Theor Appl Genet (2012) 125:185–196
DOI 10.1007/s00122-012-1825-7

ORIGINAL PAPER

Development and application of SINE-based markers for genotyping of potato varieties

Kathrin M. Seibt · Torsten Wenke · Cora Wollrab · Holger Junghans · Katja Muders · Klaus J. Dehmer · Kerstin Diekmann · Thomas Schmidt

Received: 11 November 2011 / Accepted: 11 February 2012 / Published online: 28 February 2012

Abstract Potato variety discrimination based on morphological traits is laborious and influenced by the environment, while currently applied molecular markers are either expensive or time-consuming in development or application. SINEs, short interspersed nuclear elements, are retrotransposons with a high copy number in plant genomes representing a potential source for new markers. We developed a marker system for potato genotyping, designated inter-SINE amplified polymorphism (ISAP). Based on nine potato SINE families recently characterized (Wenke et al. in Plant Cell 23:3117–3128, 2011), we designed species-specific SINE primers. From the resulting 153 primer combinations, highly informative primer sets were selected for potato variety analysis regarding number of bands, quality of the banding pattern, and the degree of polymorphism. Fragments representing ISAPs can be separated by conventional agarose gel electrophoresis; however, automation with a capillary sequencer is feasible. Two selected SINE families, SolS-IIIa and SolS-IV, were shown to be highly but differently amplified in Solanaceae, Solaneae tribe, including wild and cultivated potatoes, tomato, and eggplant. Fluorescent in situ hybridization demonstrated the genome-wide distribution of SolS-IIIa and SolS-IV along potato chromosomes, which is the basis for genotype discrimination and differentiation of somaclonal variants by ISAP markers.

Communicated by C. Gebhardt.

K. M. Seibt · T. Wenke · C. Wollrab · T. Schmidt (✉)
Institute of Botany, Dresden University of Technology, 01062 Dresden, Germany
e-mail: Thomas.schmidt@tu-dresden.de

H. Junghans · K. Muders
NORIKA GmbH, 18190 Groß Lüsewitz, Germany

K. J. Dehmer · K. Diekmann
Groß Lüsewitz Potato Collections (GLKS), Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Parkweg 3a, 18190 Groß Lüsewitz, Germany

Introduction

The Solanaceae family contains important agricultural and horticultural crops including potato, tomato, eggplant, pepper, as well as petunia and tobacco. Cultivated potato (*Solanum tuberosum* L. ssp. *tuberosum*) is tetraploid ($2n = 4x = 48$), while its wild relatives vary in ploidy from diploid to hexaploid (Spooner and Hijmans 2001; Spooner and Salas 2006). Potato breeding combines agronomic traits from established cultivars, while pre-breeding includes the introgression from old cultivars, landraces, or wild species, often maintained in genebank collections harboring tens of thousands of potato accessions. Worldwide, there are more than 4,500 potato varieties cultivated in over a 100 countries (Pieterse and Hils 2009). To differentiate cultivars phenotypically, various characteristics can be considered including morphology (e.g., sprouts, leaves and tubers), resistances, and tolerances to pathogens or stress. Nevertheless, due to the high number of varieties, unequivocal identification and differentiation of cultivars remains a laborious task. Molecular methods offer an economic and reliable alternative to the time-consuming phenotyping and furthermore are not affected by environmental conditions.

Several marker techniques have been used in potato research. Restriction fragment length polymorphism (RFLP), chloroplast DNA (Spooner et al. 2005b; Sukhotu and Hosaka 2006) as well as mitochondrial DNA polymorphisms (Hosaka and Sanetomo 2009; Scotti et al. 2007) were mainly utilized to infer the relationship of cultivated and wild potatoes. The amplified fragment length

polymorphism (AFLP) technique was applied for taxonomic studies and genome mapping (Jacobs et al. 2008, 2011; Lara-Cabrera and Spooner 2004; Li et al. 1998; Spooner et al. 2005a). Nevertheless, its commercial application is limited by potential license fees and cost-intensive equipment. Highly polymorphic simple sequence repeat (SSR) markers have been used extensively for taxonomic studies, as well as genotyping of cultivars and landraces (Ashkenazi et al. 2001; Ghislain et al. 2009, 2004; Lara-Cabrera and Spooner 2005; Moisan-Thiery et al. 2005; Reid et al. 2009; Reid and Kerr 2007; Spooner et al. 2007). Dependent on the studies' objectives and the material investigated, combinations of many SSR markers have to be used to obtain reliable results.

Retrotransposons represent a useful source for marker development because of their high abundance and genome-specific distribution. They are ubiquitous in eukaryotes, in particular in plant genomes, and characterized by family-specific conservation in structure and sequence, and transposition via *copy-and-paste* mechanisms (Bennetzen 1996; Bennetzen et al. 2005). Several PCR-based marker methods revealing insertional polymorphisms of long terminal repeat (LTR) retrotransposons have been developed. These include retrotransposon-based insertion polymorphism (RBIP), inter-retrotransposon amplified polymorphism (IRAP), retrotransposon-microsatellite amplified polymorphism (REMAP), and inter-primer binding site (iPBS) amplification (Flavell et al. 1998; Kalendar et al. 2010, 1999). Only IRAP markers have yet been applied for potato genotyping. However, inconsistencies regarding the number of bands and the general reproducibility have been reported (Lightbourn et al. 2007).

In addition to LTR retrotransposons, eukaryotic genomes harbor also retroelements lacking the long terminal repeats, so called non-LTR retrotransposons such as LINEs and SINEs (long and short interspersed nuclear elements). SINEs are up to 500 bp in size and widely dispersed along the chromosomes including gene-rich regions (Kramerov and Vassetzky 2005; Lenoir et al. 2001; Okada 1991; Schmidt 1999; Wenke et al. 2011). Primers derived from the most prominent mammalian SINE family, designated *Alu* elements, have been utilized for marker development for mammalian genomes (Kass and Batzer 1995; Kostia et al. 2000; Nelson et al. 1989). However, their application for plant genome analyses resulted in a low number of polymorphisms, low quality, and hence low information content (Alix et al. 1999; Baurens et al. 1998). Recently, Solanaceae-specific SINE families and subfamilies have been identified by the application of a bioinformatic tool called SINE-Finder (Wenke et al. 2011).

Here, we describe the development and application of the inter-SINE amplified polymorphism (ISAP) marker system which enables the rapid, robust, and cost-efficient differentiation of potato cultivars. Stability and resolution of ISAP markers were shown by the investigation of long-term in vitro cultures and mutants. Furthermore, we studied the genomic organization, interspecies, and chromosomal distribution of prominent SINE families by Southern and fluorescent in situ hybridization.

Materials and methods

Plant material and DNA preparation

Potato varieties, including a long-term in vitro culture of the cultivar 'Gala', the wild potato accessions *S. tuberosum* ssp. *andigenum* Hawkes (GLKS 34951), *S. stoloniferum* ssp. *stoloniferum* Schltdl. & Bouche (GLKS 30610), and *S. demissum* Lindley (GLKS 34951) as well as *S. melongena* accession 'SOL 1041' were provided by NORIKA GmbH (Germany), SASA (Science and Advice for Scottish Agriculture, UK), and IPK Genebank (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany), respectively. For Southern analysis of Solanaceae species, *S. lycopersicum* 'Tamina' (Quedlinburger Saatgut GmbH, Germany) and *Capsicum annuum* 'De Cayenne' (Dehner GmbH & Co. KG, Germany) were included.

Plants were grown in a greenhouse under long-day conditions (16 h of light, 25°C). Genomic DNA was prepared from young leaves according to the CTAB protocol (Saghai-Maroof et al. 1984) including an RNase A treatment.

