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ORIGINAL PAPER

A major QTL and an SSR marker associated with glycoalkaloid content in potato tubers from *Solanum tuberosum* × *S. sparsipilum* located on chromosome I

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Abstract New potato (*Solanum tuberosum*) varieties are required to contain low levels of the toxic glycoalkaloids and a potential approach to obtain this is through marker-assisted selection (MAS). Before applying MAS it is necessary to map quantitative trait loci (QTLs) for glycoalkaloid content in potato tubers and identify markers that link tightly to this trait. In this study, tubers of a dihaploid BC_1 population, originating from a cross between 90-HAF-01 (*S. tuberosum*$_1$) and 90-HAG-15 (*S. tuberosum*$_2$ × *S. sparsipilum*), were evaluated for content of α-solanine and α-chaconine (total glycoalkaloid, TGA) after field trials. In addition, tubers were assayed for TGA content after exposure to light. A detailed analysis of segregation patterns indicated that a major QTL is responsible for the TGA content in tubers of this potato population. One highly significant QTL was mapped to chromosome I of the HAG and the HAF parent. Quantitative trait loci for glycoalkaloid production in foliage of different *Solanum* species have previously been mapped to this chromosome. In the present research, QTLs for α-solanine and α-chaconine content were mapped to the same location as for TGA content. Similar results were observed for tubers exposed to light. The simple sequence repeat marker STM5136 was closely linked to the identified QTL.

Keywords α-chaconine · α-solanine · Dihaploid · Amplified fragment length polymorphism (AFLP) · Potato breeding

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Introduction

Steroidal glycoalkaloids are secondary metabolites that occur naturally in most plant organs of *Solanum* species, albeit in varying concentrations (reviewed in Smith et al. 1996). More than 90 structurally different steroidal alkaloids have been detected in *Solanum* species (summarised by Friedman and McDonald 1997). The major glycoalkaloids in domesticated potato, *Solanum tuberosum* L., are α-solanine and α-chaconine that consist of a solanidine aglycone and a trisaccharide part, i.e. solatriose and chacatriose, respectively. α-Solanine and α-chaconine are often referred to as total glycoalkaloids (TGA) as they account for 95% of the glycoalkaloids in the tubers. The average ratio of α-solanine to α-chaconine is 40:60, but deviations from this have also been reported (Griffiths and Dale 2001; Ramsay et al. 2004).

When present at low concentrations in the tubers, glycoalkaloids enhance the taste of potatoes, but in high quantities they are considered toxic to animals and humans (Morris and Lee 1984). At concentrations above 150 mg/kg fresh weight (FW) they may cause a bitter taste (van Gelder 1991). Consumption of potatoes containing more than 200 mg/kg FW induces a risk of gastrointestinal disturbances as well as neurological disorders (Friedman and McDonald 1997). In addition, aglycone forms of steroidal glycoalkaloids have been reported to have a negative effect on mice foetuses and to increase proliferation of some types of cancer cells (Friedman et al. 2003). In contrast, extracts of glycoalkaloids might have a pharmaceutical value, such as in the treatment of certain cancers (Friedman et al. 2005) and the *Herpes simplex* virus (Thorne et al. 1985), and they have other beneficial effects, as they are a part of the potato's natural defence against some pests. These pests include foliage-feeding insects like Colorado potato beetle, *Leptinotarsa decemlineata,* Say (Deahl et al. 1991) and potato leafhopper, *Empoasca fabae,* Harris (Sanford et al. 1992), and also soil-dwelling wireworms like *Agriotes obscurus* L. (Jonasson and Olsson 1994), which feed on potato tubers.

High levels of glycoalkaloids are frequently found in wild *Solanum* species (Ramsay et al. 2004). Wild germplasm has been used extensively in potato breeding programmes, particularly as a source of disease and pest resistance. However, there is a risk that high levels of toxic glycoalkaloids may be introduced into potato cultivars through introgression of wild germplasm. The cultivar 'Lenape', derived from crosses with the wild species *S. chacoense* Bitter, had to be withdrawn from the market due to high levels of glycoalkaloids (Sinden et al. 1984).

To avoid developing new potato cultivars with high glycoalkaloid levels, breeding clones are routinely analysed for TGA content. Traditionally TGA content is measured in mature tubers after comprehensive field trials resulting in expensive and laborious assays and they are therefore carried out late in the breeding process. However, even if initial concentrations usually are low in cultivated potatoes, hazardous levels of glycoalkaloid accumulation have been observed, for instance in the variety 'Magnum Bonum' grown in Sweden (Hellenäs et al. 1995). This is due to the fact that different environmental factors, post-harvest conditions and pathogen infections can affect the glycoalkaloid content in tubers. Such factors include high temperatures during the growing season (Dimenstein et al. 1997), frost damage after haulm killing (Hellenäs et al. 1995), exposure to light during storage (Dale et al. 1992; Griffiths and Dale 2001), impact damages (Olsson 1986) and attack by Colorado potato beetles (Hlywka et al. 1994).

