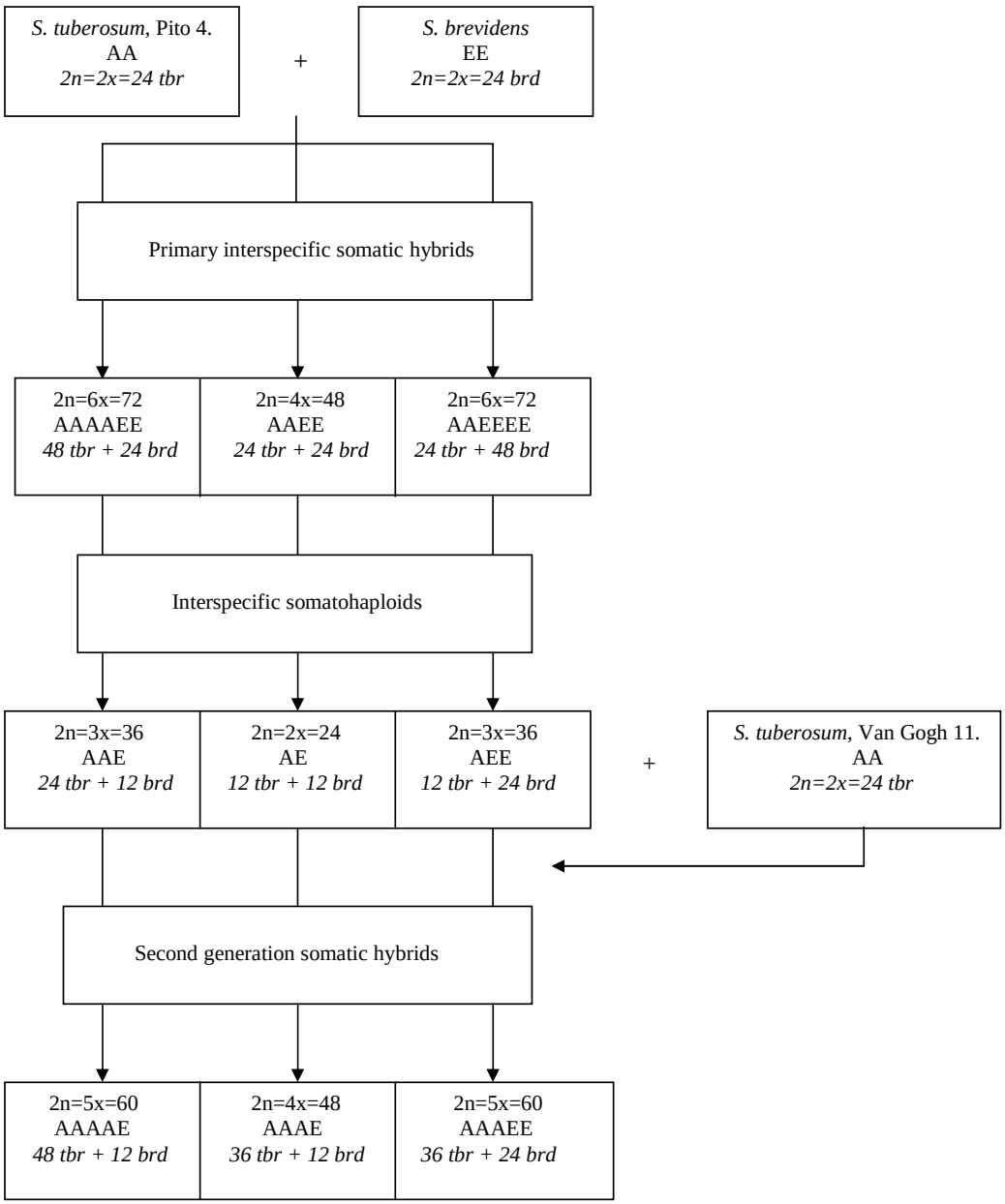

Figure 5. *Breeding scheme for production of plant material between* S. brevidens *and* S. tuberosum *using subsequent protoplast fusions and haploidisation (Gavrilenko et al. 2002).*

3.2 Glycoalkaloid aglycone analyses (I, II, III, V)

3.2.1 Sample collection

Plants were multiplied *in vitro* and then transferred to the greenhouse. Leaves were harvested either at the time of flower bud emergence (I), at various maturity stages of the leaves (II), or after 42/49 days of the growth from the middle parts of the plants, when the leaves were fully expanded (III and *Table 4*). All the plant materials were air-dried at room temperature for several days (I) or at 60–80 °C at heated cupboard in paper bags for 8-12 hours (II, III, V, and *Table 4*) and then ground to a fine powder using a table mill or mortar and pestle before the analyses.

3.2.2 Extraction and hydrolysis

Glycoalkaloids were extracted from 100 mg of leaf powder using 5% (v/v) acetic acid. In paper V and in plant material of *S. etuberosum* (*Table 4*), 200 mg samples were also used. Samples were prepared according to the modified procedure of the extraction and acid hydrolysis methods described by Gregory et al. (1981) and van Gelder (1984) (I). Minor improvements to the procedure presented in paper I were subsequently made (II). The detailed procedure described in paper II was used in the remaining studies (III, V, and *Table 4*). An internal standard (I.S.) α-amyrine (1 mg/ml in chloroform) (I) or cholesterol (1 mg/ml in chloroform) (II, III, V, and *Table 4*) was added to each sample.

3.2.3 Derivatisation of aglycone samples

The hydrolysed and dried aglycone samples with α-amyrine (I.S.) were silylated with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (I). In a combined derivatisation method, the samples with cholesterol (I.S.) were at first silylated with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) and then acylated with pentafluoropropionic acid anhydride (PFAA) (II, III, V, and *Table 4*).

3.2.4 GC-MS analyses

Derivatised samples of 1–4 µl were analysed using gas chromatography-mass spectrometry (GC-MS) in a Hewlett-Packard (HP) 5890 gas chromatograph coupled to an HP 5970 quadrupole mass selective detector operating at ionisation voltage of 70 eV (EI-mode) and electron multiplier voltage of 1800 V (I) or 1600 V (II, III, V, and *Table 4*). Before each sample set, the system performance was controlled by a tuning procedure. Chromatographic separation of glycoalkaloid aglycones was confirmed by preparing samples from pure substances (solanine, tomatine, solasodine) and also from *S. tuberosum* and *S. brevidens* materials. Detailed GC-MS analysis conditions are described in the separate papers. An injection through an HP 6890 AutoSampler was used for the materials described in paper V and for those of *S. etuberosum* described in *Table 4*.

Identification of the aglycones was based on the GC-MS spectra of silylated and combined derivatised authentic compounds and literature dealing with GC and MS data of underivatised aglycones (Budzikiewicz 1964; van Gelder et al. 1989) (I, II, III, V). Aglycones were

quantified by comparing the peak areas from total ion abundance with those of internal standards.

Repeatability of the quantitative analysis was determined from the same plant material (N=6), which was extracted, hydrolysed, derivatised, and analysed using GC-MS (I, II). Tests for recovery and linearity of the method were carried out (II). Before auto-injection and sample preparations for overnight analyses, stability of the derivatised hybrid sample was determined. Analyses were repeated five times by auto-injection during 30 hours after the sample preparation (unpublished data).

High-resolution MS and NMR analyses were carried out as described in paper II.

3.3 Bacterial ring rot analyses (IV, V)

3.3.1 *Clavibacter michiganensis* ssp. *sepedonicus* bacterial strains and inoculation (IV)

Two highly virulent strains of *C. michiganensis* ssp. *sepedonicus* (*Cms*) (3RC and R10) were used in the bacterial ring rot tests. The bacterial strains were grown on YGM medium for 4 to 5 days at 26 ºC. Bacterial suspensions for plant inoculations were prepared as described in paper IV.