Inter-SINE amplified polymorphism (ISAP)

The generation of ISAPs is based on the amplification of genomic DNA between adjacent SINE copies by PCR (Fig. 1a) and subsequent electrophoretic separation of the resulting amplicons. DNA sequences of seven potato SolSINE families and two subfamilies (Wenke et al. 2011) were used to design outward-facing primers (Table 1). Primers were 5′-extended with the arbitrary GC-rich sequence CTG ACG GGC CTA ACG GAG CG (Table 1) to allow a high annealing temperature.

A 20-µl reaction mixture consists of 10 ng template DNA, 1× PCR buffer, 0.2 mM dNTPs, 0.15 µM each primer, 0.1 g/l BSA and 0.5 U DreamTaq™ DNA Polymerase (Thermo Scientific Fermentas, Germany). The following detailed PCR conditions were used for the extended primers: initial denaturation for 5 min at 93°C, followed by three cycles of 20 s denaturation at 93°C, 30 s annealing at 60°C, and 120 s synthesis at 72°C, further 27 two step-cycles with 20 s at 93°C and 140 s at 72°C, and a final elongation of 5 min at 72°C.

The resulting products were separated on 2% LE agarose (Lonza, Switzerland) gels, prestained with ethidium bromide, in standard 1× TAE buffer for 2 h at 3 V/cm in a Sub-Cell® GT electrophoretic system (Bio-Rad, USA).

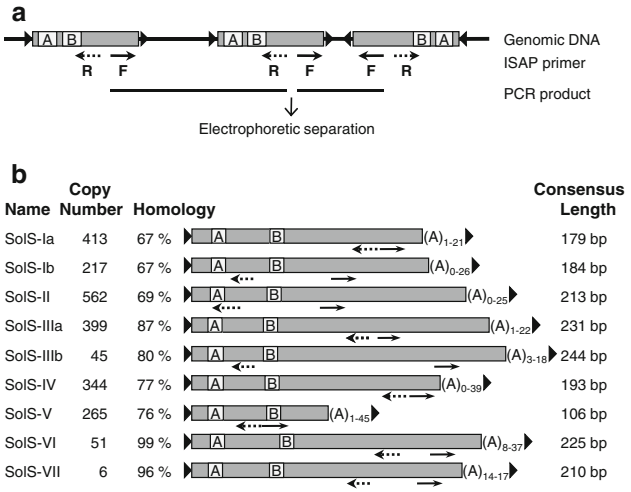


Fig. 1 ISAP principle. Short interspersed nuclear elements (SINEs) are characterized by a tRNA-related 5′ region that contains an internal promoter motif (A and B *box*). **a** The ISAP method is based on PCR using outward-facing SINE-derived primers followed by separation of the PCR product. Reverse primers (R) face the 5′ direction (*dotted arrow*), while forward primers (F) are directed to the 3′ end (*normal arrow*). **b** Characteristics and primer positions (*arrows*) of the SolS-SINE families. The average identities, copy numbers (full-length and truncated members), range of poly(A)-tail lengths, and consensus lengths are given (modified from Wenke et al. 2011)

Images were captured by the Gel Doc™ 2000 Gel Documentation System (Bio-Rad, USA).

For analysis with capillary electrophoresis on an automated sequencing device (CEQ™ 8000, Beckman Coulter, USA), one primer per PCR was 5′-labeled with Cy5 (Eurofins MWG Operon, Germany). Fragment sizing was facilitated by the application of the CEQ™ DNA Size Standard Kit-600 (Beckman Coulter, USA). For separation, the default FRAG-4 method was applied with a capillary temperature of 50°C, denaturing at 90°C for 120 s, injection with 2 kV for 30 s, and a separation at 4.8 kV for 60 min. Fragment analysis was performed according to the manufacturer's recommendations (Beckman Coulter, USA).

Data analysis and storage

To analyze the resulting polymorphisms, gel images of the ISAP patterns were processed using GelCompar II version 6.5 (Applied Maths NV, Belgium) and loaded into a database representing a potato variety catalog. Images were normalized and bands were scored, counted, and divided into classes according to their size. Subsequently, Jaccard similarity coefficients were calculated based on band presence and absence. Cluster analysis was performed for the combined banding patterns of the primer combinations

Table 1 ISAP primer sequences and their melting temperature (T_m)

Primer	Sequence	T_m (°C)
SolS-Ia-F	CTC CAA TGT GRG CTC CGG AC	62
SolS-Ia-R	TCC GAC TAA ATY CGG ATC GC	58
SolS-Ib-F	CCA ATG TGG GAC TTT CCG G	59
SolS-Ib-R	CTC TGG TTA AGG ATG AAG GAG	58
SolS-II-F	TTC CCA TCT GTT CTA GCC TTG G	60
SolS-II-R	AGT TCA TTG ACC ACT AGG CC	57
SolS-IIIa-F	CCT ATG TGG TTT GCG AGC	56
SolS-IIIa-R	TAA CCC GCA CTA GGC AAG	56
SolS-IIIb-F	ACC CGA AGG GCA GAG GC	60
SolS-IIIb-R	ATC CGT GTG GAT AAC CAC CC	59
SolS-IV-F	GTC ACA GAC GGA TTT CTC G	57
SolS-IV-R	CCC TTT GGA TCA ATC ACA GC	57
SolS-V-F	TGC GAG TTT GAG TCA CCA AG	57
SolS-V-R	AAC CTT CGG GTT GGA AGT GAG	60
SolS-VI-F	TAG TCG GGG CTC AAA GAG AC	59
SolS-VI-R	CAA GGA GTT TCC CGC AAG TG	59
SolS-VII-F	TAG TCG GGT TCG CAA AGC	56
SolS-VII-R	TCC ACC ACG GTT AGT TCC AC	59
SolS-IIIa-extended-F	CTG ACG GGC CTA ACG GAG CGC CTA TGT GGT TTG CGA GC	78
SolS-IIIa-extended-R	CTG ACG GGC CTA ACG GAG CGT AAC CCG CAC TAG GCA AG	78
SolS-IV-extended-F	CTG ACG GGC CTA ACG GAG CGG TCA CAG ACG GAT TTC TCG	78
SolS-IV-extended-R	CTG ACG GGC CTA ACG GAG CGC CCT TTG GAT CAA TCA CAG C	78

SolS-IIIa-F/SolS-IIIa-R, SolS-IIIa-F/SolS-IV-F, SolS-IIIa-F/SolS-IV-R. Trees were constructed based on Dice similarity coefficients by unweighted pair group method with arithmetic mean (UPGMA).

Southern hybridization

Genomic DNA was digested with *Fok*I (Thermo Scientific Fermentas, Germany) and separated on 1.2% agarose gels at 1.5 V/cm. Alkaline transfer of the DNA was performed using positively charged nylon membranes (GE Healthcare, UK). SINE family-specific probes were obtained by cloning and sequencing from the reference cultivar 'Gala' and labeled radioactively by PCR. Hybridization was carried out according to standard protocols (Sambrook et al. 1989) at 60°C followed by washing with 2× SSC/0.1% SDS. Signals were detected by autoradiography.

Fluorescent in situ hybridization

For the preparation of mitotic chromosomes, the meristem of young leaves of *S. tuberosum* 'Gala' was incubated in 2 mM 8-hydroxyquinoline for 3–5 h and fixed in methanol/acetic acid (3:1). Maceration was performed in an enzyme mixture containing 6% (w/v) cellulase from *Aspergillus niger* (Sigma), 0.77% (w/v) cellulase Onozuka-R10 (Serva), and 3% (v/v) pectinase from *A. niger* (Sigma) followed by the spreading of the nuclei suspension onto slides as described by Desel et al. (2001). Probes were labeled with biotin-11-dUTP by PCR (Schwarzacher and Heslop-Harrison 2000), hybridized to *S. tuberosum* chromosomes, and detected according to Schmidt et al. (1994). Chromosomes were counterstained with DAPI (4′,6′-diamidino-2-phenylindole) and mounted in antifade solution (CitiFluor). The examination of the slides was conducted with a fluorescence microscope (Zeiss Axioplan 2 imaging) equipped with Filter 15 (Cy3) and Filter 02 (DAPI). Images were captured directly with the CCD camera ASI BV300-20A coupled to the Applied Spectral Imaging software version 3.3. For contrast-optimization with Adobe Photoshop 7.0, only functions affecting the whole image equally were used.