Accumulation of glycoalkaloids in potatoes is primarily genetically controlled and therefore a selectable trait (Friedman and McDonald 1997). Identification of quantitative trait loci (QTLs) for tuber TGA content and the development of markers closely linked to this trait would suggest the use of marker-assisted selection (MAS). Markers can be scored at an early stage of crop growth and breeding clones containing high levels of glycoalkaloids can be eliminated early in the breeding programme if MAS is used, thus avoiding unnecessary work on non-useable clones and ensuring a more effective breeding process. A further economic advantage is that through this approach it might be possible to select for several traits in one test.

Previous studies have identified QTLs or markers associated with synthesis of various types of glycoalkaloids in the foliage of potato (Boluarte-Medina et al. 2002; Hutvágner et al. 2001; Ronning et al. 1999; Yencho et al. 1998). One study has been conducted on potato tubers where no QTLs were mapped, but one marker associated with TGA content was found (van Dam et al. 2003).

The aim of the present study was to map genes or QTLs and identify markers associated with α-solanine, α-chaconine and TGA content in tubers of a population from a crossing between *S. tuberosum* L. and the wild species *S. sparsipilum* Bitter. Accumulation of TGA in tubers exposed to light was also studied.

Materials and methods

Plant material

Two dihaploid BC_1 populations of $n = 186$ clones were used for phenotypic studies, linkage and QTL mapping. The populations were reciprocal crosses between 90-HAF-01 [*S. tuberosum*$_1$ (HAP 198.4 × 89-0-08-21)] and 90-HAG-15 [*S. tuberosum*$_2$ (88-0-16-02) × *S. sparsipilum* accession no. HHA 6627] and were treated as one population designated HCDHDN.

Phenotypic evaluations

Parents and all 186 clones of the population were evaluated for glycoalkaloid content of potato tubers in field trials in the years 2004 and 2005. The tubers planted in the field were grown pathogen-free in a greenhouse. In both years, field trials were performed at two localities in Denmark, i.e. at Gadbjerg (clay soil) and Vandel (sandy soil). Each trial consisted of three randomized complete blocks with three plants per plot. For comparison of glycoalkaloid content nine listed varieties were included in the trial: 'Bintje', 'Fakse', 'Folva', 'Inova', 'Jutlandia', 'Liva', 'Magnum Bonum', 'Sava' and 'Tivoli'. The plants were grown according to standard cultivation practices. Plants grown on sandy soil were irrigated but those grown on clay soil were not.

The population used had tubers of different size and shape giving different surface areas for the same tuber weight. As glycoalkaloids in tubers are mainly concentrated in the peripheral layers, it is necessary to recalculate the glycoalkaloid contents to get a surface-based figure. The tubers were sorted by size and, as most clones contained tubers in the size fraction 25–30 mm, this fraction was chosen for further analysis. If too few tubers were available in this fraction, they were supplied with tubers of size fraction 20–25 mm, as the values for glycoalkaloid content were similar in these two size fractions. The tubers were weighed for each size fraction. Linear standard curves were calculated for each fraction combining tuber length and weight and the formula $O = 4\pi \times r^2 + 2\pi \times r \times l$ (r = tuber radius and l = tuber length − tuber diameter) was used to calculate the surface of each analysed sample.

Quantification of glycoalkaloid content

Tubers were analysed for TGA content, i.e. α-solanine and α-chaconine, by high performance liquid chromatography (HPLC). Measurements were performed as single analyses. The tubers were rinsed in tap water. Each sample was finely diced and mixed and 20 g was homogenized with an Ultra Turrax homogenizer TP 18/10 with shaft 18-N (Janke & Kunkel KG, IKA-Werk, D-7813, Staufen, Germany) for 2 min with 100 ml water:acetic acid:ascorbic acid, 100:5:1 (vol/vol/wt). The volume was adjusted to 200 ml with the same solvent, clarified by centrifugation at 4°C at 10,000 rpm (Sorvall Evolution RC) for 10 min and filtered through 1F (Munktells). Ten ml of the supernatant was put onto a Sep-Pak C_{18} cartridge previously activated by acetonitrile in accordance with the method of Hellenäs and Branzell (1997), which was also used for the subsequent analytical procedure. α-Solanine and α-chaconine (Sigma Chemical Co.) were used as standards for the HPLC determination. The concentrations were given in mg/kg FW and converted to mg/cm^2.

