

## Genetic diversity analysis of *Solanum* accessions from Czech collections of potato genetic resources using nuclear SSR markers

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**Abstract:** The genus *Solanum* comprises numerous wild and cultivated species that are important for potato breeding. This pilot-scale study aimed to evaluate the genetic diversity in 44 accessions from *Solanum* sect. Petota, comprising wild species, Andean landraces, and modern cultivars, obtained from the Potato Research Institute Havlíčkův Brod, Ltd. and the Department of Crop Sciences and Agroforestry at the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague. Nuclear microsatellite markers (SSR, 29 loci) were applied via five multiplex PCR reactions and analysed using capillary electrophoresis. Binary data matrices were analysed using DARwin software to generate dendrograms reflecting allelic polymorphism. The SSR panel effectively differentiated cultivated accessions from wild types, consistent with the current taxonomy of the genus *Solanum*, with particularly clear clustering of Andean landraces and modern varieties. However, resolution among wild accessions was limited, likely due to their high genetic complexity and interspecific overlap. These results support the suitability of the SSR panel for analysing diversity in cultivated potatoes, while also highlighting the challenges in resolving wild *Solanum* taxa. This study contributes to germplasm characterisation and provides a molecular basis for future breeding programmes.

**Keywords:** DNA polymorphism; germplasm conservation; microsatellite markers; multiplex PCR; wild potatoes

The genus *Solanum* is one of the largest and most taxonomically complex plant genera, comprising approximately 2 000 species (Kaunda & Zhang 2019). The classification of the genus, particularly section

Petota, has undergone considerable revision. Earlier systems were based mainly on morphological traits, whereas current approaches rely on molecular and phylogenetic data (Spooner et al. 2014). A foun-

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dational classification was developed by Hawkes (1990), who divided section *Petota* into two subsections: *Estolonifera* (non-tuber-bearing species) and *Potatoe* (tuber-bearing species). The genus was later divided based on the outline of corolla (Berg & Jacobs 2007) into 19 series grouped under two superseries (*Stellata* and *Rotata*). However, broad intraspecies variability and overlapping phenotypic characteristics among species limited the reliability of purely morphology-based taxonomy (Bradshaw & Mackay 1994; Salas et al. 2001). The subsection *Estolonifera* was excluded from *Petota* based on cpDNA analyses, which proved a closer affinity to tomato (Spooner et al. 1993). Following taxonomic revision redefined section *Petota* to include only tuber-bearing species and reducing the number of recognised species to less than half of those listed by Hawkes (Spooner et al. 2014). This classification, grounded in extensive molecular and morphological evidence, has been widely accepted and provided the basis for current genetic and breeding studies within the genus *Solanum*. The taxonomy of wild species was reconsidered based on plastid DNA (Spooner & Castillo 1997). About twenty morphologically similar South American species, which are probably ancestors of Andean potato cultivars, considered by Hawkes (1990) as members of the *Solanum brevicaulle* complex (*brevicaule* complex), have been reconsidered synonymous with *Solanum brevicaulle* and *S. candolleanum* (Spooner et al. 2014). The taxonomy is complicated by interspecies hybridisation, an unfinished phenomenon historically occurring in section *Petota*. It often generates interspecies hybrids with species-specific or intermediate morphology (Hawkes 1990; Bedonni & Camadro 2009; Cara et al. 2013 etc.). Hybridisation is reduced by various barriers essential for maintaining species identity, which prevent crossing between species or reduce the viability of hybrids (Masuelli et al. 2009). The interspecies incompatibility in the genus *Solanum* is controlled in various ways, differing in the intensity of expression. As it is evolutionarily facilitated by mechanisms preventing self-pollination in diploid species (Spooner et al. 2014), some species exhibit a strong reproductive barrier, while in others it can be overcome (Berg & Jacobs 2007).

The cultivated potato (*Solanum tuberosum* L.) is the third most important field crop worldwide (Devaux et al. 2021), and a principal representative of the genus. Modern cultivars are relatively less genetically diverse, which results in the susceptibility of potatoes to various biotic and abiotic stresses, underscoring the

importance of incorporating novel genetic variation from wild *Solanum* species (Handayani et al. 2019). The wild species harbour valuable alleles associated with resistance to diseases, temperature extremes, and soil-related stresses. These genetic resources, preserved in gene banks, represent an essential reservoir for the breeding of resilient cultivars (Hawkes 1991; Handayani et al. 2019). The incorporation of wild *Solanum* sect. *Petota* species into potato breeding programmes is challenging due to several reproductive barriers, most notably differences in ploidy levels and incompatibility related to mismatched endosperm balance numbers (EBN) (Heřmanová et al. 2007; Hardigan et al. 2015; Tang et al. 2022).