Results

Development of ISAP markers

Recently, nine SolS-SINE families consisting of thousands of members have been characterized in potato (Wenke et al. 2011). These SINE families are highly variable in copy number and similarity between and within families. We developed molecular markers designated ISAP for differentiation and analysis of the genetic diversity of potato cultivars. The ISAP method describes the PCR amplification of genomic DNA between two neighboring SINE copies (Fig. 1a). We analyzed 2,302 SINEs falling into nine families and designed two outward-facing primers per family derived from highly conserved regions (Table 1). The main characteristics of the potato SolS families and primer positions are depicted in Fig. 1b.

All 18 single primers as well as the 153 possible primer combinations were tested on the representative cultivar 'Gala' to evaluate the number of amplicons, their size, and size distribution. Generally, applying two primers from the same or different SINE families resulted in more distinct fragments compared to a single primer reaction. Most amplicons were observed using primer combinations for SolS-II, IIIa, and IV, while SolS-VI and VII yielded only few bands. Sequence analysis of ISAP fragments showed that they were indeed flanked by SolS SINEs (not shown). For the selection of suitable primer pairs, the following criteria were considered: balanced band intensities, polymorphism rate, and amplicon sizes ranging from 100 bp to 2 kb to allow a clear separation by conventional agarose gel electrophoresis. We chose three representative primer combinations due to their high proportion of polymorphic bands: SolS-IIIa-F/SolS-IIIa-R, SolS-IIIa-F/SolS-IV-F, and SolS-IIIa-F/SolS-IV-R. Examples of typical banding patterns generated by the primer set SolS-IIIa-F/SolS-IV-R are shown in Fig. 2. The 5′ extension of primers facilitated a reduced background, more balanced band intensities, and a reduction of faint bands. In addition to these primer sets, the remaining 150 primer combinations, out of which 75% showed more than three bands for the cultivar 'Gala',

Table 2 Number of amplified bands and resulting similarity calculated from the analysis of 53 and 364 potato varieties, respectively

ISAP primer combination	Varieties analyzed	Similarity[b]			Number of bands			Percentage of polymorphic bands
		Minimum	Average	Maximum	Minimum	Average	Maximum	
SolS-IIIa-F + SolS-IIIa-R	53[a]	0.38	0.74	1.00	12	16.4	20	69
SolS-IIIa-F + SolS-IV-F	53[a]	0.29	0.66	1.00	10	15.3	22	80
SolS-IIIa-F + SolS-IV-R	53[a]	0.27	0.62	1.00	8	12.9	17	69
SolS-IIIa-F + SolS-IV-R	364	0.15	0.62	1.00	8	13.7	20	93

[a] Analyzed varieties were identical to cultivars included in Fig. 3

[b] Jaccard (1908)

provide a large resource for the selection of ISAP markers for in-depth studies of potato genetic diversity.

Analysis of the genetic diversity of potato cultivars

The selected primer combinations SolS-IIIa-F/SolS-IIIa-R, SolS-IIIa-F/SolS-IV-F, and SolS-IIIa-F/SolS-IV-R were applied to a pilot set of 53 commercially available potato cultivars. The number of bands generated by the primer sets varied from 8 to 22, while the average similarities of the banding patterns of the cultivars, represented by the Jaccard similarity coefficient, ranged from 62 to 74% (Table 2). The primer combination SolS-IIIa-F/SolS-IV-R provided the best resolution, discriminating 47 out of 53 genotypes. Hence, in three cases the patterns between two varieties were identical (maximum similarity value 100%) and could not be resolved. However, they can be clearly differentiated by a second primer combination. Application of each of the possible combinations of two ISAP primer pairs was sufficient for an unambiguous discrimination, as the maximum similarity value did not exceed 95% and enabled the discrimination of all varieties tested.

Based on the combined ISAP data of all three primer combinations, a cluster analysis was performed to visualize the genetic diversity of the 53 tested varieties (Fig. 3). In addition to *S. tuberosum* ssp. *tuberosum* cultivars, we also included the semi-cultivated *S. tuberosum* ssp. *andigenum*, and one individual each of the distantly related wild potato species *S. demissum* and *S. stoloniferum*. Cultivated and wild species were placed on separate branches and all varieties were clearly differentiated. The cultivars 'Gala' and 'Heidi' showed the lowest genetic distance according to the branch lengths in the dendrogram with a similarity of 92.7%. *S. tuberosum* ssp. *andigenum* grouped with the potato cultivars demonstrating their close relation and intensive involvement in the breeding of today's cultivars.

To explore the applicability of the ISAP markers to a large cultivar collection, we analyzed 364 commercial European varieties and breeding lines using the primer set SolS-IIIa-F/SolS-IV-R. Comparing the statistical values from the analysis of this extended variety collection to the pilot set described above, the minimum similarity decreased from 27 to 15%, while the highest number of bands increased from 17 to 20 (Table 2). The percentage of polymorphic bands increased from 69 to 93%, and the application of this primer combination alone enabled the differentiation of 237 (65%) out of 364 varieties. A GelCompar II-based catalog for the analyses and comparison of the ISAP patterns used for identification has been established (screenshot in Fig. 4). Currently, the database contains 364 commercial European varieties and breeding lines and will further be expanded with varieties and combined marker data.

We further analyzed two somaclonal variants, 'Arran Comet' and 'Pearl', which are indistinguishable with SSR markers (Reid and Kerr 2007). These two cultivars showed identical bands for the primer pairs used for cultivar differentiation. However, we identified the primer combinations SolS-II-F/SolS-II-R and SolS-V-F/SolS-VII-R that, upon application, resulted in polymorphisms between 'Arran Comet' and 'Pearl' (Fig. 5a).

To investigate the stability of the ISAP marker profiles, we applied the primer sets SolS-IIIa-F/SolS-IIIa-R, SolS-IIIa-F/SolS-IV-F, and SolS-IIIa-F/SolS-IV-R to a long-term in vitro maintained 'Gala' specimen. The cultivation of plant material in vitro can induce stress and cause transpositional activity of retrotransposons (Hirochika 1993). The comparison of the banding pattern of a