Three-week-old *in vitro* propagated plantlets were inoculated via the roots as described by Ishimaru et al. (1994) using the modifications presented in paper IV. Inoculated plantlets were transplanted to pots and transferred either to the greenhouse or to the growth chamber. The growth period and growing conditions were as described. All the tests were repeated at least twice.

3.3.2 IFAS tests for *C. michiganensis* ssp. *sepedonicus* detection (IV)

At 72 days post inoculation, stem sections of the plants were collected. An indirect immunofluorescent antibody staining (IFAS) test with slight modifications was employed to detect *Cms* bacteria. Concentrates of monoclonal antibody 9A1 and FITC were used. The bacterial slide preparations were observed using a microscope with a 100X objective, a mercury vapour light source and a filter set for fluorescein fluorescence. The number of *Cms* cells was expressed as immunofluorescing units per gram fresh weight of stem sample (IFU/g).

3.3.3 Glycoalkaloid aglycone analyses of *C. michiganensis* ssp. *sepedonicus* inoculated plants (V)

In vitro grown plant materials (*S. acaule*, *S. tuberosum* and somatic hybrids) were inoculated with bacterial suspension (*Cms*-inoculated plants) or they were mock inoculated with sterile phosphate buffer (negative control plants). Leaves of the mock inoculated and *Cms*-inoculated plants were harvested at the end of the bacterial ring rot testing period (72 days). Comparison between the aglycone concentrations of the mock-inoculated and *Cms*-inoculated plants was carried out to determine the effect of *Cms* on aglycone contents. GC-MS analyses were carried out as described above.

3.4 Statistical analyses (I, III, IV, V)

Analyses of variance and correlation (Pearson's correlation coefficient) were used to statistically analyse differences among the relative levels of the aglycones (I). Non-parametric tests of Kruskal-Wallis and Mann-Whitney (SPSS Base 8.0, SPSS Inc., Chicago, USA) were used to analyse the proportions of the aglycones and total aglycone content in terms of the different genome constitutions (III). GENMOD and LOGISTIC procedures (SAS 8.1, SAS Institute Inc. Cary, NC, USA) were performed to analyse differences in numbers of *Cms* bacteria and to compare the differences based on the number of infected plants per number of plants inoculated (IV). Comparison of the aglycone concentrations between mock- and *Cms*-inoculated plants was carried out using a MIXED procedure (SAS 8.1, SAS Institute Inc. Cary, NC, USA) (V). P-values of less than 0.05 were considered to indicate statistical significance.

4 RESULTS AND DISCUSSION

The *Solanum* glycoalkaloids have been intensively studied during recent decades and as a result of substantial research efforts, thousands of articles concerning various aspects of glycoalkaloids have been published. The use of wild *Solanum* species in potato improvement has stimulated interest in the glycoalkaloids formed by wild *Solanum* species and their interspecific hybrids. To introgress desirable resistance traits into the cultivated potato genome, various interspecific somatic hybrids have been produced between *S. tuberosum* and wild *Solanum* species, such as *S. acaule* (Yamada et al. 1997; Rokka et al. 1998b,c; Kozukue et al. 1999), *S. brevidens* (Barsby et al. 1984; Austin et al. 1985; Fish et al. 1987; Rokka et al. 1994), and *S. etuberosum* (Novy & Helgeson 1994b; Thieme & Thieme 1998). For example, viral disease resistance (PLRV, PVY, PVX) and bacterial disease resistance (*Erwinia* soft rot) were expressed in the somatic hybrids between *S. brevidens* and *S. tuberosum* (Austin et al. 1985; Helgeson et al. 1986; Austin et al. 1988; Gibson et al. 1988; Pehu et al. 1990a; Helgeson et al. 1993; Rokka et al. 1994; McGrath et al. 2002), although *S. brevidens* (E genome species) and *S. tuberosum* (A genome species) are distantly related. In addition, the somatic hybrids between *S. etuberosum* (E genome) and *S. tuberosum* have carried many desirable traits, such as resistance to viral disease (PVY) and aphids (*M. persicae* and *A. solani*) (Novy & Helgeson 1994a,b; Thieme et al. 1999, 2003; Gavrilenko et al. 2003). Due to the wild species *S. acaule* and *S. tuberosum* being closely related A genome species, certain desirable characters derived from *S. acaule* (e.g. resistance to PVX) were successfully transferred to cultivated potato germplasm (Ross 1986). *Solanum acaule* also expresses many other desirable disease and nematode resistance traits as well as beneficial physiological characters, such as tolerance to frosts and cold (Ross 1986; Hawkes 1994).

Glycoalkaloids are generally considered to be an undesirable character of potato, which is why analyses are of such interest. However, high glycoalkaloid content or certain glycoalkaloids are linked to some desirable traits, such as resistance to Colorado potato beetle (Stürckow & Löw 1961; Sinden et al. 1986a; Sinden et al. 1988; Deahl et al. 1991; Silhavy et al. 1996; Sanford et al. 1997), certain fungal diseases (Fewell & Roddick 1993, 1997; Fewell et al. 1997) and tolerance to cold (Burton 1989). According to the literature, the foliar or tuber glycoalkaloid content of at least 70 *Solanum* species has already been determined (Osman et al. 1978; Gregory et al. 1981; van Gelder et al. 1988b; Deahl et al. 1993). Glycoalkaloid concentrations in a wide range of species have been analysed and many other glycoalkaloids than those present in *S. tuberosum* have been found. Therefore, in the present study, variation was investigated in qualitative and quantitative glycoalkaloid aglycone contents in interspecific *Solanum* hybrids of various genome constitutions.

4.1 Optimisation of the GC-MS method for glycoalkaloid aglycone analyses (I, II)

4.1.1 Sample preparation (I, II)

In the present study, glycoalkaloids were extracted and hydrolysed according to the modified procedure (I, Figure 1). Laborious evaporation of acetic acid extracts by nitrogen flow (I) was replaced by a freeze-drying process (II). In addition, after alteration of the hydrolysis solvent 2M HCl and methanol in study I, in which solanthrene was found in small amounts, to 1M HCl in methanol (II), no solanthrene was detected in the *S. brevidens* + *S. tuberosum* somatic hybrids or in the *S. tuberosum* parental lines. The result was consistent with earlier reports that acid hydrolysis with ethanol or methanol compared with aqueous hydrolysis prevented

the dehydration of solanidine to solanthrene (Coxon et al. 1979; King 1980; van Gelder 1984; Lawson et al. 1992).

4.1.2 Silylation of aglycone samples (I)

Due to their high molecular weight and low volatility, glycoalkaloids and their aglycones are problematic in GC analyses. Derivatisation can, however, be used to improve the thermal stability and volatility of the compounds (Willard et al. 1988). Derivatisation of glycoalkaloids and their aglycones using different methods, for example permethylation (Herb et al. 1975; Osman et al. 1978; Gregory et al. 1981) and trimethylsilylation (Juvik et al. 1982; Gaffield & Keeler 1996; Griffiths et al. 2000) has been done, but is not a very widely used technique in glycoalkaloid analyses. Glycoalkaloid aglycones can be analysed without derivatisation (e.g. van Gelder et al. 1988, 1989; Lawson et al. 1992), but then quite high analysis temperatures have to be used, which may decrease column age and cause decomposition of compounds. In the present study, after silylation with BSTFA + 1% TMCS, trimethylsilyl (TMS) derivatives of solanidine- and tomatidine-type aglycones were successfully analysed using GC-MS (I, Figure 2). Repeatability of the method was 5–7% (CV) for the principal aglycones (I, Table 1). The molecular ions *m/z* 469 and 471 showed that monoTMS derivatisation of solanidine and demissidine occurred because the molecular weights of silylated solanidine and demissidine increased by 72 a.m.u. (atom mass unit), which indicated attachment of a TMS group to the molecule. The base peaks (*m/z* 150 and 204) of silylated solanidine and demissidine were identical to the base peaks of underivatised solanidine and demissidine (Budzikiewicz 1964; van Gelder et al. 1989). However, solanidine and demissidine can be separated using an appropriate column without an MS apparatus. The base peak *m/z* 125 and the molecular ion *m/z* 559 of tomatidine indicated diTMS derivatisation. In this study, the procedure was established especially for the analyses of solanidane-based aglycones as TMS derivates by GC-MS.