Detailed characterisation of germplasm stored in gene banks is essential to conserve valuable alleles and to guide effective utilisation of genetic resources in potato breeding. The genetic diversity of germplasm has been studied in various ways, whilst the microsatellite markers (SSRs – simple sequence repeats) are widely used due to their high polymorphism, codominant inheritance, and genome-wide distribution. Their application enables standardisation, reliable genotyping, and the comparison of accessions across different species and populations (Vieira et al. 2016).

In recent years, the gene banks adopted the classification system by Spooner et al. (2014), which reduced the number of species and worsened the orientation in the gene bank databases. This issue markedly influenced orientation in *Solanum brevicaulle* complex and *S. tuberosum* Andigena group, which now contains a range of former wild species important for breeding on resistance against pathogens. The identification of accessions and the management of germplasm collections should be supported by reliable molecular techniques, as recently accented by germplasm conservation initiatives (Zedek et al. 2023). The research presented in this manuscript was aimed to support these activities. The main hypothesis was that a panel of 30 nuclear SSR markers, designed previously for the identification of cultivated potato landraces, is suitable for characterising the genetic variability across a broad range of species of the genus *Solanum* sect. *Petota*, and that the resulting structure of genetic diversity is consistent with current taxonomy.

## MATERIAL AND METHODS

**Plant material.** In cooperation with Potato Research Institute Havlíčkův Brod, Ltd. (PRI) and

the Department of Crop Sciences and Agroforestry of the Faculty of Tropical AgriScience of the Czech University of Life Sciences Prague (FTA CZU), a set of 44 *in vitro* genotypes (Table 1) was obtained for pilot-scale analysis of polymorphisms in nuclear microsatellite markers (nSSR). The collection included all *Solanum* species classified according to Hawkes (1990), which were available in the potato gene bank *in vitro* of PRI and in the collection of the FTA CZU. Several species were represented by multiple genotypes to partially assess the intraspecies variability; these individual *in vitro* plants originated from different populations, as the original seeds used for culture production were provided by different sources at different times. The *in vitro* plants were previously transferred to field conditions, widely evaluated and morphologically verified by Zeka (2014) and Zeka et al. (2015) based on descriptions published by Correll (1962). The collection finally consisted of 26 wild tuber-bearing *Solanum* species, specifically originating from South (SA) and North America (NA), and cultivated potatoes represented by nine Andean landraces and nine random European varieties of cultivated potato (*Solanum tuberosum* L., Chilotanum group; Spooner et al. 2014).

**DNA extraction.** Nuclear DNA of each sample was extracted from 100 mg of plant material subjected to liquid nitrogen freezing and homogenisation. Isolation and purification of DNA were performed using the DNeasy® Plant MiniKit (Qiagen, Germany) according to the provider's instructions. The DNA quantity and purity were determined using NanoPhotometer™ UV/Vis Spectrophotometer (Implen, Germany). The DNA samples were subsequently diluted to a concentration of 5 ng per µL.

**Primer selection, multiplex design and amplification.** Thirty SSR primer pairs were selected from published sources (Milbourne et al. 1998; Ghislain et al. 2004, 2009; Feingold et al. 2005; Sedlák et al. 2022), whilst 24 of them were adopted from the methodology developed by CIP (the International Potato Center Lima, Peru) for the assessment of genetic diversity in potato landraces (Ghislain et al. 2009), and six other markers were associated with genes involved in tuber starch content. The markers were arranged into five multiplex PCR panels. The collection of markers thoroughly covered the whole *Solanum* genome (Figure 1). The sequences of all markers were compared with the reference genome ASM982715v1 (*Solanum tuberosum* cultivar P8) in the

NCBI database (National Centre for Biotechnology Information, USA). It was verified that all markers occur only once in the annotated genome except for STM0031, which was duplicated on chromosomes III and VII (Table S1 in Electronic Supplementary Material (ESM)). Detailed characterisation of markers and relevant information about compositions of multiplexes is provided in Supplementary material (Table S1 in ESM).

The multiplex PCR mixture consisted of 10 ng of template DNA, 1× Multiplex PCR Master Mix (Qiagen, Germany), and a specific mixture of primers (Table S1 in ESM). Multiplex PCR was performed in a C1000 Thermal Cycler with Dual 48-Well Block (Bio-Rad, USA). The thermal profile consisted of an initial denaturation (95 °C, 10 min), followed by 35 cycles of denaturation (95 °C, 30 s), annealing (56 °C, 90 s), and extension (72 °C, 60 s). A final extension step was carried out at 60 °C for 80 min.

**Capillary electrophoresis.** Amplified fragments were diluted 1:19 with ddH<sub>2</sub>O followed by mixing of one µL of diluted PCR product with 12 µL of Hi-Di™ formamide and 0.2 µL of GeneScan™ 600 LIZ size standard (Thermo Fisher Scientific, USA). Samples were denatured at 95 °C for 15 min using a Biometra T-gradient thermal cycler (Analytik Jena, Germany). PCR products were then analysed by means of capillary electrophoresis ABI PRISM 310 Genetic Analyser (Applied Biosystems, USA) using a 36-cm capillary. The separation of fragments was carried out at 15 kV for 28 min at 60 °C.