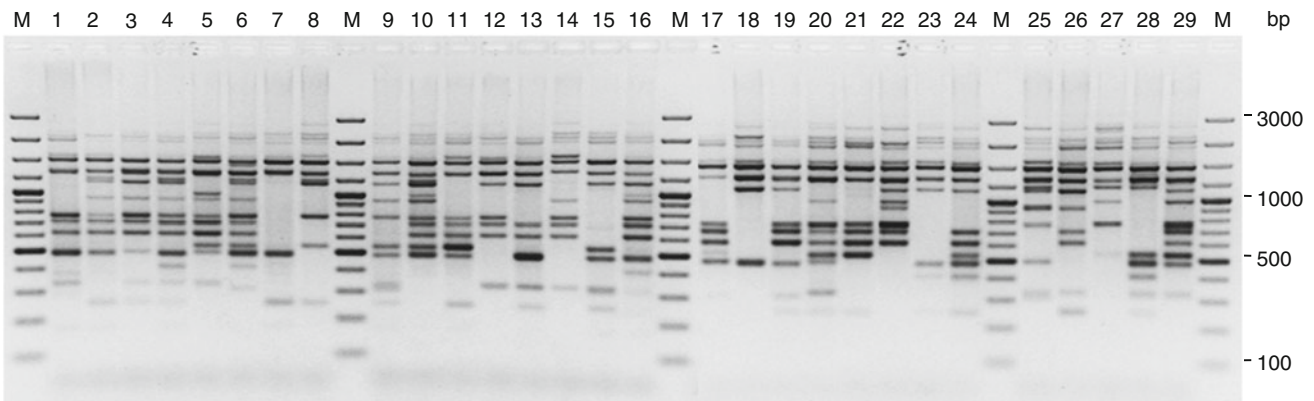
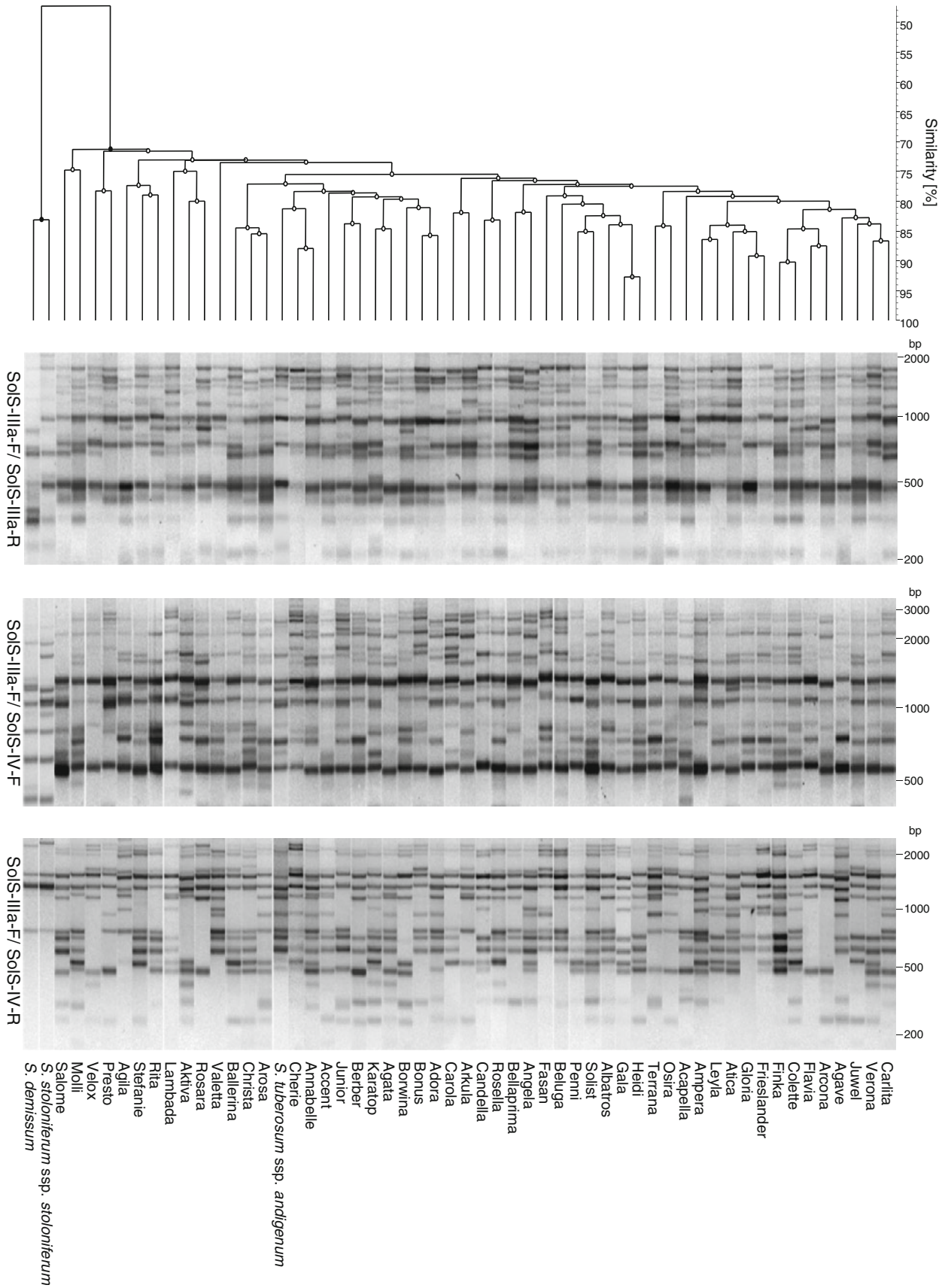


Fig. 2 Examples of the ISAP banding patterns for potato varieties generated by the primer combination SolS-IIIa-F/SolS-IV-R. Bands are highly polymorphic and well resolvable by gel electrophoresis. The following varieties were analyzed: 'Acapella' (1), 'Accent' (2), 'Adora' (3), 'Agata' (4), 'Angela' (5), 'Annabelle' (6), 'Arcona' (7), 'Arkula' (8), 'Arosa' (9), 'Atica' (10), 'Ballerina' (11), 'Bellaprima' (12), 'Berber' (13), 'Bonus' (14), 'Borwina' (15), 'Carlita' (16), 'Rita' (17), 'Rosara' (18), 'Salome' (19), 'Solist' (20), 'Stefanie' (21), 'Valetta' (22), 'Velox' (23), 'Verona' (24), 'Terrana' (25), 'Agave' (26), 'Agila' (27), 'Aktiva' (28), 'Ampera' (29), size marker (M). Fragments were separated on 2% agarose gels


Similarity [%]
50
55
60
65
70
75
80
85
90
95
100
SolS-IIIa-F/ SolS-IIIa-R
SolS-IIIa-F/ SolS-IV-F
SolS-IIIa-F/ SolS-IV-R
bp
2000
1000
500
200
3000
S. demissum
S. stoloniferum ssp. stoloniferum
Salome
Molli
Velox
Presto
Agila
Stefanie
Rita
Lambada
Aktiva
Rosara
Valetta
Ballerina
Christa
Arosa
S. tuberosum ssp. andigenum
Cherie
Annabelle
Accent
Junior
Berber
Karatop
Agata
Borwina
Bonus
Adora
Carola
Arkula
Candella
Rosella
Bellaprima
Angela
Fasan
Beluga
Penni
Solist
Albatros
Gala
Heidi
Terrana
Osira
Acapella
Ampera
Leyla
Atica
Gloria
Frieslander
Finka
Colette
Flavia
Arcona
Agave
Juwel
Verona
Carlita

◀ **Fig. 3** Cluster analysis of cultivated potato varieties and three *Solanum* species based on three ISAP banding patterns. The wild potato species, *S. demissum* and *S. stoloniferum*, are separated from the cultivated forms. The tree was constructed by UPGMA analysis (Dice similarity coefficient) based on the three ISAP banding patterns displayed. Primer combinations are indicated on the left

field-grown plant to that of the 'Gala' plantlet propagated in vitro continuously since 2000 showed no differences, verifying the stability of ISAP markers (Fig. 5b).

For a higher resolution, the ISAP markers can also be analyzed on a capillary sequencer (Fig. 6). For this approach, only labeling of one primer with a fluorescent dye and an adequate size standard are required. This enables automation and analyses of large numbers of varieties in parallel, the generated banding patterns can be directly captured by the software and analyzed in the pattern catalog.

Chromosomal and genomic organization of the SINE families and their distribution within the Solanaceae

High copy numbers and location in PCR amplifiable distances result in a relatively large number of ISAP bands and indicate a dispersed distribution of the potato SINEs families SolS-IIIa and SolS-IV (Fig. 1b). In order to investigate the genome coverage of SolS-IIIa and SolS-IV, we studied the abundance and distribution of these SINE families in the potato genome and investigated their conservation across related Solanaceae species. Fragments derived from the tRNA-unrelated regions of SolS-IIIa and SolS-IV were used as probes.