4.1.3 Combined derivatisation method (II)

Although silylation was used, broadening of the peaks was sometimes observed and the resolution was inadequate, especially when the compounds of tomatidine-type at high concentrations were analysed. Furthermore, the base peak of tomatidine (*m/z* 125) was quite unspecific. In the present study a novel combined derivatisation method, including trimethylsilylation using MSTFA reagent followed by acylation using pentafluoropropionic acid anhydride PFAA, was developed to improve GC resolution and to produce more specific and abundant fragments for the aglycones of the tomatidine-type. The repeatability of the method ranged between 1 and 6% (CV) for the main aglycones (II, Table 3). According to a recovery test, the mean percent recovery of tomatidine was 113%. Notable decomposition of derivatised aglycones was not detected in re-analyses of the hybrid sample (CV from 7.5 to 12.3% for the main aglycones), when an automatic sampler was used during 30 hours (unpublished data). When the combined derivatisation technique was employed, no broadening of the peaks was evident (II, Figure 3).

Solanidanes. The combined derivatisation method did not affect the fragmentation of solanidine and demissidine compared with the base peaks of TMS-silylated solanidine and demissidine, which were presented in paper I. In the structure of solanidine and demissidine there is only one hydroxyl group available for a TMS-group and no position for a

pentafluoropropionyl group. Thus, the major fragments *m/z* 150 ($C_{10}H_{16}N$) and 204 ($C_{14}H_{22}N$) and the molecular ions *m/z* 469 and 471 of solanidine and demissidine remained unchanged.

Spirosolanes. The major alterations and improvements were seen in the fragmentation of tomatidine-type structures. After addition of MSTFA, a TMS-group was attached to positions 3 and 16 in tomatidine-type structures. A pentafluoropropionyl group was attached to nitrogen after addition of the acylation reagent PFAA. The combined derivatisation technique formed specific fragments for spirosolanes (II, Tables 1 and 2, Figure 2). The fragment *m/z* 269 ($C_{11}H_{12}NOF_5$) was common for all spirosolanes and was formed by the attachment of a pentafluoropropionyl group. Furthermore, specific fragments *m/z* 417 ($C_{24}H_{41}O_2Si_2$) of solasodine and tomatidenol, and *m/z* 419 ($C_{24}H_{43}O_2Si_2$) of tomatidine and assumed soladulcidine were formed. The presence or absence of a double bond at the 5,6 position of the aglycone can now be seen as the difference between the base peak fragments *m/z* 417 and 419. Even though the two spirosolanes had the same base peak, retention times of all these four spirosolanes could be differentiated (II, Figure 4).

Specific fragments *m/z* 417 and 419 and individual retention times obtained by the combined derivatisation technique enabled more reliable detection and quantification of the aglycones of spirosolane-types. Using this derivatisation technique, solasodine and tomatidine differing from each other by the existence of a double bond at the position 5,6, formed distinct peaks (II, Figure 2 and 4). Tomatidine and tomatidenol were also able to be separated from each other. In total ion analyses (TIC, total ion chromatogram), tomatidine was superimposed on tomatidenol, but using ion *m/z* 417 tomatidenol was identified (II, Figure 4). Solasodine and tomatidenol were also distinguished from each other even though they had the same molecular weights and the same base peaks (*m/z* 417). This was because they had the opposite configurations at C-22 and C-25 in their structures, which produced clearly separate peaks. Not only solasodine and tomatidenol, but also tomatidine and soladulcidine form another isomeric pair with the same molecular weight but opposite configurations at C-22 and C-25. The peaks of tomatidine and putative soladulcidine were clearly separated from each other despite having the same base peak fragment (*m/z* 419) (II, Figure 4). In conclusion, opposite configurations at C-22 and C-25, but not double bond differences, gave clearly distinct peaks.

The silylation of spirosolanes presented in paper I also became clearer in paper II. Although tomatidine had only one hydroxyl group in its structure, the formation of the diTMS structure (the molecular ion *m/z* 559 and the base peak *m/z* 125, $C_8H_{15}N$) after silylation was proposed (I) and later confirmed (II). The first TMS-group was attached to the hydroxyl group at position 3 of the aglycone. According to the NMR analysis (II), the second TMS-group was attached to the hydroxyl group, which was formed after the opening of the tetrahydrofuran ring. Opening the tetrahydrofuran ring seemed to be related to the occurrence of the nitrogen ring.

The new derivatisation method combined with the SIM (selected ion monitoring) technique enabled the analyses of the same aglycones both in adult primary hybrid plants and in two-week-old in vitro grown hybrid plants (II, Figure 5). The GC-MS technique is therefore well suited for plant breeding and screening purposes, when minor and novel aglycones are expected and large numbers of samples have to be screened. The validity of GC-MS technique appears in the identification of new compounds as a result of the specificity and sensitivity of it. Low concentrations of compounds or low sample amounts are not the restrictive factors in GC-MS analyses. Furthermore, known aglycone profiles can even be sufficiently analysed by GC. However, the lack of knowledge of the glycoalkaloid glycosides

is considered to be a limitation of GC-MS technique. Analysis of entire glycoalkaloid glycosides is impossible by GC-MS technique. The need of hydrolysis of glycoalkaloids and also derivatisation of aglycones require prolonged sample preparation and analysis times. In addition, due to the described works and expensive investments, GC-MS analysis technique is regarded as a relatively costly method.

4.2 Glycoalkaloid aglycone contents in the hybrids between A and E genome *Solanum* species (I, III)

4.2.1 Formation of demissidine (I)

The main glycoalkaloid aglycone of *S. brevidens* was tomatidine and of *S. tuberosum* was solanidine. Both parental-derived aglycones were also found in all the somatic hybrids between *S. brevidens* and *S. tuberosum* (I, Figure 2). Furthermore, a novel aglycone, demissidine, was identified in each interspecific hybrid between the A and E genome species.

In the present study, a hypothetical pathway was proposed for the formation of demissidine aglycone in the somatic hybrids (I, Figure 6). According to this pathway, a hydrogenase enzyme encoded by *S. brevidens* mediated the production of tomatidine through hydrogenation of the double bond of the teinemine precursor. In the somatic hybrids the proposed enzyme hydrogenated the double bond of solanidine leading to the production of demissidine. The only difference between solanidine and demissidine is the presence of a double bond at position 5,6 in solanidine, which is absent in demissidine. This hypothetical pathway for demissidine formation was based on the view that teinemine was the precursor of solasodine, tomatidine, and solanidine (Kaneko et al. 1976; van Gelder 1991).