**Data analysis.** Capillary electrophoresis data were evaluated using GeneMapper® Software Ver. 4.1 (Applied Biosystems, USA). Allele sizes were automatically assigned by the software, followed by manual curation. Alleles falling outside predefined bin ranges were manually corrected, and false-positive peaks were removed. The curated allelic data were exported as a CSV file and converted into a binary matrix (1/0). Descriptive genetic diversity parameters per locus, such as the number of alleles, expected and observed heterozygosity, and PIC were calculated using Microsoft Excel 365.

Genetic structure was evaluated using unweighted neighbour-joining (UNJ) algorithm (Saitou & Nei 1987) based on Jaccard's similarity coefficients (Jaccard 1901), with 1 000 bootstraps. Genetic structure and distances were presented as dendrograms. All computations and final graphic visualisations to assess genetic structure were performed using DARwin v6.0.21 (Perrier & Jacquemoud-Collet 2006).

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Table 1. List of genotypes analysed by nSSR

Sample	Hawkes (1990)	Spooner et al. (2014)	GRIN accession code	Ploidy <sup>b</sup>	Category <sup>c</sup>
saca_27	<i>Solanum acaule</i> Bitter	<i>S. acaule</i> Bitter	07S0300027	4×	SA
sber_260	<i>S. berthaultii</i> Hawkes	<i>S. berthaultii</i> Hawkes	07S0300260	2×	SA
sajan_6886	<i>S. ajanhuiri</i> Juz. & Bukasov	<i>S. ajanhuiri</i> Juz. & Bukasov	BOL6886TR-2n <sup>a</sup>	2×	AL
sajan_6985	<i>S. ajanhuiri</i> Juz. & Bukasov	<i>S. ajanhuiri</i> Juz. & Bukasov	BOL6985TR-2n <sup>a</sup>	2×	AL
sand_108	<i>S. tuberosum</i> subsp. <i>andigenum</i> Hawkes	<i>S. tuberosum</i> Andigenum group	07S0300108	4×	AL
sbol_15217	<i>S. boliviense</i> Dunal in DC.	<i>S. boliviense</i> Dunal in DC.	BOL15217-2n <sup>a</sup>	2×	SA
sblb_240	<i>S. bulbocastanum</i> Dunal in Poir.	<i>S. bulbocastanum</i> Dunal in Poir.	07S0300240	2×	NA
sblb_17	<i>S. bulbocastanum</i> Dunal in Poir.	<i>S. bulbocastanum</i> Dunal in Poir.	07S0300322	2×	NA
sfind_275	<i>S. fendleri</i> A. Gray	<i>S. stoloniferum</i> Schltdl.	07S0300275	4×	NA
sgonio_109	<i>S. stenotomum</i> Juz. & Bukasov subsp. <i>gontocalyx</i> (Juz. & Bukasov) Hawkes	<i>S. tuberosum</i> Andigenum group	07S0300109	2n	AL
sgour_43	<i>S. gourlayi</i> Hawkes	<i>S. brevicaule</i> Bittner	07S0300043	2×	SA, BC
sgour_45	<i>S. gourlayi</i> Hawkes	<i>S. brevicaule</i> Bittner	07S0300045	2×	SA, BC
sguer_280	<i>S. guerrieroense</i> Correll	<i>S. guerrieroense</i> Correll	07S0300280	6×	NA
schac_37	<i>S. chacoense</i> Bitter	<i>S. chacoense</i> Bitter	07S0300037	2×	SA
schac_230	<i>S. chacoense</i> Bitter	<i>S. chacoense</i> Bitter	07S0300230	2×	SA
sincam_47	<i>S. incanayoense</i> K.A. Okada & A.M. Clausen	<i>S. brevicaule</i> Bittner	07S0300047	unspecified	SA, BC
slepto_48	<i>S. leptophyes</i> Bitter	<i>S. brevicaule</i> Bittner	07S0300048	2×	SA, BC
smicro_49	<i>S. microdontum</i> Bittner	<i>S. microdontum</i> Bittner	07S0300049	2×	SA
smoch_50	<i>S. mochiquirense</i> Ochoa	<i>S. mochiquirense</i> Ochoa	07S0300050	2×	SA
sphu_308	<i>S. phureja</i> Juz. & Bukasov	<i>S. tuberosum</i> Andigenum group Hawkes	07S0300308	2×	AL
spin_51	<i>S. pinnatisectum</i> Dunal	<i>S. pinnatisectum</i> Dunal	07S0300051	2×	NA
spoly_290	<i>S. polyadenium</i> Greenm.	<i>S. polyadenium</i> Greenm.	07S0300290	2×	NA
spotri_53	<i>S. polytrichon</i> Rydb.	<i>S. stoloniferum</i> Schltdl.	07S0300053	4×	NA
sspars_71	<i>S. sparsipilum</i> (Bitter) Juz. & Bukasov	<i>S. brevicaule</i> Bitter	07S0300071	2×	SA, BC
sspega_60	<i>S. spegazzinii</i> Bitter	<i>S. brevicaule</i> Bitter	07S0300060	2×	SA, BC
ssten_212	<i>S. stenotomum</i> Juz. & Bukasov	<i>S. tuberosum</i> Andigenum group	07S0300212	2×	AL
ssten_4850	<i>S. stenotomum</i> Juz. & Bukasov	<i>S. tuberosum</i> Andigenum group	BOL4850TR-2n <sup>a</sup>	2×	AL
ssten_6821	<i>S. stenotomum</i> Juz. & Bukasov	<i>S. tuberosum</i> Andigenum group	BOL6821TR-2n <sup>a</sup>	2×	AL
sstol_295	<i>S. stoloniferum</i> Schltdl.	<i>S. stoloniferum</i> Schltdl.	07S0300295	4×	NA
ssucr_62	<i>S. sucrense</i> Hawkes	<i>S. brevicaule</i> Bitter	07S0300062	4×	SA, BC