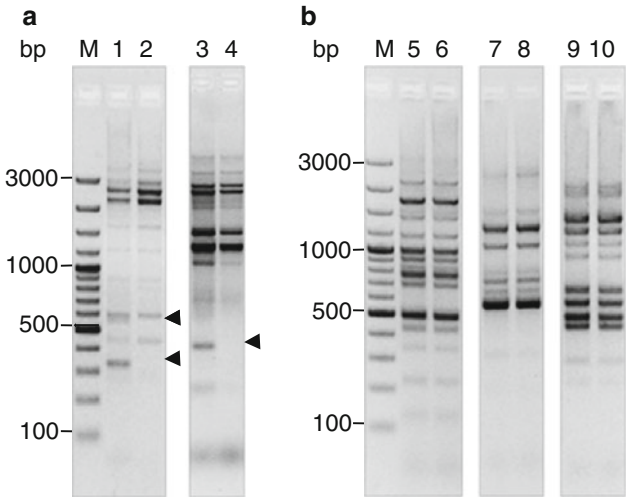


Fig. 5 Differentiation of somaclonal mutants and pattern stability during in vitro propagation. **a** 'Arran Comet' (1, 3) and 'Pearl' (2, 4) can be distinguished by the ISAP primer combinations SolS-II-F/SolS-II-R (1, 2) and SolS-V–F/SolS-VII-R (3, 4). Polymorphic bands are indicated by *arrowheads*. **b** Analyses with the three genotyping primer sets SolS-IIIa-F/SolS-IIIa-R (5, 6), SolS-IIIa-F/SolS-IVF (7, 8), and SolS-IIIa-F/SolS-IV-R (9, 10) show stable banding patterns and no differences between field-grown 'Gala' plants (5, 7, 9) and a 'Gala' plant propagated in vitro since 2000 (6, 8, 10). Fragments and size marker (M) were separated on 2% agarose gels

The chromosomal distribution was visualized by fluorescent in situ hybridization (FISH) to mitotic metaphase chromosomes of the cultivar 'Gala' (Fig. 7a, b). For both SolS families, scattered fluorescence signals were detected along all 48 chromosomes reflecting their dispersed organization, high abundance, and genome coverage in potato.

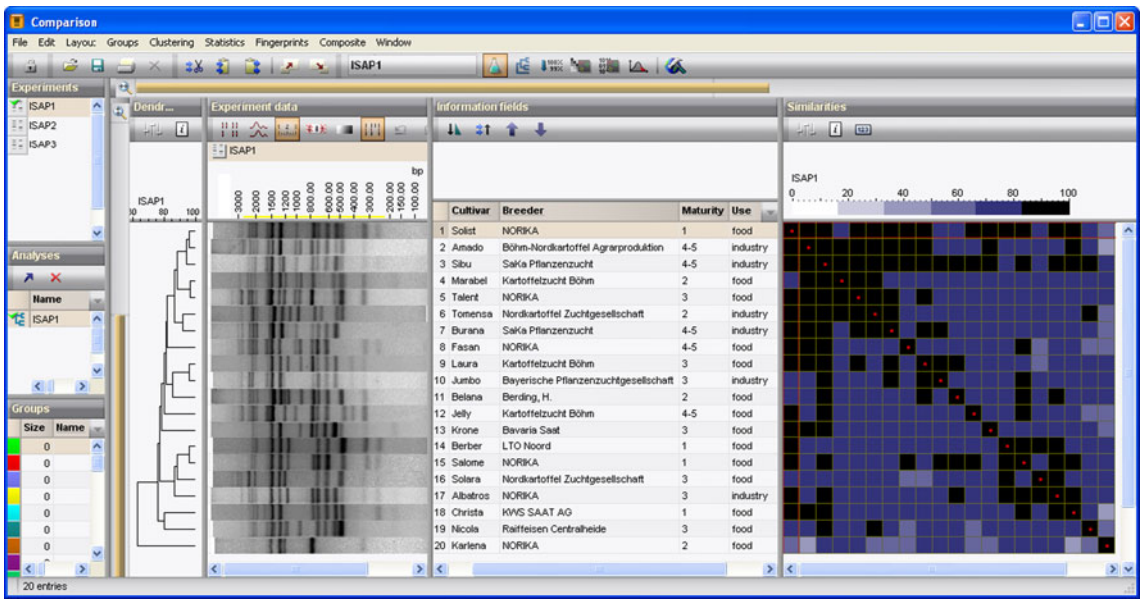


Fig. 4 GelCompar II screenshot of the ISAP marker catalog of selected registered varieties. The catalog contains variety information (*Information fields*) and ISAP banding patterns (*Experiment data*). *Comparison* enables cluster analyses to determine similarity values (*Similarities*) between varieties displayed as color-coded matrix useful for identification and selection

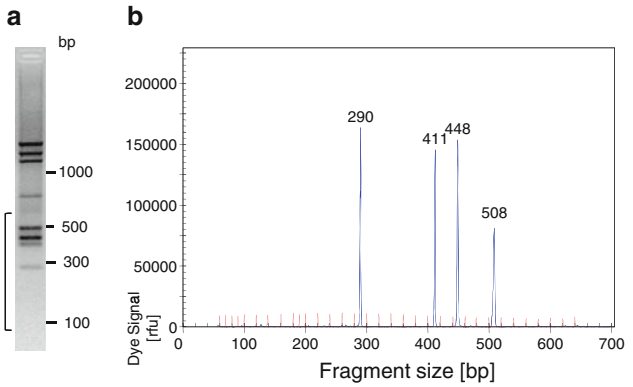


Fig. 6 ISAP fragment separation by capillary electrophoresis. Banding pattern of the cultivar 'Target' generated using the SolS-IV-R and the Cy5-labeled SolS-IIIa-F primer. Fragments were separated by 2% agarose gels (**a**) and on the Beckman CEQ^{TM} 8000 capillary sequencer (**b**). Unassigned small peaks refer to the internal size standard. The *bracket* indicates the analyzed size range