In addition to its presence in the *S. brevidens* + *S. tuberosum* hybrids, demissidine was also found as a novel aglycone in the *S. etuberosum* + *S. tuberosum* hybrids (*Table 4*). Similarly, demissidine was analysed earlier in the somatic hybrids between *S. circaeifolium* and *S. tuberosum*, in addition to the parental glycoalkaloid aglycones tomatidine and solanidine (Mattheij et al. 1992). In contrast to the results of the present study, Vallin et al. (1996) reported that the *S. brevidens* + *S. tuberosum* somatic hybrid contained identical glycoalkaloids to the parental species (solanine and chaconine in *S. tuberosum* and two uncharacterised glycoalkaloids in *S. brevidens*). It can be speculated that the reason why no novel glycoalkaloid was detected in that hybrid may be due to the analytical method used or to the genotype of *S. brevidens*, PI 218228. It can be supposed, however, that the unknown compounds detected in *S. brevidens* and in the hybrid (Vallin et al. 1996) were tomatine-type structures because tomatine was not identified in their study. Furthermore, intergeneric potato + tomato somatic hybrids contained the glycoalkaloids of parental origin (solanine, chaconine, and tomatine) (Roddick & Melchers 1985), but additional uncharacterised compounds that resembled glycoalkaloids were also detected. Also, non-parental glycoalkaloids have been identified in progenies derived from crosses between various *S. chacoense* genotypes (McCollum & Sinden 1979).

4.2.2 Aglycone composition (I, III)

Similarly to *S. brevidens*, the main glycoalkaloid aglycone in *S. etuberosum* was tomatidine (*Table 4*). As a result of the improvement in the analysis technique due to the combined

derivatisation, tomatidenol was successfully identified and separated from tomatidine in both *S. brevidens* (II, Figure 4) and *S. etuberosum* using ion *m/z* 417. Recently, due to the improved analysis technique, dehydrotomatine (tomatidenol) was also detected as an impurity in commercial tomatine chemical products and separated from tomatine (tomatidine) in tomato plants (Bushway et al. 1994; Friedman et al. 1994; Ono et al. 1997). In addition, putative soladulcidine was supposed to be one of the glycoalkaloid aglycones in *S. brevidens* and *S. etuberosum*. Also in paper I using the silylation, the proposed soladulcidine was most probably detected in *S. brevidens* (I, Figure 2, peak 4). However, in paper I, *S. brevidens* was suggested to contain tomatidine and three unidentified aglycones.

Table 4. *Mean concentrations (mg/kg dw) and proportions (%) of the main glycoalkaloid aglycones in the somatic hybrids between S. etuberosum and S. tuberosum and their backcross (BC_1 and BC_2) progenies.*

			Solanidine			Demissidine			Tomatidine			Total	
Genotype	**Genome ratio***	**N**	**Mean**	**Sd**	**%**	**Mean**	**Sd**	**%**	**Mean**	**Sd**	**%**	**Mean**	**Sd**
Parental lines													
S. etuberosum k-9141	EE	10							18470	2655	100	18470	2655
S. tuberosum T67	AA	7	1607	271	100							1607	271
S. tuberosum N90	AAAA	6	1023	259	100							1023	258
Somatic hybrids													
26/2/11/1	nd.	7	315	105	40	318	107	41	144	43	19	777	238
27/2/12/1	AAAAEE	8	291	53	34	285	57	33	284	36	33	860	138
27/2/14/1	AAEEEE	9	51	17	1	93	29	3	3783	575	96	3927	603
8/1/2/1	AAEEEE	8	25	11	1	40	15	1	3342	430	98	3407	451
6/1/2/1	AAEEEE	8	79	29	2	136	54	3	3935	658	95	4150	731
BC_1 progeny													
64/10	AAAEE	8	925	160	45	628	132	31	516	183	24	2069	312
BC_2 progenies													
64/10/1/1	AAA(A)(E)	5	913	52	74	288	48	23	37	17	3	1230	104
64/10/1/3	nd.	7	1680	326	65	261	57	10	661	172	25	2602	437
64/10/2/1	nd.	6	220	76	72	70	18	23	12	4	5	302	93
64/10/4/1	AAA(A)(E)	5	1234	341	72	395	97	24	70	18	4	1699	370
64/10/7/1	nd.	8	820	83	72	227	18	20	91	47	8	1138	109
64/10/8/1	nd.	10	1051	203	74	350	60	25	22	7	1	1419	259
64/10/16/1	nd.	6	962	251	64	426	142	28	122	71	8	1510	398
64/10/23/4	nd.	4	1003	278	68	446	121	30	24	8	2	1473	406

* Gavrilenko et al. 2003
nd., not determined

The principal glycoalkaloid aglycones solanidine, demissidine, and tomatidine were found in leaves of all the primary somatic hybrids between *S. brevidens* and *S. tuberosum* and in their second generation hybrids (I, Figure 2; III, Figure 1) and in the *S. etuberosum* + *S. tuberosum* primary somatic hybrids and in their backcross (BC) progenies (*Table 4*). Also tomatidenol and putative soladulcidine were identified in most of the *S. brevidens* + *S. tuberosum* (II, Figure 4) and *S. etuberosum* + *S. tuberosum* primary hybrids. Similarly, tomatidenol was also detected in the somatic hybrids between *S. circaeifolium* and *S. tuberosum* (Mattheij et al. 1992). The main aglycones in the second generation (III) and in the BC hybrids (*S.*

etuberosum, *Table 4*) were identical to the aglycones found in the primary hybrids. However, the glycoalkaloid profiles can also be different in BC hybrids than in the primary hybrids (Sanford et al. 1998; Veilleux & Miller 1998; Carputo et al. 2003).

Identification of tomatidenol in parallel with tomatidine in *S. brevidens* and *S. etuberosum* species and in the somatic hybrids was consistent with the observations that plants usually produce two glycoalkaloids (e.g. potato: solanine–chaconine, tomato: tomatine–dehydrotomatine [tomatidine–tomatidenol aglycones]) (Friedman & McDonald 1999; Friedman 2002). Due to sample hydrolysis for GC-MS analysis, information on the composition of all the glycoalkaloid glycosides in hybrids was lost. However, it can be speculated that solanidine is derived from solanine and chaconine, tomatidine from tomatine, demissidine from demissine or commersonine, tomatidenol from α-solamarine, β-solamarine or dehydrotomatine, and the putative soladulcidine is derived from soladulcine.

4.2.3 The effect of genome constitution on aglycone contents (I, III)

Primary hybrids. Tomatidine concentration in *S. brevidens* was high and ranged from 3852 mg/kg (III) to 8173 mg/kg dw (I). Tomatidine levels analysed in *S. brevidens* in the present study were not consistent with the very low total glycoalkaloid contents earlier determined in *S. brevidens* (233 mg/kg dw) (Vallin et al. 1996). The mean tomatidine concentration (18 470 mg/kg) in *S. etuberosum* (*Table 4*) was higher than that in *S. brevidens*. However, the differences in tomatidine concentrations between *S. brevidens* and *S. etuberosum* cannot be directly compared because they were grown at different periods and probably under slightly different conditions.