Table 1 to be continued

Sample	Hawkes (1990)	Spooner et al. (2014)	GRIN accession code	Ploidy <sup>b</sup>	Category <sup>c</sup>
svrn_69	<i>S. vernei</i> Bitter & Wittm.	<i>S. vernei</i> Bitter & Wittm.	07S0300069	2×	SA
svrn_234	<i>S. vernei</i> Bitter & Wittm.	<i>S. vernei</i> Bitter & Wittm.	07S0300234	2×	SA
svru_299	<i>S. verrucosum</i> Schltldl.	<i>S. verrucosum</i> Schltldl.	07S0300299	2×	NA
sxchau_134	<i>S. chaucha</i> Juz. & Bukasov	<i>S. tuberosum</i> Andigenum group	07S0300134	3×	AL
syung_70	<i>S. yungasense</i> Hawkes	<i>S. chacoense</i> Bitter	07S0300070	2×	SA
Anuschka	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	-	4×	var
Carrera	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	-	4×	var
Colette	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0101780	4×	var
Colomba	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0102266	4×	var
Eurostarch	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0102267	4×	var
Finka	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0102065	4×	var
Impala	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0101538	4×	var
Verdi	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0102086	4×	var
Verne	<i>S. tuberosum</i> subsp. <i>tuberosum</i> Hawkes	<i>S. tuberosum</i> L. Chilotanum group	07S0102139	4×	var

<sup>a</sup>Samples provided by FTA CZU Prague; <sup>b</sup>ploidy determined by Zeka (2014); <sup>c</sup>SA – South American species; AL – Andean landraces; NA – North American species; BC – member of *brevicaule* complex; var – variety; GRIN – germplasm resources information network



Figure 1. Positions of SSR loci on chromosomes of *Solanum* sect. Petota by Ghislain et al. (2009)  
Red box identifies markers from the CIP identification kit; blue box identifies markers from the fifth supplementary multiplex panel

After analyses, the marker STG0010 was excluded due to monomorphism. The remaining 29 markers detected a total of 319 alleles. All markers exhibited high expected heterozygosity ( $H_e$ ) and polymorphic information content (PIC) values greater than 0.5. A high average  $H_e$  of 0.798 and a mean PIC value of 0.787 indicated strong discriminatory power of the marker set. The frequency of the most common allele was below 0.6 for all markers, with an average value of 0.321. Observed heterozygosity ( $H_o$ ) values were

**Genetic diversity and structure in collection of germplasm.** A dendrogram in Figure 2 reveals two distinctly separated clusters. One cluster represented germplasm comprising the wild species exclusively, whilst the second cluster included Euro-

pean varieties of cultivated potato (labelled green) and Andean potato landraces belonging to various cultivated tuber-bearing species (labelled orange). Interestingly, *S. goniocalyx* (*sgonio\_109*) accession was placed in the cluster of European tetraploid varieties, although it was expected to be in the cluster of the Andean landraces. The cluster of cultivated potato species showed relatively short distances and high homogeneity within groups, which indicates relatedness and a reduced genetic diversity due to selection.