Light exposure

Half the tubers from each clone of the field trials in 2005 were placed on white plastic, in a greenhouse, at 15°C and in continuous light for four days and nights. Greenhouse lamps (Osram Powerstar HQI-E 400 W/D, qy3) were placed 50–60 cm directly above the tubers, so that the tubers received approximately 950 Lux. Tuber TGA content was quantified as described above.

DNA extraction

Parents and progeny for DNA extraction were maintained pathogen-free in vitro on Murashige and Skoog medium (Murashige and Skoog 1962) containing 3% (w/v) sucrose and 0.05% (w/v) MES (2-[N-Morpholino]ethanesulfonic acid). The plants were grown at 20°C with a 16/8 h day/night period. DNA was extracted from young leaves using the method of Dellaporta et al. (1983).

Simple sequence repeat (SSR) assay

Previously mapped markers were used for aligning and orienting linkage groups to chromosomes. A total of six markers were used for chromosome I. Primer pairs for STWIN12G were obtained from Provan et al. (1996) and for LeatpacA, STM1049 and STM2030 from Milbourne et al. (1998). Primer pair sequences for the markers STM5127 (F: TTCAAGAATAGCCAAAACCA, R: CTTTTTCTGACTGAGTTGCCTC) and STM5136 (F: GGGAAAAGGAAAAGCTCAA, R: GTTTATATGAACCACCTCAGGCAC) were kindly provided by Gerry Saddler (Scottish Agricultural Science Agency, Scotland).

For the SSR markers STM1049, STM2030 and STWIN12G the polymerase chain reactions (PCRs) were performed as described in Provan et al. (1996) and Sørensen et al. (2006). The PCR amplification products were analysed on 6% polyacrylamide gels after staining with ethidium bromide. For LeatpacA, STM5127 and STM5136 amplification was carried out using 20 ng of genomic DNA in a final volume of 10 µl containing 1× PCR buffer, 2.5 mM $MgCl_2$, 250 µM dNTP, 0.4 U AmpliTaq (all Applied Biosystems) and 0.3 pmol of forward and reverse primers. Forward primers were fluorescence-labelled. A short programme described in Sørensen et al. (2006) was used for PCR amplification with the addition after the final cycle of a period of 45 min at 60°C and finally 20 min at 20°C. The PCR amplification products were separated on an ABI 310 Genetic Analyzer (Applied Biosystems). Multiple alleles of SSR markers were labelled with their respective band sizes in base pairs (bp) after the marker designation.

Amplified fragment length polymorphism (AFLP) assay

Amplified fragment length polymorphism assays were carried out according to the protocol described in Vos et al. (1995). Respectively ten *Eco*RI/*Mse*I and eight *Hin*dIII/*Mse*I primer combinations were used for map construction of chromosome I. Amplification products were separated using 5% polyacrylamide gel-electrophoresis and detected using auto-radiographic procedures. Nomenclature of AFLP markers is as presented by Sørensen et al. (2006) except HA (*Hin*d-AAC/*Mse*-CAC) and HF (ATG/CAC). Multiple polymorphic fragments from the same AFLP primer were labelled with a number after the primer designation. Autoradiograms were scored manually, twice.

Linkage analysis

Linkage map construction was performed using JoinMap® 3.0 (Van Ooijen and Voorrips 2001). Mapping was based on the presence or absence of SSR and AFLP marker bands. The marker data were split into an HAF and an HAG set containing 252 and 293 polymorphic marker loci, respectively, and a map was made for each of the parents. Three clones were removed from the dataset before map construction because one of them had too many missing values and the other two displayed a tetraploid segregation pattern. Haldane's mapping function was used for map construction. Deviations from the expected Mendelian ratio of 1:1 were calculated with a goodness-of-fit χ^2 test with one degree of freedom. Chromosome numbering was according to Gebhardt et al. (1991).

Data analysis

The data for the glycoalkaloid content were adjusted for lack of normality and for the possible effects of year and location by fitting a suitable generalized linear model. Means of Pearson residuals were used for further analyses. This yielded a response variable with stabilized variance and a sample distribution not distinguishable from the normal distribution (when adjusting for the effect of clone also). The generalized linear model used was defined using an identity link and a Compound Poisson distribution (Jørgensen et al. 1996) with a power coefficient (estimated by maximum likelihood) equal to 1.25 and factors representing the location and the year. A principal component analysis was used to characterize the distribution of the information among the individual measurements of α-chaconine and α-solanine contents for the two locations and years.