There was also a wide variation in the total aglycone content in the tetraploid and hexaploid primary somatic hybrids between *S. brevidens* and *S. tuberosum* (I, III). In paper I, the total aglycone levels in the hybrids were closer to the mid-parent levels than to the lower parental level of *S. tuberosum*. However, in paper III, the total aglycone concentrations in the somatic hybrids and their (somato)haploids were considerably lower compared with the tomatidine content of *S. brevidens*, but the concentrations were at a similar level to the solanidine concentration of the *S. tuberosum* parental line, Pito 4. Moreover, in all the primary somatic hybrids between *S. etuberosum* and *S. tuberosum* (*Table 4*), the mean total aglycone content was much lower than the mid-parent level, which may be an indication of the dominance effect of the *S. tuberosum* parent for low glycoalkaloid synthesis (Ross 1966). The inheritance of glycoalkaloids is not clear and there are studies (Sanford & Sinden 1972; van Dam et al. 1999) that do not support the conclusion of Ross (1966). Sanford et al. (1994, 1995) observed lower total glycoalkaloid content than the mid-parent mean content in the F_2 hybrids derived from a cross between *S. tuberosum* and *S. chacoense*. This was in agreement with the hypothesis of the dominance effect of the low level synthesis presented by Ross (1966). On the other hand, the amounts were supposed to be too high to indicate complete dominance. There are also results published that have shown intermediate or even higher glycoalkaloid concentrations in hybrids compared with the parental lines (Louwes et al. 1992; Mattheij et al. 1992; Kozukue et al. 1999). In the present study, the genome constitution of the hybrids also affected the total aglycone amounts in the *S. etuberosum* + *S. tuberosum* hybrids. The hexaploid 2x *etb* + 4x *tbr* (AAAAEE) hybrid (27/2/12/1) and the hybrid 26/2/11/1 (unknown genome composition) had significantly lower ($p<0.001$) total aglycone levels than the hexaploid 4x *etb* + 2x *tbr* (AAEEEE) hybrids.

The genome constitution of the hybrids also affected the relative proportions of aglycones. The relative proportion of tomatidine was significantly lower in the *S. brevidens* + *S. tuberosum* hexaploid hybrids (2x *brd* + 4x *tbr*, AAAAEE), in which one third of the genome consisted of *S. brevidens*, in comparison with the tetraploid 2x *brd* + 2x *tbr* (AAEE) and hexaploid 4x *brd* + 2x *tbr* (AAEEEE) hybrids (I). The highest solanidine proportion was detected in the 2x *brd* + 4x *tbr* (AAAAEE) hybrids. It was noticed that in the hybrids the relative proportion of tomatidine correlated positively with the proportion of the genome of *S. brevidens* (I, Figure 4). Furthermore, there was a positive correlation between the relative proportions of solanidine and demissidine in the *S. brevidens* + *S. tuberosum* somatic hybrids (I, Figure 3), but the relative proportions of tomatidine and demissidine correlated negatively. In paper III, the relative proportion of tomatidine was higher than the relative proportion of solanidine and demissidine in the entire *S. brevidens* + *S. tuberosum* hybrid material (III, Table 1). The relative proportion of demissidine was also high in the hexaploid 4x *brd* + 2x *tbr* (AAEEEE) hybrids and the triploid anther-derived (somato)haploid. The relative proportion of solanidine was similar in both tetraploid 2x *brd* + 2x *tbr* (AAEE) and hexaploid 4x *brd* + 2x *tbr* (AAEEEE) hybrids. Similarly to the *S. brevidens* + *S. tuberosum* hybrids, the genome constitution also affected the aglycone proportions in the *S. etuberosum* + *S. tuberosum* hybrids. The relative proportions of all the aglycones in the 2x *etb* + 4x *tbr* (AAAAEE) hybrid (27/2/12/1) and in 26/2/11/1 hybrid (unknown genome composition) were significantly different ($p<0.001$) from the relative aglycone proportions the 4x *etb* + 2x *tbr* (AAEEEE) hybrids (*Table 4*). The mean tomatidine proportion of the three 4x *etb* + 2x *tbr* (AAEEEE) hybrids was as high as 96%, while the mean solanidine and demissidine proportions were only about 2%. The reason for the high tomatidine proportions in the 4x *etb* + 2x *tbr* (AAEEEE) hybrids is not known, but it may be inherited from *S. etuberosum* because there was already a high tomatidine concentration in *S. etuberosum*. Finally, it can be concluded that the higher the genome portion of *S. tuberosum* the more enhanced production of solanidine was expressed in the somatic hybrids.

The tetraploid and hexaploid *S. brevidens* + *S. tuberosum* primary somatic hybrids did not exhibit differences from their anther-derived diploid and triploid (somato)haploids in terms of their relative proportions of glycoalkaloid aglycones (III). However, the diploid *S. chacoense* genotypes produced significantly higher mean leptine and total glycoalkaloid concentrations in their foliage than the corresponding tetraploid genotypes but no differences between proportions were found (Sanford et al. 1997). Also, in *S. acaule* the ploidy level influenced the amount of tomatine but not of demissine (Kozukue et al. 1999).

The relative proportions of aglycones in the *S. brevidens* + *S. tuberosum* hybrids presented in paper I differed from those presented in paper III, although the genome constitutions of the hybrids were similar in both studies. In the hexaploid 4x *brd* + 2x *tbr* (AAEEEE) hybrids (I), the tomatidine proportion was higher and the demissidine proportion was lower than in the corresponding hexaploid (AAEEEE) and triploid (AEE) hybrids in paper III. The proportion of solanidine was similar in the hexaploid hybrids with the same genome constitution. In the tetraploid 2x *brd* + 2x *tbr* hybrids, the proportion of solanidine varied (papers I and III), but the proportions of demissidine and tomatidine were equivalent. The aglycone proportions in the 2x *etb* + 4x *tbr* (AAAAEE) hybrid (27/2/12/1) and in the 26/2/11/1 hybrid (unknown genome composition) were at similar levels as the mean proportions of 2x *brd* + 4x *tbr* (AAAAEE) hybrids (I), but the aglycone proportions of the 4x *etb* + 2x *tbr* (AAEEEE) hybrids were different from those of the 4x *brd* + 2x *tbr* (AAEEEE) hybrids (I, III). However, the mean total aglycone content in *S. etuberosum* + *S. tuberosum* hybrids was more similar to the level in *S. brevidens* + *S. tuberosum* hybrids presented in paper I, but much higher than

presented in paper III. *Solanum brevidens* and *S. etuberosum* share the same E genome (Perez et al. 1999), but obviously the reason for the variation in the aglycone proportions between the *S. brevidens* + *S. tuberosum* and *S. etuberosum* + *S. tuberosum* primary somatic hybrids was due to the difference between the E genome species. In addition, the difference in the aglycone proportions within the 2x *brd* + 2x *tbr* (AAEE) hybrids (I, III) could be explained by the different parental genotypes of *S. tuberosum*. As previously described, the choice of nonleptine-producing parents was an important factor in determination of the amount and proportion of leptines synthesised in the hybrids (Sanford et al. 1996b; Veilleux & Miller 1998). Also different growth conditions may be another important factor affecting aglycone proportions and amounts (van Gelder & Scheffer 1991; Nitithamyong et al. 1999).

Second generation somatic hybrids and BC hybrids. The effect of genome constitution of the hybrids on aglycone proportions was clearly seen in the *S. brevidens* + *S. tuberosum* second generation somatic hybrids (AAAEE) (III) and in the *S. etuberosum* + *S. tuberosum* BC_1 (AAAEE) and BC_2 progeny [AAA(A)(E)] (*Table 4*). In the second generation somatic hybrids the mean total concentrations were at an approximately similar level compared with the primary *S. brevidens* + *S. tuberosum* hybrids and their (somato)haploids. Selection for reduced glycoalkaloid content should be possible in the BC hybrids (Sanford et al. 1998; Veilleux & Miller 1998; Sarqúis et al. 2000; Carputo et al. 2003), even though it was not consistently observed in the second generation and BC hybrids in the present study. Alteration of the genome constitution in the second generation hybrids (AAAEE) closer to the *S. tuberosum* type promoted a significantly higher relative proportion of solanidine (average 29%) compared with the *S. brevidens* + *S. tuberosum* primary hybrids and their (somato)haploids (10%). A similar trend towards a higher solanidine proportion was also seen in the 2x *brd* + 4x *tbr* hybrids (I), which had a higher A genome dose, as described by Sanford et al. (1998). A more marked modification of the glycoalkaloid type of *S. tuberosum* was observed in the BC_1 (AAAEE) and BC_2 hybrids containing *S. etuberosum* genome. There was a progressive elimination of the alien E genome, but increase of the A genome, which caused reduced tomatidine but increased solanidine expression. The mean solanidine proportion in the BC_2 hybrids was as high as 70%. Demissidine proportions were increased in the BC_1 and BC_2 hybrids compared with those of demissidine in the parental hybrids earlier selected for backcrossing. Moreover, the glycoalkaloids of *S. commersonii* rapidly disappeared in the BC population derived from crosses between *S. commersonii* and *S. tuberosum* (Carputo et al. 2003).