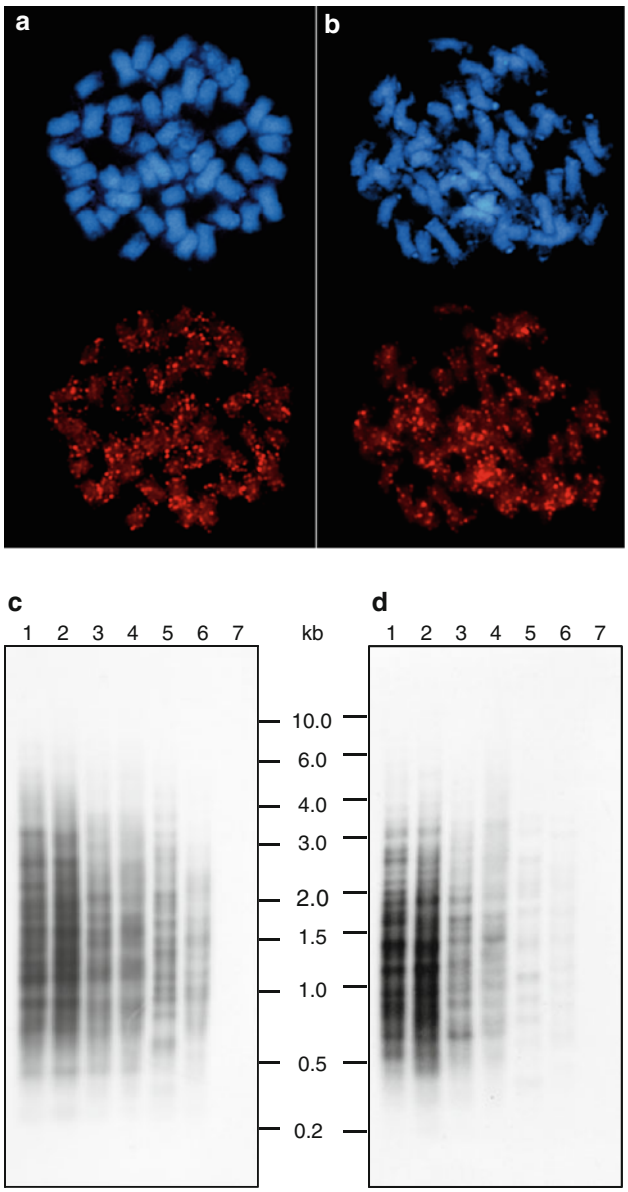


Fig. 7 Chromosomal organization and species distribution within the Solanaceae of the SINE families SolS-IIIa (**a**, **c**) and SolS-IV (**b**, **d**). Fluorescent in situ hybridization (FISH) for the reference potato cultivar 'Gala' shows the dispersed localization of the SINE families on mitotic metaphase chromosomes. In each panel, the chromosomal morphology is shown as DAPI-stained DNA (*top*) as well as the hybridization pattern for SolS-IIIa (**a**) and SolS-IV (**b**) analyzed with biotin-labeled probes detected as Cy3-fluorescence (*bottom*). For Southern analysis (**c**, **d**) genomic DNA was digested with *Fok*I, electrophoretically separated and hybridized with the SINE families SolS-IIIa (**c**) and SolS-IV (**d**). Both families are present in all species of the genus *Solanum* with varying abundance. The following species were included: *Solanum tuberosum* ssp. *tuberosum* 'Gala' (1), *S. tuberosum* ssp. *andigenum* (2); the wild potatoes *S. stoloniferum* ssp. *stoloniferum* (3), *S. demissum* (4); *S. lycopersicum* 'Tamina' (5); *S. melongena* 'SOL 1041' (6); and *Capsicum annuum* 'De Cayenne' (7)

The signal distribution indicates an almost uniform dispersal extending to the distal chromosomal regions (Fig. 7a). Strong signals suggest a local accumulation of SINEs, while in some regions of the genome the SINE copy number is reduced but they are not completely absent.

Subsequently, we examined the presence of SolS-IIIa and IV in cultivated and wild potatoes and related Solanaceae crops. *Fok*I-restricted genomic DNA of two cultivated potato subspecies, *S. tuberosum* ssp. *tuberosum* 'Gala' and *S. tuberosum* ssp. *andigenum,* the wild potato species *S. stoloniferum* and *S. demissum,* the Solanaceae crops tomato (*S. lycopersicum*), and eggplant (*S. melongena*), as well as pepper (*Capsicum annuum*), was used for Southern hybridization (Fig. 7c, d). Both SINE families hybridized strongly to the genus *Solanum* (Fig. 7c, d, lanes 1–6), particularly to the four potato species (Fig. 7c, d, lanes 1–4). The hybridization over a wide range of molecular weight in all *Solanum* species verifies the dispersed genomic organization and high abundance of both SINE families. The strongest hybridization signals were detected within the two cultivated *S. tuberosum* subspecies *tuberosum* and *andigenum* (Fig. 7c, d, lanes 1 and 2). A lower abundance was observed for *S. demissum* and *S. stoloniferum* (Fig. 7c, d, lanes 3 and 4). In tomato and eggplant (Fig. 7c, d, lanes 5 and 6), hybridization has also been observed, however, the signal intensity of SolS-IV was lower compared to SolS-IIIa. The occurrence of SolS families in the distantly related pepper (Fig. 7c, d, lane 7) was only detectable after an increased exposure time (data not shown).

Discussion

We developed ISAP markers for the analysis and differentiation of potato varieties. For the ~850 Mbp potato genome (Arumuganathan and Earle 1991; Huang et al. 2011), approximately 6.500 SINE copies were calculated falling into nine families and subfamilies (Wenke et al.

2011). ISAP markers are based on the amplification of genomic sequences between adjacent SINEs. Assuming an equidistant distribution, the average distance between two SINE copies is 131 kb. However, SINEs show a tendency to cluster (Jurka et al. 2005) and smaller distances between SINEs can be bridged by PCR. Fluorescent in situ hybridization confirmed the genome-wide distribution of regions with higher SINE density along all potato chromosomes.

Southern analyses demonstrated the high abundance of the SINE families, SolS-IIIa and IV, in *Solanum* species. Both SolS-families showed a stronger hybridization for the cultivated *S. tuberosum* subspecies *tuberosum* and *andigenum* compared to the wild potatoes, *S. demissum* and *S. stoloniferum*, which indicates an amplification of these SINE families after radiation of *S. tuberosum* species. Amplification most likely occurred after *S. tuberosum* and *S. lycopersicum* split from the last common ancestor less than 7.3 Mya (Wu and Tanksley 2010).

Repetitive DNA is rapidly evolving and different types of repeated sequences including satellite DNA families, retrotransposons, and transposable elements vary considerably in sequence, copy number, and species distribution (Kubis et al. 1998). This sequence divergence often results in species-specific amplification of particular sequence motifs. (Kamm et al. 1995; Schmidt et al. 1990). Transposable elements including retrotransposons such as Ty3-*gypsy*, Ty1-*copia*, LINEs, and SINEs facilitate genome evolution and lineage divergence (Oliver and Greene 2009). Accordingly, the activity of transposable elements contributed to genome divergence as observed in *Oryza* species and subspecies (Ma and Bennetzen 2004; Zuccolo et al. 2007).

The creation of new retrotransposon copies immediately before, during, or after speciation events and their evolutionary fixation are a source for insertional polymorphisms within and between species (Hamon et al. 2011; Kumar et al. 1997). Consistently, a better phylogenetic resolution of closely related species was observed for markers derived from active retrotransposon families (Schulman et al. 2004). Accordingly, SolS-IIIa and SolS-IV yielded highly polymorphic ISAP banding patterns in potato. However, transposition of these SINEs has not been studied yet and genomic rearrangements, priming site mutations, as well as internal insertions or deletions, can also cause polymorphisms (Bannikova et al. 2005).

Several non-retrotransposon markers have been applied for potato genome analysis including the marker techniques RAPD (randomly amplification of polymorphic DNA), SSR, ISSR (inter-simple sequence repeat), and AFLP. An obvious advantage of retrotransposon-based marker systems over markers based on single nucleotide polymorphisms (SNPs), RAPDs and AFLPs, is the unidirectionality of changes by retrotransposon integration. While the ancestral state of SNPs is unknown, it is always the 'empty site' state for retrotransposon insertions. The analysis of potato cultivars with LTR retrotransposon-derived IRAP markers revealed a number of bands which was in a similar range compared to ISAP markers described here (Nováková et al. 2009). However, ISAP provided notably fewer faint bands and reduced background smear. Although RAPD and ISSR markers provide similar informative banding patterns and numbers of polymorphic fragments compared to ISAP, they are very limited due to problems of reproducibility (McGregor et al. 2000). AFLP markers are more informative than ISAPs; however, they are more costly and require sophisticated equipment often not available for plant breeders. SSR markers detecting the variable copy number of microsatellite motifs are routinely used for potato genotyping using high-resolution fragment analysis systems (Reid et al. 2009, 2011; Reid and Kerr 2007). The clustering based on six SSR markers for 121 varieties yielded maximum similarities of 90–93% (Reid and Kerr 2007), which is in a similar range as obtained in this study based on only three ISAP reactions (92.7%, resolved on agarose gels). Less primer combinations and a less sophisticated fragment analysis system are required, which is an advantage of ISAPs. In addition, the number of SSR markers required rises quickly if the number of samples is increased, as has been shown for the differentiation of 856 out of 892 genotypes where already nine SSRs were needed (Reid et al. 2011). Complex mutations at potato SSR loci were reported by Lara-Cabrera and Spooner (2005). In contrast, we showed the stability of SINE markers by analysis of the long-term in vitro propagated potato cultivar 'Gala' where no stress induced SINE transposition was observed. However, SINE transposition cannot completely be ruled out, because novel copies might escape detection after insertion into distant genomic regions which are not amplifiable by PCR.