The cluster of wild germplasm was internally differentiated into three sub-branches, where the genetic profiles of North American species were well distinguishable from those of South America. The sub-branch of South American species (*schac\_37*, *schac\_230*, *syung\_70*, *svrn\_234*, *svrn\_69* and *smicro\_49*; labelled black) included some South American species currently classified as members of the *S. brevicaulis* (*spega\_60*, *sgour\_43*, *sgour\_45* and *sincam\_47*; labelled blue). The second sub-branch included exclusively representatives of North American diploids

Table 2. Descriptive statistics of genetic diversity detected for SSR markers across sample of potato germplasm

Locus	Chromosome	$N_a$	Allele size range (bp)	$H_e$	$H_o$	PIC	Frequency of most common allele
STG0001	XI	12	122–139	0.880	0.545	0.878	0.1579
STG0016	I	14	116–154	0.887	0.682	0.886	0.2471
STG0025	X	7	183–201	0.669	0.432	0.655	0.5079
StI001	IV	10	172–196	0.820	0.500	0.814	0.3284
StI003	VIII	8	112–154	0.746	0.364	0.732	0.3521
StI004	VI	9	66–99	0.748	0.523	0.721	0.3425
StI012	IV	11	160–205	0.809	0.477	0.801	0.3623
StI014	IX	9	108–129	0.760	0.568	0.752	0.4000
StI030	XII	17	84–117	0.875	0.591	0.874	0.2184
StI032	V	9	104–131	0.831	0.523	0.821	0.2308
StI033	VII	10	100–133	0.830	0.500	0.828	0.2703
StI022	VIII	7	109–128	0.775	0.432	0.765	0.3729
StI028	XI	10	158–190	0.840	0.455	0.838	0.2206
StI043	I	9	119–142	0.771	0.523	0.751	0.3636
StI058	V	17	66–98	0.859	0.659	0.855	0.2500
STM0003	XII	18	98–151	0.909	0.682	0.908	0.1548
STM0019	VI	25	111–229	0.893	0.318	0.893	0.2708
STM0031	VII	11	130–252	0.845	0.318	0.843	0.2727
STM0037	XI	16	67–101	0.895	0.659	0.894	0.2105
STM1043	VII	4	208–226	0.589	0.045	0.510	0.5000
STM1052	IX	8	206–235	0.644	0.250	0.642	0.5600
STM1053	III	9	156–171	0.675	0.250	0.668	0.5094
STM1064	II	9	178–191	0.783	0.341	0.778	0.3167
STM1104	VIII	13	151–181	0.858	0.409	0.854	0.1970
STM1106	X	11	102–201	0.806	0.136	0.802	0.3611
STM5114	II	10	278–306	0.819	0.614	0.811	0.2927
STM5121	XII	4	282–288	0.670	0.114	0.621	0.3947
STM5127	I	9	224–267	0.830	0.477	0.822	0.2817
STPoAc58	V	13	223–244	0.826	0.523	0.818	0.3467
Mean		11	NA	0.798	0.445	0.787	0.3205

$N_a$  – number of alleles per locus;  $H_e$  – expected heterozygosity;  $H_o$  – observed heterozygosity (Nei & Roychoudhury 1974); PIC – polymorphic information content (Serrote et al. 2020)

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(*sblb\_17*, *sblb\_240*, *spoly\_290*, *spin\_51* and *sveru\_29*, labelled red). In the third sub-branch, South American polyploid species (*sstol\_295*, *sfend\_275*, *spotri\_53*,

and *sguer\_280*; labelled violet) prevailed, followed by other *brevicaule* complex species (*sspars\_71*, *slepto\_48*, *ssucr\_62*) and other South American wild