4.3 The somatic hybrids between two A genome species (*S. acaule* and *S. tuberosum*) (IV, V)

4.3.1 Bacterial ring rot immunity tests in the *S. acaule* + *S. tuberosum* somatic hybrids (IV)

The wild species *S. acaule* expresses desirable resistance traits to diseases and nematodes that affect cultivated potato and it also possesses beneficial physiological characters (Ross 1986; Hawkes 1994). As *S. acaule* and *S. tuberosum* are both A genome species, they are closely related. The genome formula of tetraploid *S. acaule* is proposed to be AAA^aA^a (Matsubayashi 1982) or $A_2A_2A_3A_3$ (Hawkes 1994), while tetraploid *S. tuberosum* has the genome formula of AAAA or AAA^tA^t (Hawkes 1990, 1994). Some desirable characters of *S. acaule* (e.g. resistance to PVX) have been successfully transferred into cultivated potato germplasm (Ross 1986). One desirable character of *S. acaule* acc. PI 472655.8 (7-8) was proposed to be

immunity to bacterial ring rot disease (Ishimaru et al. 1994; Kriel et al. 1995a,b) following screening of a range of wild *Solanum* species for potential immunity to infection by *C. michiganensis* ssp. *sepedonicus* (Kurowski & Manzer 1992; Kriel et al. 1995a). The immunity trait was thought to be encoded by two dominant genes (Kriel et al. 1995b).

The goal of this study was to incorporate immunity to *Cms* into the interspecific somatic hybrids between *S. acaule* and *S. tuberosum* (IV). However, all the *S. acaule* + *S. tuberosum* somatic hybrids in this study exhibited typical ring rot symptoms and were susceptible to infection by *Cms* in the greenhouse (IV, Figure 1). If the trait were due to dominant genes, it would be expected to be expressed in the somatic hybrids (Wenzel et al. 1979). However, no evidence of dominance of the trait in the somatic hybrids was found. The genome composition of the somatic hybrids, however, influenced bacterial titres. The 4x *acl* + 2x *tbr* somatic hybrids supported reduced replication of *Cms* compared with the 2x *acl* + 4x *tbr* or 2x *acl* + 2x *tbr* hybrids in all the other replications except one (IV, Figure 1). The 2x *acl* + 4x *tbr* and 2x *acl* + 2x *tbr* hybrids did not statistically differ from each other. Thus, a higher proportion of *S. acaule* genome enhanced the lower replication of *Cms*. In addition, interactions between the genomes of *S. acaule* and *S. tuberosum* may also have affected replication of *Cms*. The occasional chromosome loss observed in the *S. acaule* + *S. tuberosum* hybrids (Yamada et al. 1998a) is unlikely to have been responsible for the suppression of resistance to *Cms*, because most of the hybrids were euploid (V).

4.3.1.1 Temperature-dependent immunity of *S. acaule*

In the present study, none of the *S. acaule* lines exhibited visible ring rot symptoms as a result of infection by *Cms*. The dihaploid *S. acaule* lines 10 and 119 were immune to infection, but tetraploid *S. acaule* 7-8 and dihaploid *S. acaule* 86 contained bacteria when grown in the greenhouse (IV, Figure 1). All the other dihaploid lines were infected by *Cms* (IV, Table 3), but there were also uninfected plants in each *S. acaule* line. Based on "fixed heterozygosity" of *S. acaule*, variation in responses to bacterial ring rot between different dihaploids was expected because of the higher level of heterozygosity of the dihaploids compared with tetraploid *S. acaule* (Yamada et al. 1998a).
Due to the variation in the number of infected and non-infected plants in the material derived from *S. acaule* and because ring rot immunity in tetraploid *S. acaule* 7-8 to *Cms* was not observed under greenhouse conditions, additional studies concerning *S. acaule* 7-8 were carried out. *Solanum acaule* produces tubers only under short day (12 h) and cool conditions (15 °C) and thus did not form tubers in the greenhouse at 18-22 °C (Rokka et al. 1998c). To investigate the influence of temperature on immunity, plants of *S. acaule* 7-8 were inoculated and placed in the growth chamber set at either 15 or 21 °C. Symptoms of ring rot were not observed at either temperature. Based on the IFAS tests, at 21 °C *S. acaule* 7-8 plants were immune to *Cms* (IV, Table 2), but at 15 °C the plants were infected and contained *Cms* bacteria. Thus, temperature had a highly significant effect on the response of *S. acaule* 7-8 to ring rot.

Temperature-dependent resistance of plants is a common phenomenon. Studies with fungal diseases have shown that in some cases resistant plants have become susceptible at lower temperature (Gousseau et al. 1985; Islam et al. 1989; Judelson & Michelmore 1992; Balass et al. 1993; Ge et al. 1998). Similarly, resistance can also be induced by lowering the temperature (Fraser & Loughlin 1982; Kaul & Shaner 1989; Roderick et al. 2000). The effect of temperature on resistance to viral diseases has been reported (Valkonen 1997; Valkonen &

Watanabe 1999). Several mechanisms for temperature-dependent resistance were proposed (Islam et al. 1989; Judelson & Michelmore 1992; Valkonen et al. 1998; Roderick et al. 2000). For example, it was suggested that temperature may have altered the expression of either avirulence or resistance genes or temperature may have affected their interactions (Judelson & Michelmore 1992). In addition, it was suggested that there is interaction between resistance genes and genes affected by temperature (Roderick et al. 2000). Valkonen et al. (1998) concluded that the temperature-dependent modifier gene of the potato cultivar Pito altered symptom expression in PVY^{o} infected potatoes. However, in the present study, the mechanism for temperature-dependent response in the case of *S. acaule* remains unknown.

4.3.2 The effect of genome constitution of *S. acaule* + *S. tuberosum* somatic hybrids on glycoalkaloid aglycone contents (V)

Solanum acaule contained demissidine, tomatidine, and trace amounts of solanidine aglycones (V, Table 4) as reported by van Gelder et al. (1988b). In contrast to the present study, Kozukue et al. (1999) using various analytical methods did not find solanine and chaconine (solanidine) in their *S. acaule* lines. In the studies with *S. brevidens* and *S. etuberosum* species (II and *Table 4*), tomatidenol aglycone was masked by tomatidine. However, in total ion analyses of *S. acaule* lines the specific fragment of tomatidenol (*m/z* 417) was not found, but putative soladulcidine was detected. All the somatic hybrids between *S. acaule* and *S. tuberosum* contained solanidine, demissidine, and tomatidine aglycones (V, Table 4) and also putative soladulcidine was found in some hybrid plants.