Quantitative trait locus (QTL) analysis

The QTL analysis of mean values of α-solanine, α-chaconine and TGA was performed with MapQTL® 4.0 (Van Ooijen et al. 2002). The QTL mapping was based on 172 clones as only the clones developed in both test years in the field were used. LOD thresholds for declaring a QTL significant were estimated from permutation tests ($P < 0.05$, 1,000 permutations). The genome wide threshold for glycoalkaloid content in the HAF and the HAG parent was 7.8 and 7.7 (α-solanine), 8.8 and 8.7 (α-chaconine) and 9.1 and 9.2 (TGA), respectively. The QTL mapping of glycoalkaloid accumulation in tubers exposed to light was based on 151 clones and measured as the difference in TGA content between non-light and light-treated tubers. The genome wide threshold was 4.5 (HAF) and 4.7 (HAG). The LOD threshold per linkage group was used to indicate presence of suggestive QTLs. QTL analysis used a non-parametric Kruskal-Wallis test as well as simple interval and multiple-QTL model (MQM) mapping. Estimates of the percentage of variation, explained by the most significant marker for each QTL, were obtained by MapQTL. Graphic representation of both the map and LOD score plots from the QTL-mapping used the software MapChart 2.1 (Voorrips 2002).

Results

Phenotypic evaluations

Statistical analysis showed a significant difference ($P < 0.0001$) between clones of the HCDHDN population regarding α-solanine, α-chaconine and TGA content. Therefore, the population was suitable for mapping the traits above. In addition, a significant difference ($P < 0.001$) was observed between locations but no significant differences between years or significant interactions between location and year were detected (all P-values were larger than 0.30). The TGA content was higher in potatoes grown on clay soil than those grown on sandy soil (Table 1).

Elevated levels of glycoalkaloid content were, furthermore, observed in tubers after light exposure, as the mean TGA contents in the HCDHDN population were 0.36 and 0.47 mg/cm^2 in non-light and light-treated tubers, respectively. This was also observed in 'Magnum Bonum', which had TGA contents of 0.06 and 0.12 mg/cm^2 in non-light and light-treated tubers, respectively. However, the corresponding mean levels of all the tested varieties were 0.09 and 0.19 mg/cm^2. Thereby the present results could not confirm an increased sensitivity to environmental conditions resulting in high TGA accumulation in 'Magnum Bonum', although this has previously been observed in this variety (Hellenäs et al. 1995).

The glycoalkaloid data (content of α-solanine, α-chaconine and TGA) displayed a bimodal distribution with the offspring of the HCDHDN population segregating into two distinct groups of high and low TGA content with means close to the values of each of the two parents (Fig.1). The pattern was similar for light-treated tubers (data not shown). The TGA range of the *S. tuberosum* material (HAF parent and the nine listed varieties) was 0.06–0.18 mg/cm^2 (mean of locations). For the HAG parent the TGA content was 0.52 mg/cm^2 (Table 1). The α-solanine content (mean of locations) in the *S. tuberosum* material except 'Tivoli' was 33–41% (Table 1). 'Tivoli' contained 50% α-solanine and this elevated percentage is similar to the HAG parent that contained 53%.

Principal components were formed with four variables representing the results of α-solanine and α-chaconine in the non-light and the light-treated tubers. In both locations