Two outward-facing primers derived from the nine potato SINE families and subfamilies enable 153 possible primer combinations. To obtain highly informative ISAP markers, the selection of suitable PCR primer pairs is crucial and influenced by several criteria: (1) abundance and distribution of the particular SINE family, (2) primer position within the SINE, (3) homologies within and between SINE families, and (4) the conservation of the priming sites. We recommend selecting primers located at the 3′ terminus, because SINEs are mobilized by LINEs by target-primed reverse transcription which is prone to abortion resulting in a large fraction of 5′ truncated SINE copies (Luan et al. 1993).

We combined the data of three primer sets to maximize the information content resulting in a clear separation of the varieties analyzed. The varieties 'Gala' and 'Heidi' with the highest similarity (92.7%) show a close relation in the dendrogram. This is consistent with data from potato

breeding: 'Heidi' is a direct progeny of 'Gala' (NORIKA GmbH, personal comm.). Furthermore, the pairs with the second and third highest similarities, 'Colette' and 'Finka' (90.2%) and 'Gloria' and 'Frieslander' (89.2%), are also parent and progeny (Potato Pedigree Database, Van Berloo et al. 2007) and were closely arranged in the dendrogram. Similarly in SSR analysis of 892 varieties, a maximal similarity of 91% was observed for one cultivar and its parent (Reid et al. 2011). Nevertheless, there are also related cultivars situated on different branches. For example, 'Leyla' is parent of 'Gala' and 'Finka', but positioned on a separate branch even though for 'Leyla' and 'Gala' the similarity is high (87.5%). This might reflect the complex breeding history of varieties that is often either not well documented or not accessible. Therefore, we want to emphasize the clear differentiation of the varieties rather than their relationship. The information and ISAP markers stored in the GelCompar II database (Fig. 4) have been already successfully applied for variety identification and selection of breeding lines by a breeding company (NORIKA GmbH, personal comm.). The database can be supplemented and combined with additional information (e.g., on agronomic traits such as flowering time, maturity or resistances against plant pathogens) as well as other molecular marker types.

This study included the two cultivars 'Arran Comet' and 'Pearl', most likely arisen by somaclonal variation. Using conventional SSR markers, these two cultivars cannot be distinguished from each other (Reid and Kerr 2007). However, by extending the ISAP primer combinations, we were able to identify at least three polymorphic bands between these two varieties. Thus, retrotransposon-based ISAP markers can facilitate the identification of very closely related cultivars which have been already shown for somaclonal variants in barley using LTR retrotransposon markers (Campbell et al. 2011).

Solanum tuberosum ssp. *andigenum* was grouped with cultivated varieties in the ISAP-based cluster analysis indicating genomic similarities or shared genome fractions. Consistently, RFLP markers placed *S. tuberosum* ssp. *andigenum* and ssp. *tuberosum* in the same cluster (Sukhotu et al. 2004). These assignments reflect the close relationship of these two subspecies resulting in the recent reclassification where Chilotanum (*S. tuberosum* ssp. *tuberosum*) and Andigenum (*S. tuberosum* ssp. *andigenum*) are two cultivar groups of the botanical species *S. tuberosum* (Ovchinnikova et al. 2011; Spooner et al. 2007). Moreover, *S. tuberosum* ssp. *andigenum* was used as a crossing partner to introduce resistance genes (Simko et al. 2007).

In summary, the ISAP method is rapid, robust and not confined to cultivated potato. ISAP markers could also be useful to identify redundant accessions in potato germplasm collections. Nevertheless, the resolution regarding wild potatoes remains to be investigated in detail as heterogeneity in wild species is likely. Based on the accelerating number of plant genome sequences, it can be transferred to other crops as the identification of plant SINEs is straightforward (Wenke et al. 2011). As described here, ISAP markers are suitable for variety differentiation and applicable by breeders to verify the identity and purity of potato varieties which is important regarding consumers' rights.

Acknowledgments We gratefully acknowledge the German Federal Ministry of Education and Research for funding this project (KMU-Innovativ, grant no. 0315425). We thank the Science and Advice for Scottish Agriculture Organization (SASA, UK) for generously providing seed and plant material and I. Walter for technical support.

References

Alix K, Paulet F, Glaszmann JC, D'Hont A (1999) Inter-*Alu*-like species-specific sequences in the *Saccharum* complex. Theor Appl Genet 99:962–968

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Ashkenazi V, Chani E, Lavi U, Levy D, Hillel J, Veilleux RE (2001) Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. Genome 44:50–62

Bannikova AA, Lavrenchenko LA, Kramerov DA (2005) Phylogenetic relationships between Afrotropical and Palaearctic *Crocidura* species inferred from inter-SINE-PCR. Biochem Syst Ecol 33:45–59

Baurens FC, Noyer JL, Lanaud C, Lagoda PJL (1998) Inter-*Alu* PCR like genomic profiling in banana. Euphytica 99:137–142

Bennetzen JL (1996) The contributions of retroelements to plant genome organization, function and evolution. Trends Microbiol 4:347–353

Bennetzen JL, Ma J, Devos KM (2005) Mechanisms of recent genome size variation in flowering plants. Ann Bot 95:127–132

Campbell B, LeMare S, Piperidis G, Godwin I (2011) IRAP, a retrotransposon-based marker system for the detection of somaclonal variation in barley. Mol Breed 27:193–206

Desel C, Jung C, Cai DG, Kleine M, Schmidt T (2001) High-resolution mapping of YACs and the single-copy gene Hs1(pro)-1 on *Beta vulgaris* chromosomes by multi-colour fluorescence in situ hybridization. Plant Mol Biol 45:113–122

Flavell AJ, Knox MR, Pearce SR, Ellis THN (1998) Retrotransposon-based insertion polymorphisms (RBIP) for high throughput marker analysis. Plant J 16:643–650

Ghislain M, Spooner DM, Rodriguez F, Villamon F, Nunez J, Vasquez C, Waugh R, Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor Appl Genet 108:881–890

Ghislain M, Núñez J, del Rosario HerreraM, Pignataro J, Guzman F, Bonierbale M, Spooner D (2009) Robust and highly informative microsatellite-based genetic identity kit for potato. Mol Breed 23:377–388

Hamon P, Duroy PO, Dubreuil-Tranchant C et al (2011) Two novel Ty1-*copia* retrotransposons isolated from coffee trees can effectively reveal evolutionary relationships in the *Coffea* genus (Rubiaceae). Mol Genet Genomics 285:447–460

Hirochika H (1993) Activation of tobacco retrotransposons during tissue-culture. EMBO J 12:2521–2528

Hosaka K, Sanetomo R (2009) Comparative differentiation in mitochondrial and chloroplast DNA among cultivated potatoes and closely related wild species. Genes Genet Syst 84:371–378

Huang SW, Xu X, Pan SK et al (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:U189–U194

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat 44:223–270

Jacobs MM, van den Berg RG, Vleeshouwers VG, Visser M, Mank R, Sengers M, Hoekstra R, Vosman B (2008) AFLP analysis reveals a lack of phylogenetic structure within *Solanum* section *Petota*. BMC Evol Biol 8:145

Jacobs MMJ, Smulders MJM, van den Berg RG, Vosman B (2011) What's in a name; genetic structure in *Solanum* section *Petota* studied using population-genetic tools. BMC Evol Biol 11:42

Jurka J, Kohany O, Pavlicek A, Kapitonov VV, Jurka MV (2005) Clustering, duplication and chromosomal distribution of mouse SINE retrotransposons. Cytogenet Genome Res 110:117–123

Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 98:704–711

Kalendar R, Antonius K, Smykal P, Schulman AH (2010) iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theor Appl Genet 121:1419–1430