The mean total aglycone contents in the *S. acaule* + *S. tuberosum* somatic hybrids were at similar levels to those of the *S. acaule* lines (V, Table 4). In the *S. acaule* + *S. tuberosum* somatic hybrids of Kozukue et al. (1999), the glycoalkaloid contents in tubers were intermediate between values for total amount in the parental species. Additionally, in the present study, the total aglycone concentrations among the somatic hybrids were similar, although the total amounts in 2x *acl* + 2x *tbr* hybrid were significantly lower than those in the 2x *acl* + 4x *tbr* hybrid. However, the genome constitution of the somatic hybrids affected the concentrations and the relative proportions of aglycones (V, Table 4). A higher proportion of *S. tuberosum* genome in the 2x *acl* + 4x *tbr* hybrid enhanced production of solanidine (amount and proportion) compared with the remaining hybrids. The effect of the higher proportion of *S. tuberosum* genome on aglycone production has also been detected previously (Sanford et al. 1998; I, III, and *Table 4*). Correspondingly, a higher proportion of *S. acaule* genome in the 4x *acl* + 2x *tbr* hybrid increased the proportion of demissidine. This may be due to the high demissidine proportion in the *S. acaule* parental line. In the *S. brevidens* + *S. tuberosum* and *S. etuberosum* + *S. tuberosum* (A + E genome) hybrids (I and *Table 4*) the increase of wild species genome raised the tomatidine proportion. The increase of demissidine proportion was a result of a higher proportion of wild species DNA in the *S. acaule* + *S. tuberosum* hybrids and is consistent with the results presented in paper III. On the other hand, the basis for aglycone proportions/contents in the *S. acaule* + *S. tuberosum* hybrids differs from the *S. brevidens* + *S. tuberosum* and *S. etuberosum* + *S. tuberosum* hybrids because the *S. acaule* parental lines originally contained demissidine while *S. brevidens* and *S. etuberosum* only formed tomatidine.

4.3.3 Influence of *C. michiganensis* ssp. *sepedonicus* on glycoalkaloid aglycone accumulation (V)

The preliminary results of the present study showed that the mean solanidine, demissidine, and total aglycone concentrations in leaves of *S. acaule* + *S. tuberosum* somatic hybrids infected by *Cms* were significantly lower than in leaves of the corresponding mock-inoculated plants (V, Figure 1). The decrease in aglycone concentration after *Cms*-inoculation was also observed in the lines of *S. acaule* 7-8 and *S. acaule* 86 (V, Figure 2). In Pito, the effect of *Cms*-infection on solanidine content was reversed when compared with any other lines tested. Increased solanidine levels in *Cms*-infected Pito were consistent with results from a study of *C. michiganensis* ssp. *michiganensis* in which tomatine was accumulated in the leaves of a susceptible tomato cultivar after bacterial infection (Beimen et al. 1992). Also Lachman et al. (2001) reported that high levels of glycoalkaloids were found in potatoes in response to rotting caused by fungi or bacteria. However in tomato, tomatine levels decreased in the leaves after infection by *Xanthomonas campestris* pv. *vesicatoria* bacteria (Kumar & Prasad 1989) and in shoots of susceptible varieties after infection by *Pseudomonas solanacearum* (Smith) Smith bacteria (Mohanakumaran et al. 1969).

The decrease in aglycone levels in the *S. acaule* + *S. tuberosum* hybrids after *Cms*-infection was similar to the results of earlier studies, in which suppression of glycoalkaloid accumulation after different elicitor or fungal pathogen inoculation/treatment was reported (Shih et al. 1973; Tjamos & Kuć 1982; Zook & Kuć 1987; Choi et al. 1994; Mucharromah et al. 1995; Dimenstein et al. 1997; Andreu et al. 2001). Furthermore, in a study of *P. infestans*, reduction of glycoalkaloid accumulation was greater after inoculation with an incompatible rather than a compatible race of *P. infestans* (Shih et al. 1973). During the suppression of glycoalkaloid accumulation, inhibition of squalene synthetase redirected biosynthetic pathways from sterol/steroid glycoalkaloid synthesis towards sesquiterpenoid phytoalexin synthesis (Stermer & Bostock 1987; Vögeli & Chappell 1988; Zook & Kuć 1991). However, in the present study, sesquiterpenoid compounds were not measured. Even though the *S. acaule* + *S. tuberosum* hybrids were susceptible to *Cms* and showed ring rot symptoms (IV), the hybrids responded to inoculation with *Cms* in a similar way to the tolerant *S. acaule* by reduced glycoalkaloid accumulation. The suppressed glycoalkaloid concentrations may be a trait derived from the *S. acaule* wild potato that also becomes expressed in interspecific hybrids during interaction with *Cms*. It can be concluded that infection by *Cms* affected glycoalkaloid accumulation in the potato material analysed. Overall, the results raised two questions: 1) are glycoalkaloids involved in defence mechanisms against *Cms*? 2) do the glycoalkaloids have an essential role in the interaction between plant and bacteria?

Many secondary metabolites produced by plants are known to be defensive compounds against plant pathogens and pests (Osbourn 1996a,b; Dixon 2001; Wittstock & Gershenzon 2002). In common with other secondary metabolites, glycoalkaloids may be strongly linked to plant defence mechanisms, especially to resistance responses to various fungal diseases and attack by insects (Stürckow & Löw 1961; Sinden et al. 1986a, 1988; Deahl et al. 1991; Fewell & Roddick 1993, 1997; Silhavy et al. 1996; Fewell et al. 1997; Sanford et al. 1997). Due to the preformed nature of glycoalkaloids, they are sometimes termed phytoanticipins in order to separate them from phytoalexin compounds (VanEtten et al. 1994). Although there has been a strong interest in elucidating the role of glycoalkaloids in interactions between plants and fungal diseases (Fewell & Roddick 1993, 1997; Fewell et al. 1997), their function in plant-bacteria interactions has been little studied. Prokaryotes are known to lack sterols in their cell membranes and therefore glycoalkaloids were considered to play at most only a minor

protective role against bacterial diseases (Jadhav et al. 1981; Hostettmann & Marston 1995; Roddick 1996), although some contradictory opinions have been expressed (Bobeica et al. 1996; Lachman et al. 2001; Dr. Anne Osbourn, personal communication). In the place of sterol compounds, prokaryotes have complex molecules termed hopanoids, which are structurally similar to sterols. Hopanoids are assumed to function in prokaryotic cytoplasm as stabilising agents as with the eukaryotic sterols in membranes (Brock et al. 1994). Glycoalkaloids are known to have membrane disruptive effects as a result of their interaction with membrane sterols.

In *in vitro* tests many plant secondary metabolites have expressed activity against bacteria, particularly against gram positive bacteria (Tegos et al. 2002). *Cms*, which was studied here, is one of the very few gram positive plant pathogens. *Solanum acaule*, immune to infection by *Cms*, showed decreased glycoalkaloid accumulation after inoculation, the reverse of the susceptible *S. tuberosum* cultivar. Whether the variation in the glycoalkaloid levels of different genotypes is related to a defence response against bacteria or to regular metabolic alterations caused by infection remains yet uncertain in the present study. Earlier it was noted that bacterial ring rot resistance in potato was not related to tuber glycoalkaloid content (Paquin 1966). Similar observations were made with *Streptomyces scabies* (Thaxter) Waksman & Henrici (gram positive bacterium) (Frank et al. 1975) and *E. carotovora* ssp. *atroseptica* (van Hall 1902) Dye 1969 (gram negative bacterium) (Andrivon et al. 2003). However, tomatine was shown to be bactericidal, inhibiting development of bacterial black spot (caused by *X. vesicatoria*) (Bobeica et al. 1996) and *P. solanacearum* (Mohanakumaran et al. 1969) in tomato.