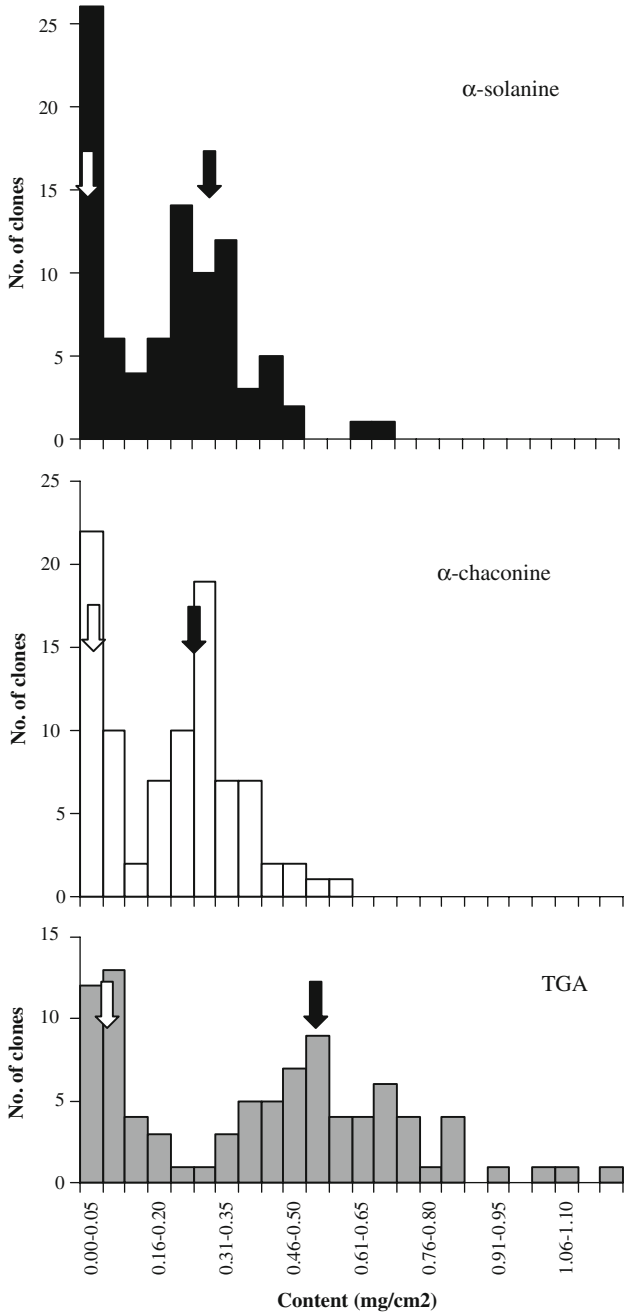

Fig. 1 Frequency distributions of α-solanine, α-chaconine and total glycoalkaloid (TGA) content in tubers of the HCDHDN population. Mean values are indicated for the parents: 90-HAF-01 (*white arrow*) and 90-HAG-15 (*black arrow*)

the first principal component represented the great majority of the total variation (95% for Vandel and 94% for Gadbjerg), suggesting that this principal component contained essentially all the information on these variables and therefore should be used in a joint analysis. The coefficients of the first principal components were all approximately equal (less than 0.5% variation), indicating that these principal components represented the mean of these variables.

Linkage map

A total of 15 linkage groups were constructed for both the HAG and the HAF parent. They comprised a total of 158 and 137 markers, and they covered 882 and 1099 cM, respectively. The linkage groups were assigned to the 12 chromosomes using SSR markers previously mapped in other potato populations. This study concentrated on linkage group I of the HAG parent. Linkage group I of the HAF parent was divided into two and this was, therefore, used as supplementary information. In the HAG parent, linkage group I was created at a LOD threshold of 12.0. In the HAF parent, thresholds of LOD 8.0 were used. The map of HAG's chromosome I was composed of a total of 19 markers, of which six were SSR markers and 13 were AFLP markers and it had a length of 90 cM (Fig. 2a). The two linkage groups representing chromosome I of the HAF parent consisted of a total of 21 markers, of which three were SSR markers and 18 were AFLP markers. Linkage groups I and Ia had map lengths of 82 and 13 cM, respectively (Fig. 3a). Distorted segregation was pronounced for chromosome I in the HAF parent.

Solanum sparsipilum produced bands for four out of the six SSR marker loci that were mapped to chromosome I of the two parents, i.e. Leatpac (size 230 and 233 bp), STM2030 (size 176 and 205 bp), STM5127 (size 237 and 243 bp) and STM5136 (size 218 and 235 bp). Only SSR marker allele STM2030_205 was specific for *S. sparsipilum*.

Quantitative trait locus analysis of glycoalkaloid content

Results from the Kruskal-Wallis test strongly indicated the presence of a QTL for TGA content on chromosome I in the HAG parent in the region between AFLP marker EN8 and SSR marker STM1049_180 ($P < 0.0001$) with SSR marker STM5136 (_223 and _232) having the highest K* test statistic of 88.0 (Fig. 2c). This indication was confirmed by the interval mapping method, which detected a significant region ($P < 0.0001$) between AFLP marker ED4 and STM1049_180 covering 54 cM (Fig. 2b). The highest LOD score of 32.3 was located close to STM5136 and this explained 75.7% of the phenotypic variance. Similar values for LOD score and phenotypic variance were detected after MQM mapping (Fig. 2b). However, this method narrowed the significant ($P < 0.0001$) region down to 19 cM with STM5136 (_223 and 232) and STM2030_205 as flanking markers.