Kamm A, Galasso I, Schmidt T, Heslop-Harrison JS (1995) Analysis of a repetitive DNA family from *Arabidopsis arenosa* and relationship between *Arabidopsis* species. Plant Mol Biol 27:853–862

Kass DH, Batzer MA (1995) Inter-*Alu* polymerase chain reaction: advancements and applications. Anal Biochem 228:185–193

Kostia S, Ruohonen-Lehto M, Vainola R, Varvio SL (2000) Phylogenetic information in inter-SINE and inter-SSR fingerprints of the Artiodactyla and evolution of the Bov-tA SINE. Heredity 84:37–45

Kramerov DA, Vassetzky NS (2005) Short retroposons in eukaryotic genomes. Int Rev Cytol 247:165–221

Kubis S, Schmidt T, Heslop-Harrison JS (1998) Repetitive DNA elements as a major component of plant genomes. Ann Bot 82:45–55

Kumar A, Pearce SR, McLean K, Harrison G, Heslop-Harrison JS, Waugh R, Flavell AJ (1997) The Ty1-*copia* group of retrotransposons in plants: genomic organisation, evolution, and use as molecular markers. Genetica 100:205–217

Lara-Cabrera SI, Spooner DM (2004) Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Plant Syst Evol 248:129–142

Lara-Cabrera SI, Spooner DM (2005) Taxonomy of Mexican diploid wild potatoes (*Solanum* sect. *Petota*): morphological and microsatellite data. Monogr Syst Bot Mo Bot Gard 104:199–205

Lenoir A, Lavie L, Prieto JL, Goubely C, Cote JC, Pelissier T, Deragon JM (2001) The evolutionary origin and genomic organization of SINEs in *Arabidopsis thaliana*. Mol Biol Evol 18:2315–2322

Li X, van Eck HJ, Rouppe van der Voort JNAM, Huigen D-J, Stam P, Jacobsen E (1998) Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theor Appl Genet 96:1121–1128

Lightbourn GJ, Jelesko JG, Veilleux RE (2007) Retrotransposon-based markers from potato monoploids used in somatic hybridization. Genome 50:492–501

Luan DD, Korman MH, Jakubczak JL, Eickbush TH (1993) Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site—a mechanism for non-LTR retrotransposition. Cell 72:595–605

Ma JX, Bennetzen JL (2004) Rapid recent growth and divergence of rice nuclear genomes. Proc Natl Acad Sci USA 101:12404–12410

McGregor CE, Lambert CA, Greyling MM, Louw JH, Warnich L (2000) A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica 113:135–144

Moisan-Thiery M, Marhadour S, Kerlan M, Dessenne N, Perramant M, Gokelaere T, Le Hingrat Y (2005) Potato cultivar identification using simple sequence repeats markers (SSR). Potato Res 48:191–200

Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis R, Webster TD, Ledbetter DH, Caskey CT (1989) *Alu* polymerase chain reaction: a method for rapid isolation of human-specific sequences from complex DNA sources. Proc Natl Acad Sci USA 86:6686–6690

Nováková A, Šimáčková K, Bárta J, Čurn V (2009) Potato variety identification by molecular markers based on retrotransposon analyses. Czech J Genet Plant Breed 45:1–10

Okada N (1991) SINEs. Curr Opin Genet Dev 1:498–504

Oliver KR, Greene WK (2009) Transposable elements: powerful facilitators of evolution. Bioessays 31:703–714

Ovchinnikova A, Krylova E, Gavrilenko T, Smekalova T, Zhuk M, Knapp S, Spooner DM (2011) Taxonomy of cultivated potatoes (*Solanum* section *Petota*: Solanaceae). Bot J Linn Soc 165:107–155

Pieterse L, Hils U (2009) World Catalogue of Potato Varieties 2009/10 Agrimedia

Reid A, Kerr EM (2007) A rapid simple sequence repeat (SSR)-based identification method for potato cultivars. Plant Genet Resour 5:7–13

Reid A, Hof L, Esselink D, Vosman B (2009) Potato cultivar genome analysis. In: Burns R (ed) Plant pathology: techniques and protocols. Humana, New York, pp 295–308

Reid A, Hof L, Felix G et al (2011) Construction of an integrated microsatellite and key morphological characteristic database of potato varieties on the EU Common Catalogue. Euphytica 182:239–249

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schmidt T (1999) LINEs, SINEs and repetitive DNA: non-LTR retrotransposons in plant genomes. Plant Mol Biol 40:903–910

Schmidt T, Junghans H, Metzlaff M (1990) Construction of *Beta procumbens*-specific DNA probes and their application for the screening of *B.vulgaris* × *B.procumbens* ($2n = 19$) addition lines. Theor Appl Genet 79:177–181

Schmidt T, Schwarzacher T, Heslop-Harrison JS (1994) Physical mapping of rRNA genes by fluorescent in situ hybridization and structural analysis of 5S rRNA genes and intergenic spacer sequences in sugar beet (*Beta vulgaris*). Theor Appl Genet 88:629–636

Schulman AH, Flavell AJ, Ellis THN (2004) The application of LTR retrotransposons as molecular markers in plants. In: Miller WJ, Capy P (eds) Mobile genetic elements. Humana, Totowa, pp 145–174

Schwarzacher T, Heslop-Harrison P (2000) Practical in situ hybridization. BIOS Scientific Publishers Ltd, Oxford

Scotti N, Cozzolino S, Cardi T (2007) Mitochondrial DNA variation in cultivated and wild potato species (*Solanum* spp.). Genome 50:706–713

Simko I, Jansky SH, Stephenson S, Spooner DM (2007) Genetics of resistance to pests and disease. In: Vreugdenhil D, Bradshaw J, Gebhardt C, Govers F, MacKerron DKL, Taylor MA, Ross HA (eds) Potato biology and biotechnology—advances and perspectives. Elsevier, Amsterdam, pp 117–155

Spooner DM, Hijmans RJ (2001) Potato systematics and germplasm collecting, 1989–2000. Am J Potato Res 78:237–268

Spooner DM, Salas A (2006) Structure, biosystematics, and genetic resources. In: Gopal J, Khurana SMP (eds) Handbook of potato production, improvement, and postharvest management. Haworth's Press, Binghamton, pp 1–39

Spooner DM, McLean K, Ramsay G, Waugh R, Bryan GJ (2005a) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. Proc Natl Acad Sci USA 102:14694–14699

Spooner DM, Nunez J, Rodriguez F, Naik PS, Ghislain M (2005b) Nuclear and chloroplast DNA reassessment of the origin of Indian potato varieties and its implications for the origin of the early European potato. Theor Appl Genet 110:1020–1026

Spooner DM, Núñez J, Trujillo G, Del Rosario Herrera M, Guzmán F, Ghislain M (2007) Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proc Natl Acad Sci USA 104:19398–19403

Sukhotu T, Hosaka K (2006) Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers. Genome 49:636–647

Sukhotu T, Kamijima O, Hosaka K (2004) Nuclear and chloroplast DNA differentiation in Andean potatoes. Genome 47:46–56

Van Berloo R, Hutten RCB, Van Eck HJ, Visser RGF (2007) An online potato pedigree database resource. Potato Res 50:45–57

Wenke T, Döbel T, Sörensen TR, Junghans H, Weisshaar B, Schmidt T (2011) Targeted identification of short interspersed nuclear element families shows their widespread existence and extreme heterogeneity in plant genomes. Plant Cell 23:3117–3128

Wu F, Tanksley SD (2010) Chromosomal evolution in the plant family Solanaceae. BMC Genomics 11:182

Zuccolo A, Sebastian A, Talag J, Yu Y, Kim H, Collura K, Kudrna D, Wing RA (2007) Transposable element distribution, abundance and role in genome size variation in the genus *Oryza*. BMC Evol Biol 7:152