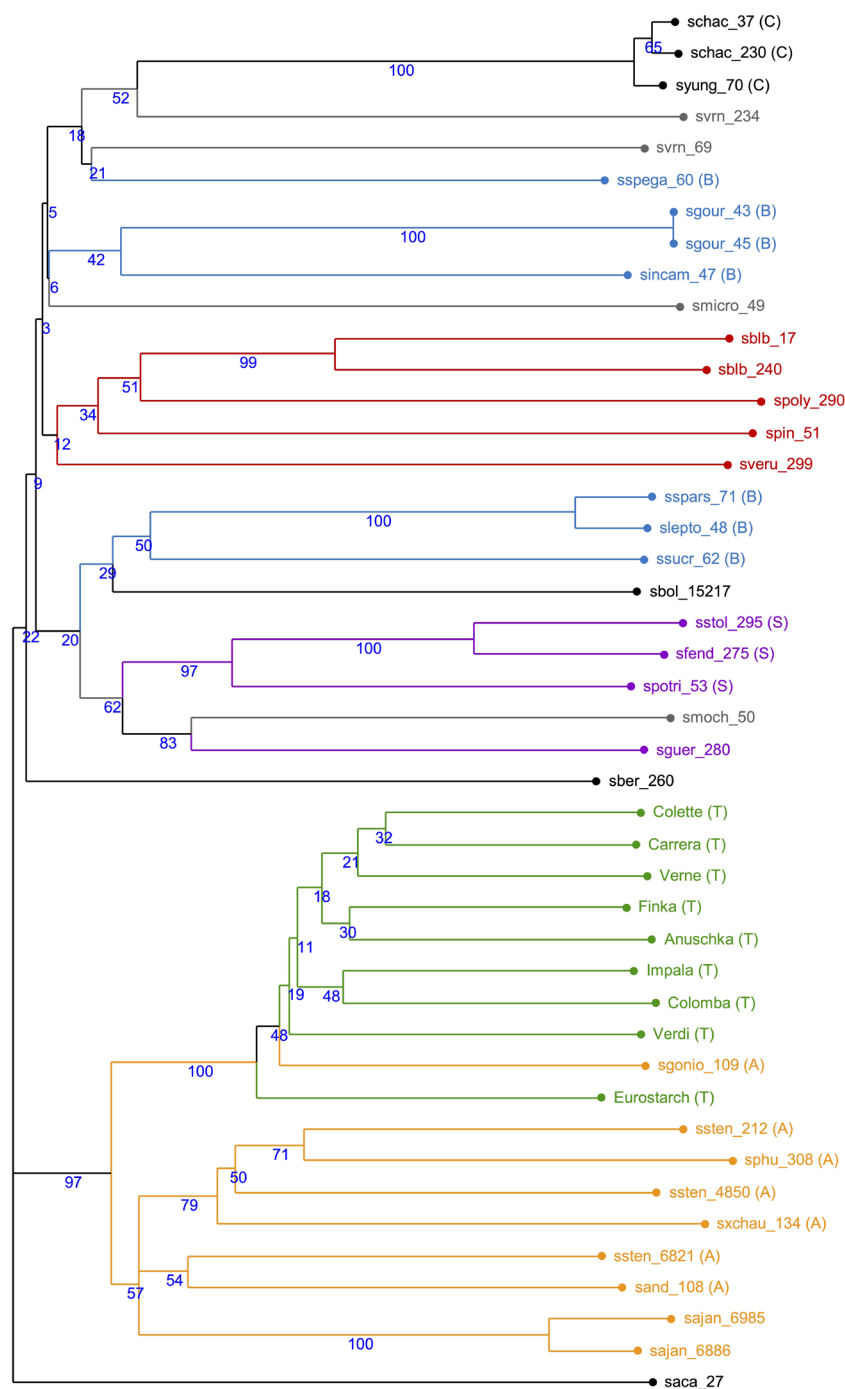


Figure 2. Comprehensive dendrogram of 44 germplasm accessions produced using DARwin software based on allelic data of 29 SSR markers

The colours refer to the category of clones (Table 1): black – South American species (SA); blue – *brevicaule* complex (BC); red – diploid North American species (NA); violet – polyploid NA; green – modern variety; orange – Andean landrace; taxonomy according to Spooner et al. (2014): (A) – *S. tuberosum* Andigenum group, (B) – *S. brevicaule*, (C) – *S. chacoense*, (S) – *S. stoloniferum*, (T) – *S. tuberosum* Chilotanum group



diploids (*sbol\_15217* and *smoch\_50*). Two South American species (*sber\_260* and *saca\_27*) were more distant from the main cluster of wild accessions, with *saca\_27* tending to be genetically closer to the cluster of cultivated potato than to wild species.

## DISCUSSION

This is the first report on the analysis of interspecies diversity of nSSR across the wild potato germplasm maintained in the Czech collections of potato genetic resources. We consider the research relevant to the strategy of the National Programme on Conservation and Utilization of Plant, Animal and Microbial Genetic Resources Important for Food and Agriculture (Zedek et al. 2023) and the international strategy of the FAO: Commission on Genetic Resources for Food and Agriculture (<https://www.fao.org/cgrfa/en>). These programmes suggest the implementation of molecular methods for assessing variability, identification, and evaluation of germplasms. The capacity of the Czech *in vitro* collection is limited, which results in uneven representation of different *Solanum* species. Most of the wild accessions are samples with breeding potential of higher tolerance or resistance to abiotic and biotic stress. This naturally reduces genetic diversity within the collection towards the genotypes having a breeding value, whilst most of the species in the bank are represented by a few individuals. More specifically, within the 29 species according to the outdated classification by Hawkes (1990) listed in Table 1, seven species were in the PRI bank represented by only one genotype, ten by up to five genotypes, and the other ten by a higher number of genotypes; they were siblings originating from the same berry with reduced intraspecies diversity. Two species missing in the PRI (*S. ajanhuiri* and *S. boliviense*) were obtained from FTA CZU. The reduced availability of genetic resources disabled wider evaluation of intraspecies diversity. Our collection (Table 1) represented both, all valid taxonomic groups across the section *Petota* subjected to the recent taxonomical revisions and all substantial species with potential for breeding. The way of sampling described in the methodology minimised the risk of selecting related individuals in the species represented by multiple accessions. The initial work collection also included the *S. demissum*, which was, however, discarded from final evaluations, as during analyses did not produce reliable data in most loci due to hexaploidy.