The existence of a major QTL or gene involved in glycoalkaloid synthesis located to chromosome I was also indicated through mapping of the HAF parent. Results from interval and MQM mapping located a significant QTL ($P < 0.0001$) for TGA between LeatpacA_230 and

Table 1 Mean glycoalkaloid content (mg/cm^2) in potato tubers of parents (90-HAF-01 and 90-HAG-15) and progeny of the HCDHDN population and nine listed varieties

	α-Solanine		α-Chaconine		TGA		α-Solanine: α-chaconine	
	Gadbjerg	Vandel	Gadbjerg	Vandel	Gadbjerg	Vandel	Gadbjerg	Vandel
90-HAF-01	0.03	0.02	0.04	0.03	0.06	0.05	41:59	41:59
90-HAG-15	0.27	0.28	0.24	0.26	0.51	0.54	53:47	52:48
HCDHDN	0.22	0.18	0.21	0.19	0.43	0.37	50:50	46:54
Range	0.01–0.77	0.01–0.62	0.01–0.62	0.01–0.57	0.02–1.26	0.02–1.14	39:61–61:39	35:65–58:42
Varieties	0.05	0.04	0.06	0.06	0.11	0.10	40:60	37:63
Range	0.02–0.11	0.02–0.08	0.04–0.10	0.03–0.10	0.06–0.21	0.05–0.16	35:65–52:48	30:70–48:52

The tubers were grown at two localities, i.e. Gadbjerg (clay soil) and Vandel (sandy soil). Values of α-solanine, α-chaconine and total glycoalkaloid (TGA) content are means of measurements in the years 2004 and 2005

STM1049_190 with a LOD-score of 17.5 and 17.2 (Fig. 3b), respectively, and it explained 72.3 and 16.8% of the TGA content, respectively. The Kruskal–Wallis test confirmed the presence of a QTL on this part of chromosome I ($P < 0.0001$; Fig. 3c). In addition, this test suggested the existence of a QTL on the other part of this chromosome in the vicinity of STM5136 (_218 and _226) with $K^* = 11.4$ ($P < 0.001$; Fig. 3c). Though a peak with a LOD-score of 2.6 was identified by interval mapping in this region of the chromosome this method did not significantly identify the putative QTL. The location and nature of a QTL on chromosome I of the HAF parent is most likely affected by the fact that this chromosome is divided into two parts, but the results might be used as indications of QTLs in this part of the genome.

There were also weak indications of a QTL in the other distal region of chromosome I. The Kruskal–Wallis test suggested that a QTL was located close to marker EB8 in the HAG parent ($P < 0.0001$; Fig. 2c) and EM32 in the HAF parent ($P < 0.01$; Fig. 3c). Moreover the interval mapping method detected a significant peak ($P < 0.0001$) between the markers HA6 and EI43 (Fig. 3b) of the HAF parent, which is in line with the finding of the Kruskal–Wallis test. However, there is a discrepancy between the magnitude of the QTL as detected using the Kruskal–Wallis test and the interval mapping method. These results should be interpreted with caution as implementation of the applied interval mapping uses a system of five neighbouring markers to model putative QTLs. Therefore it is not to be expected that this method is reliable in the extremes of a chromosome, which can explain discrepancies between the results of the interval mapping and the other method.

The results of chromosome I were similar for α-solanine, α-chaconine and TGA content in the HAG as well as the HAF parent. Furthermore, the QTL results from the tubers exposed to light showed the same pattern as those for non-light-treated tubers.

No other chromosomal positions revealed QTLs for any of the investigated traits.

Discussion

It has been reported that glycoalkaloid production in potatoes is highly heritable (Sinden et al. 1984; van Dam et al. 1999). However, it is not clear whether it is quantitatively controlled or based on a few major genes. In *S. tuberosum* clones TGA content has been found to be a polygenic trait (Sinden et al. 1984) based on a relatively low number (three to seven) of genes (van Dam et al. 1999), whereas results involving other species have indicated that it is based on one or a few closely linked major gene(s) (Boluarte-Medina et al. 2002; Hutvágner et al. 2001; Ronning et al. 1998, 1999).

In this study the bimodal segregation pattern of the data for α-solanine, α-chaconine and TGA content in tubers with and without exposure to light suggested that a major QTL in the investigated clones controls these traits (Fig. 1). Every analysis of marker association with glycoalkaloid content performed in this research indicated the existence of a major QTL involved in the synthesis of these compounds on chromosome I. However, it cannot be ruled out that several minor QTLs are involved in the production of TGA and it is highly likely that other QTLs or genes are present in other *Solanum* species, especially the non-domesticated potato relatives. Such QTLs could be involved in the synthesis of other types of steroidal glycoalkaloids that have been detected in tubers of wild potato relatives (Osman et al. 1978; van Gelder et al. 1988). In *S. sparsipilum*, which is the wild species used in the present study, solanine and chaconine have previously been identified as the major types (van Gelder et al. 1989; Ramsay et al. 2004).