It is also important to point out that some phytopathogenic fungi have specific methods of tolerating antimicrobial compounds produced by plants (Osbourn 1996a,b; Morrissey & Osbourn 1999). This may be a result of modification of the membrane composition or the ability to enzymatically reduce the toxic effects of the defensive compounds (Osbourn 1996a,b; Morrissey & Osbourn 1999). An interesting example is tomatine (tomato glycoalkaloid), which can be detoxified by fungal pathogens (Pegg & Woodward 1986; Lairini & Ruiz-Rubio 1997, 1998; Sandrock & VanEtten 1998; Quidde et al. 1998). In addition, it was reported that potato glycoalkaloids are also detoxified by fungi (Weltring et al. 1997; Becker & Weltring 1998; Oda et al. 2002). Whether bacteria are able to detoxify the antimicrobial compounds metabolised by plants is not yet well studied.

Overall, since various glycoalkaloids have synergistic effects, a broader spectrum of glycoalkaloids in commercial potato cultivars may be beneficial in order to improve resistance to diseases and pests (Lachman et al. 2001). As already noted, cultivated potato produces only solanidine, but the main glycoalkaloid aglycone of E genome *Solanum* species (*S. brevidens* and *S. etuberosum*) is tomatidine. Moreover, in *S. acaule* the proportion of tomatidine is considerably high. This is interesting since according to results from the most recent studies, tomatine may not be toxic to humans (Friedman et al. 2000a,b). Tomatine and green tomatoes with high tomatine levels induced a significant reduction in plasma LDL cholesterol and triglyceride levels in hamsters in comparison with ripened red tomatoes (Friedman et al. 2000a,b; Kozukue & Friedman 2003). Furthermore, the less-toxic effect of tomatine might also have been demonstrated by Keukens et al. (1995) by illustrating that tomatine disrupts the membrane in a different manner to the toxic chaconine. Tomatidine aglycone had no relative teratogenic capacity in hamsters either (Gaffield & Keeler 1996). It can be suggested that formation of solanine and chaconine, which are both toxic, could be replaced by tomatine. This may represent a novel desirable trait that could be incorporated

from either wild potato species or from tomato into cultivated potato (Friedman 2002; Dr. Tatjana Gavrilenko, personal communication).

The research relating to the non-toxicity of tomatine has highlighted further interesting possibilities. In the genetic enhancement of cultivated potato using interspecific hybridisation, it is possible to eliminate undesirable wild characters, such as wild type glycoalkaloids, using sexual backcrosses and back-fusion methodology. However, another possibility also exists through creating interspecific somatic hybrids. A broader range of glycoalkaloids in potato cultivars could also be a desirable novel trait. The formation of tomatine instead of solanine and chaconine could prove beneficial to humans because of its health promoting properties. Variation in glycoalkaloids could also increase the level of resistance to plant diseases. Interestingly, glycoalkaloids have been shown to have broad beneficial properties in human health care, such as tomatine as an adjuvant in malaria vaccine development (Heal et al. 2001) and solamargine glycoalkaloid, which has anti-cancer properties (Kuo et al. 2000).

5 CONCLUSIONS

In the present study, the analyses of glycoalkaloid aglycones were improved using a combined derivatisation i.e. silylation-acylation and GC-MS. The method described can now be used for identification of the tomatidine-type aglycones in particular due to their more specific fragmentation by MS.

In addition to the parental type glycoalkaloid aglycones, a novel aglycone, demissidine, was also recorded in the interspecific somatic hybrids between cultivated potato and E genome *Solanum* (*S. brevidens* and *S. etuberosum*) species. Accordingly, for the formation of demissidine a hypothetical pathway was proposed based on the expression of a hydrogenase enzyme originating from the E genome *Solanum* species.

The genome constitution had a strong effect on the relative proportions of the aglycones (solanidine, tomatidine and demissidine) in each interspecific hybrid. The higher genome portion of *S. tuberosum* enhanced the production of solanidine in the progenies produced by backcrossing or repeated somatic fusions with *S. tuberosum*. In contrast, the portions of alien glycoalkaloids were reduced. This is important because through synergism different glycoalkaloids may have higher toxicity than solanidine alone. However, it is also worth noting that tomatine derived from wild *Solanum* species may have certain health enhancing properties. Therefore, replacement of solanine and chaconine by tomatine production may be worth studying in the near future.

The present study also included bacterial ring rot (caused by *Cms*) tests of the somatic hybrids between cultivated potato and *S. acaule* wild potato. The hybrids were susceptible to *Cms*. However, expression of the temperature-dependent immunity in *S. acaule* was examined. Therefore, *S. acaule* was subsequently considered tolerant rather than immune to bacterial ring rot. The *Cms* infection decreased glycoalkaloid aglycone levels in *S. acaule* and in interspecific hybrids. The *Cms*-infected *S. tuberosum* lines showed either significantly higher accumulations or no alteration in their aglycone concentrations. Whether the decreased accumulation of aglycones is linked to the mechanism of tolerance to *Cms*, remains unknown according to the results from this study and therefore needs further work.

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APPENDIX

Trivial and systematic names of glycoalkaloids mentioned in this study are listed below.

Trivial name	Systematic name	CAS registry number
α-Chaconine	β-D-glucopyranoside, (3β)-solanid-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[6-deoxy-α-L-mannopyranosyl-(1→4)]- (9CI)	20562-03-2
Commersonine	β-D-galactopyranoside, (3β, 5α)-solanidan-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	60776-42-3
Dehydrocommersonine	β-D-galactopyranoside, (3β)-solanid-5-en-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	65428-74-2
Dehydrotomatine	β-D-galactopyranoside, (3β, 22β, 25S)-spirosol-5-en-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	157604-98-3
Δ^5-Demissine	β-D-galactopyranoside, (3β, 5α)-solanid-5-en-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	195433-57-9
Demissine	β-D-galactopyranoside, (3β, 5α)-solanidan-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	6077-69-6
Leptine I	β-D-glucopyranoside, (3β, 23β)-23-(acetyloxy)solanid-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[6-deoxy-α-L-mannopyranosyl-(1→4)]- (9CI)	101030-83-5
Leptine II	β-D-galactopyranoside, (3β, 23β)-23-(acetyloxy)solanid-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]- (9CI)	101054-39-1
Leptinine I	β-D-glucopyranoside, (3β, 23β)-23-hydroxysolanid-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[6-deoxy-α-L-mannopyranosyl-(1→4)]- (9CI)	101009-59-0
Leptinine II	β-D-galactopyranoside, (3β, 23β)-23-hydroxysolanid-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]- (9CI)	100994-57-8
Sisunine	β-D-galactopyranoside, (3β, 5α, 22β, 25S)-spirosolan-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	85547-35-9
Soladulcine B	β-D-galactopyranoside, (3β, 5α, 22α, 25R)-spirosolan-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	90366-11-3
Solamargine	β-D-glucopyranoside, (3β, 22α, 25R)-spirosol-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[6-deoxy-α-L-mannopyranosyl-(1→4)]- (9CI)	20311-51-7
α -Solamarine	β-D-galactopyranoside, (3β, 22β, 25S)-spirosol-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]- (9CI)	20318-30-3
β-Solamarine	β-D-glucopyranoside, (3β, 22β, 25S)-spirosol-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[6-deoxy-α-L-mannopyranosyl-(1→4)]- (9CI)	3671-38-3
α -Solanine	β-D-galactopyranoside, (3β)-solanid-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]- (9CI)	20562-02-1
Solasonine	β-D-galactopyranoside, (3β, 22α, 25R)-spirosol-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]- (9CI)	19121-58-5
α-Tomatine	β-D-galactopyranoside, (3β, 5α, 22β, 25S)-spirosolan-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)- (9CI)	17406-45-0

Source: CAS Registry File [online-database]. Columbus (OH, U.S.A.): Chemical Abstracts Service [cited 26.3.2004]. Updated daily. Available from STN International, Karlsruhe (Germany).