The genotype collection allowed us to verify the set of markers for systematic revisions, species identification, and the management of germplasm collections. The last two points are particularly significant given the recent transition of the gene bank to the new taxonomical system proposed by Spooner et al. (2014). This has generally complicated navigation within database germplasm resources information network (GRIN) due to the reclassification of many species to the *S. brevicaulle* and *S. tubero-sum* Andigenum group, when the original names by Hawkes (1990) were removed. Consequently, the need of integrating SSR data into germplasm management is highlighted.

The results indicate that the optimised analysis is applicable in all the mentioned scenarios. Descriptive characteristics in Table 2 indicate very good informativeness of markers; high value of  $H_e$ , high number of alleles ( $N_a$ ) and PIC values exceeding 0.5 together indicate a broad range of polymorphism per locus observed in the germplasm sample. With the low frequency of the most frequent allele, this suggests good potential to find specific differences within or between species and populations, and to identify individuals or clones precisely using clustering analysis. This is supported by the finding that specific alleles were detected repeatedly across a phylogenetically variable set of species. Because the collection of genotypes is a heterogeneous selection of different species representatives, the results do not represent intrapopulation data. The greatest contribution of this work is in the robustness and the multiplexing of the microsatellite panel with 29 nSSR markers. In previous studies, Tiwary et al. (2019) and Poulsen Hornum and Camadro (2024) used lesser number of singleplex SSR markers, 22 and 6 respectively, detected using capillary and polyacrylamide gel electrophoresis. Our approach used a higher number of multiplexed markers detected simultaneously by capillary electrophoresis, which may be more informative and cost-effective for the germplasm management, and more accurate for evaluations of diversity in natural populations of wild potato species.

Although the study was not aimed at detecting the intraspecies variability, some species were represented by multiple genotypes provided by different subjects at different times. Lower intraspecies variability was detected in *S. chacoense*, *S. gourlayi*, *S. bulbocastanum* and *S. ajanhuiri* (Figure 2), which may indicate reproductive incompatibility (Cara et al. 2013). On the contrary, Poulsen Hornum and

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Camadro (2024) identified high intraspecies variability for *S. chacoense* using SSR markers. This is associated with the number of analysed samples, and our results are only indicative for the purposes of the administrator of collections. Higher intraspecies variability was detected in *S. vernei*, which was consistent with previous morphological observations (Zeka et al. 2015). The variability may be a consequence of intensive interspecies hybridisation (Spooner et al. 2014). Intraspecies diversity was also observed among accessions of *S. stenotomum*, *S. phureja* and *S. andigenum*, which were recently merged into *S. tuberosum* Andigenum group based on polymorphisms of cpDNA (Spooner et al. 2014). Our results, although based on the nuclear SSR, directly support this reclassification (Figure 2). In terms of interspecies variability, the results of cluster analysis based on the allelic data aligned with four main germplasm groups: (i) modern cultivars of *S. tuberosum* Chilotanum group, (labelled green), (ii) cultivated Andean landraces (labelled orange), (iii) North American diploid wild species (labelled red), and (iv) other wild species. The result clearly reflects genetic differentiation between domesticated and wild germplasm. The close genetic relationship between the Andean landraces and modern cultivars supports the hypothesis that Chilotanum-type potatoes originated through adaptation of Andigenum group accessions to lowland conditions (Grun 1990; Hawkes 1990; Hosaka et al. 2018). The clustering aligns with the classification based on restriction fragment length polymorphism (RFLP) analysis presented in Bradshaw and Mackay (1994) and with the taxonomy of Spooner et al. (2014): the representatives of *S. chacoense* and *S. yungasense* (newly united into *S. chacoense*) clustered together, like the species *S. andigenum*, *S. stenotomum*, *S. phureja*, *S. goniocalyx* and *S. × chaucha* recently assembled into *S. tuberosum* Andigenum group. The clustering of *S. ajanhuiri* (2×, 2 EBN) into the same group (Figure 2) is consistent with its phylogeny, which is based on interspecies hybridisation between *S. stenotomum* and *S. megistacrolobum* (Rodríguez et al. 2010). The only discrepancy from the expected pattern was observed in *sgonio\_109* (*S. goniocalyx*), a diploid Andean landrace accession considered a subspecies of *S. stenotomum*, which clustered unexpectedly together with modern cultivars. The genotyping of the accession, revealing three alleles at ten SSR loci, indicated polyploidy, as the duplications combined with a change in allele size would unlikely occur at so many

loci. This suggests an interspecies hybrid or sample misidentification during collections. As polyploidy does not pose a difficulty for identifying *S. stenotomum* based on species-specific combinations of alleles, the genotype could represent a triploid hybrid of Andean cultivated species, expressing *S. stenotomum* morphology and private alleles, which resulted in its similarity to modern cultivars. Research of the European database of potato pedigrees ([www.europotato.org](http://www.europotato.org)) did not provide evidence of the genotype being used in breeding programmes; excluding a potential mismatch requires subsequent research.