Quantitative trait loci for glycoalkaloid content in tubers of potato have not previously been mapped. However, the

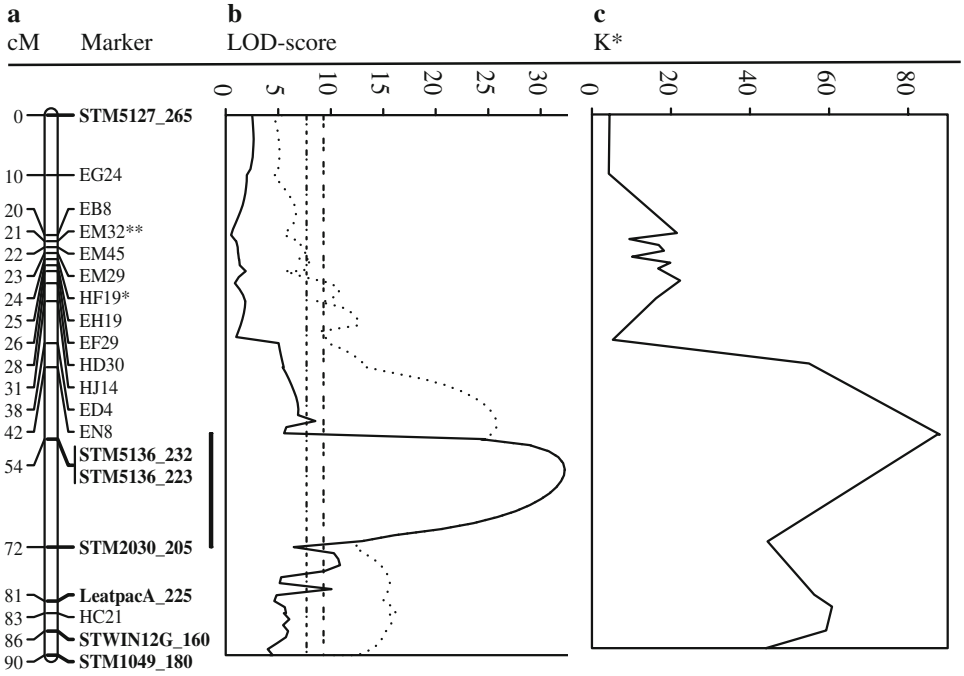

Fig. 2 **a** Linkage map of chromosome I of 90-HAG-15. Map distances are indicated in centiMorgan (cM). The SSR markers are indicated in bold and the remaining are AFLP markers. *Asterisks* following the marker name indicate segregation distortion, with the extent of the deviation from the expected ratio shown as: *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$. **b, c** Likelihood maps of a QTL for total glycoalkaloid content in tubers. **b** Log of odds (LOD), interval (*dotted line*) and Multiple-QTL Model (MQM) mapping (*straight line*), chromosome (*dashed dotted line*) and genome (*dashed line*) wide threshold. **c** Kruskal–Wallis (KW) test statistic *K** (*straight line*)

foliage of different *Solanum* species has been used in studies looking for QTLs and markers associated with the synthesis of various glycoalkaloids or their aglycones, i.e. solasodine and solanidine (Yencho et al. 1998), leptine (Ronning et al. 1999; Boluarte-Medina et al. 2002; Sagredo et al. 2006) and leptinine (Hutvágner et al. 2001). Yencho et al. (1998) identified several minor QTLs involved in solasodine accumulation. Likewise Sagredo et al. (2006) mapped two loci involved in the synthesis of leptinidine and acetyl-leptinidine to chromosomes II and VIII, respectively. But most prominent is a major locus or a cluster of closely linked genes mapped to chromosome I for solanidine (Yencho et al. 1998), leptine (Ronning et al. 1999) and leptinine (Hutvágner et al. 2001). Leptine is foliar-specific, but other glycoalkaloids are synthesised in both tubers and foliage of different potato species, i.e. α-solanine and α-chaconine (Friedman and Dao 1992; Sanford et al. 1996) and solanidine as well as solasodine glycosides (van Gelder et al. 1988), and therefore the putative QTL located at the top of chromosome I in this study might correspond to these previously mapped loci. On the other hand, the highly significant QTL reported here is located at the bottom half of chromosome I, far away from the putative QTL at the top of the chromosome, suggesting that these are distinct QTLs.