The most interesting group of “pseudospecies” synonymous with *S. brevicaule* showed internal structure, splitting into two distinctive branches: one included *S. gourlayi* and *S. incamayoense*, the other *S. sparsipillum*, *S. leptophyes*, and *S. sucrense*. *S. spegazzinii* clustered separately of those previous species and appeared near to *S. chacoense*, *S. vernei*, and *S. microdontum*, which indicates genetic ambiguity and transitional position between groups. Such placement variability may result from intermediate allele profiles, which prevent stable clustering (Felsenstein 2004; Jacobs et al. 2008). However, our results agree well with those of Achakkagari et al. (2024), who placed *S. spegazzinii*, *S. vernei*, and *S. microdontum* within the same nuclear phylogenetic clade. This pattern also aligns with cpDNA results by Yan et al. (2023), which showed scattered placement of *S. spegazzinii* and other *brevicaule* complex members. These findings directly suggest that the status of *Solanum brevicaule* is disputable, an artificial solution of the *brevicaule* complex, comparable with the systematic revision of *Scrophulariaceae* based on molecular data (Albach et al. 2005). Our results highlight the complexity of species boundaries within the *S. brevicaule* complex, supporting the suggestion that synonymous species in the group may be understood rather as a part of a genetic continuum shaped by hybridisation, ecological adaptation, and historical taxonomy, as stated by Rouhan and Gaudeul (2014) or Berg and Jacobs (2007). While traditional classification has emphasised clear species boundaries, more recent molecular evidence suggests these may be artificial in groups with high phenotypic plasticity and evolutionary dynamics (Hardigan et al. 2015; Jacobs et al. 2011; Spooner et al. 2018). For practical applications such as breeding or germplasm conservation, however, the flexible recognition of genetically distinctive groups using molecular tools may be more meaningful than morphological taxonomic distinctions.

North American diploids (*S. bulbocastanum*, *S. polytrichon*, *S. pinnatisectum* and *S. verucosum*) consistently formed a separate clade, regardless of EBN differences. However, *S. verucosum* differs from the 1EBN species in both, EBN = 2 and cpDNA haplotype (Spooner & Castillo 1997). Its clustering with 1EBN diploids in this study is possibly caused by convergent nuclear profiles. In contrast, North American polyploids (*S. stoloniferum*, *S. fendleri* and *S. polytrichon*) formed a separate cluster consistent with their genomic makeup distinct from 2× 1EBN species. This agrees with previous findings by Hosaka et al. (2025) and is fully consistent with the taxonomy of Spooner et al. (2014), which merges these three original species together into *S. stoloniferum* (Figure 2).

*S. berthaulti* and *S. accaule* appeared as isolated outliers due to the presence of private alleles, which supports their phylogenetic divergence as distinct lineages within *Solanum* sect. *Petota*. In Figure 2, the *saca\_27* genotype represents a bridge between uncultivated and cultivated tuber-bearing *Solana-ceous* species.

The clustering generally appeared to be influenced, as expected, by the geographic distribution of species. Many accessions sharing natural ranges (e.g. *S. chacoense*, *S. vernei*, *S. spagazzinii*, and *S. microdontum* from northern Argentina) grouped together, possibly reflecting gene flow and adaptation to shared environments. However, exceptions occurred when *S. mochiuense* (Peru) clustered with *S. guerreroense* (Mexico), which can indicate that geographic proximity was not the sole driver of genetic similarity. In contrast, the clustering was not likely influenced by ploidy level, although variations in ploidy were observed in multiple alleles per locus. In many loci, even polyploids showed two alleles, making them indistinguishable from diploids in matrices.

## CONCLUSION

This study confirmed that nuclear SSR markers are an effective tool for analysing genetic diversity within wild accessions of *Solanum* sect. *Petota*, because the multiplexed SSR markers were successfully used for studying the genetic relations within a collection of 44 cultivated and wild potato germplasm accessions. The markers demonstrated high polymorphism and proved suitable for distinguishing and identifying the evaluated genetic resources. The resulting dendrograms enabled the classification of accessions into

four major groups: modern cultivars (*S. tuberosum* Chilotanum group), cultivated Andean types, North American diploid species, and other wild species, consistent with the current taxonomy of the *Solanum* genus. While the resolution among wild species was limited, reflecting ongoing taxonomic challenges within the section, the results align with previous SSR-based studies on potato diversity and genetic structure, and are applicable in potato breeding and conservation management.

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