The QTL or cluster of loci on chromosome I may code for enzymes that catalyse reactions in the biosynthesis pathway after the branching of solasodine and solanidine from cholesterol but before the conversion of solanidine to glycoalkaloids. Solanidine is hypothesised to be the precursor of α-solanine and α-chaconine (reviewed in Friedman and McDonald 1997) as well as leptinine and leptine (Osman et al. 1987). Therefore the repeated mapping of chromosome I located gene(s) associated with production of solanidine, leptinine and leptine as described above and with α-solanine as well as α-chaconine as found in this study suggests that the gene(s) may control metabolism before the conversion of solanidine. Furthermore, the biosynthetic step where this locus or cluster of loci is active might be after the branching of solasodine and solanidine from cholesterol as no QTLs for accumulation of the solasodine aglycone have been mapped to chromosome I, but to chromosomes IV, VI, VIII, XI and XII instead (Yencho et al. 1998).

The QTL for α-solanine, α-chaconine and TGA content in potato tubers exposed to light was located at exactly the same position on chromosome I as the QTLs in non-light-treated tubers. This in accordance with the results of the principal component analyses, which showed that essentially the same information is contained in the content of α-solanine and α-chaconine measured in tubers with or without light exposition. Our results therefore suggest that the effect of the reported QTL is not due to modifications in the response of the production of α-solanine and α-chaconine to light.

The QTL, which is mapped to chromosome I in this research, is very interesting for potato breeding as it is highly significant and explains a major proportion of the TGA content in both light and non-light-treated potato tubers of the investigated population. The QTL is flanked by STM5136 (_223 and _232) and STM2030_205, and as these markers are of the SSR type, they are fairly simple to

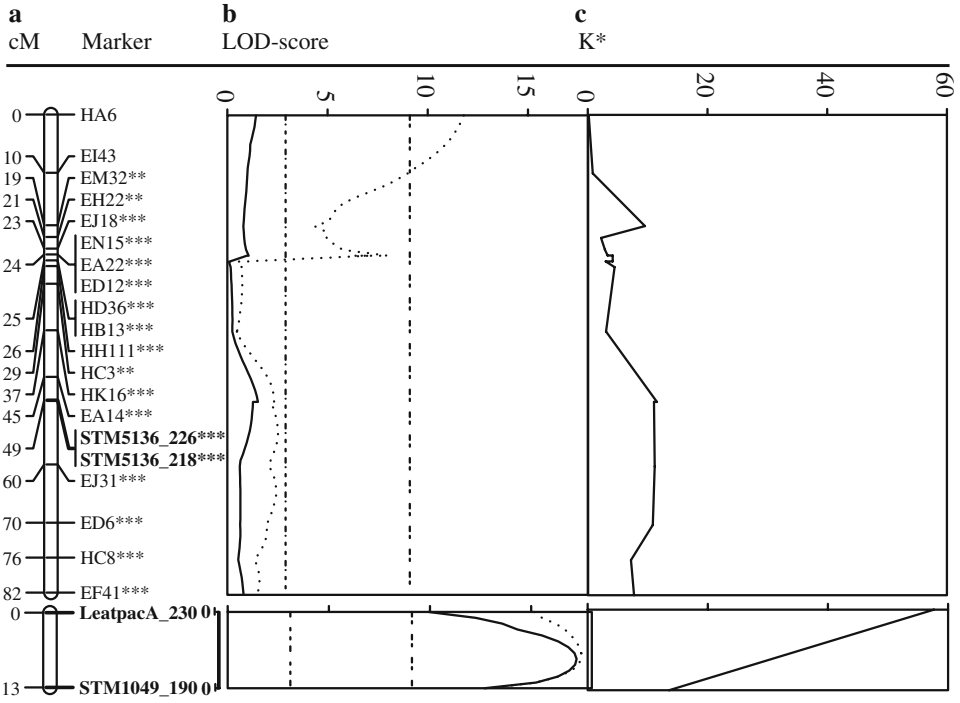

Fig. 3 **a** Linkage map of chromosome I (*top*) and Ia (*bottom*) of 90-HAF-01 with the two parts located in their most likely orientation. **b, c** Likelihood maps of a QTL for total glycoalkaloid content in tubers. See Fig. 2 for legend

use and very attractive to apply in MAS. Furthermore STM5136 (_223 and 232) is relatively tightly linked to the QTL for TGA content, indicating that the risk of recombination between marker and gene is minimal, which is important for the applicability of the marker in MAS. Future studies concerning development of markers that are even more closely linked to the target gene would further improve the benefits of the identified QTL in the breeding of new varieties with low glycoalkaloid content.

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