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RESEARCH ARTICLE

A microsatellite and morphological assessment of the Russian National cultivated potato collection

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Abstract The germplasm collections of the Vavilov Institute of Plant Industry, Russia represent the first germplasm collection made for potatoes, now numbering 8,680 accessions. It has tremendous historical and practical importance and a rich history, having been used to document a polyploid series in the cultivated species, to formulate initial taxonomic hypotheses in potato, for studies of interspecific hybridization, and serving as the germplasm base for Russian breeding efforts. Despite its importance and size, there has never been a study of its molecular diversity, and there were many gaps in its passport data. The purpose of the present study is to obtain morphological, ploidy, and microsatellite (SSR) data needed to set up a useful subset of the collection of cultivated potatoes and closely related wild species,

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and to use this collection to study cultivated potato taxonomy and phylogeny. Through assessments of viability, passport data, and chromosome counts, we selected a subset of 238 cultivated and 54 wild accessions. A morphological and nuclear SSR study of these collections distinguished only three cultivated species: *Solanum curtilobum*, *S. juzepczukii* and *S. tuberosum*, not the many more cultivated potato species of prior taxonomic treatments. The SSR study supports the ideas of *S. acaule* as one of the parental species for *S. curtilobum* and *S. juzepczukii*. The morphological and SSR results are very similar to other recent studies of cultivated species, and show the need to reclassify the collection of cultivated potatoes by modern taxonomic criteria.

Keywords Cultivated potato · Gene bank collection · Morphology · Nuclear SSRs · *Solanum* · Taxonomy

Introduction

The germplasm collections of the Vavilov Institute of Plant Industry, Russia (VIR) have a rich history (Loskutov 1999). They were initiated by Professor R. E. Regel of the Bureau of Applied Botany in St. Petersburg in 1890 and were greatly expanded under the direction of Nikolay I. Vavilov (Alexanyan and Krivchanko 1991; Loskutov 1999). The potato collection is especially significant. It was initiated by a series of collections to Central America led by Yuriy Voronov (Sergey Bukasov took part in this expedition) from 1925 to 1926; by Sergey Juzepchuk from 1926 to 1928; and by Nikolay Vavilov from 1930 to 1933 (Loskutov 1999). These collections were widely used for plant breeding (Bukasov and Kameraz 1959), for studies of crop evolution (e.g., Vavilov 1928), and for studies of potato systematics and evolution (e.g., Juzepczuk and Bukasov 1929; Rybin 1929, 1933; Bukasov 1933, 1939, 1978).

Despite the critical importance of the VIR potato collection, it almost did not survive as it encountered almost insurmountable problems beginning as early as 1941 during World War II. The main activities of the potato collection were in Leningrad (now St. Petersburg) and the surrounding field stations of Pushkin and Pavlovsk 30 km to the south, and these areas were subject to the Siege of Leningrad from 1941 to 1944. During this time many scientific and technical staff were sent to the front, and destruction of the field stations in the south of St. Petersburg led the collection to be moved to the Central Building of VIR in St. Isaac's Square, Leningrad. Despite widespread starvation in Leningrad, curators of the VIR collection did not use the collections for food (Alexanyan and Krivchanko 1991; Loskutov 1999).

The collection has tremendous historical and practical importance. Rybin (1929, 1933), a colleague of Juzepczuk and Bukasov, first demonstrated the existence of a polyploid series in cultivated potato from 2×, 3×, 4×, and 5×, and in wild potatoes all these levels in addition to 6×. Ploidy became an important taxonomic and evolutionary character in potato. Russian monographers were the first to describe the diversity of cultivated potato species, first recognizing 12 cultivated species (Juzepczuk and Bukasov 1929), then 18 (Bukasov 1937) and finally 17 (Bukasov 1978). In addition to these species there are descriptions of hundreds of subspecies, "convarieties," varieties, and forms (Lekhnovich 1971). In the systems of Bukasov and later of Hawkes (1990), each cultivated species had a single ploidy level. This differs from systems of Dodds (1962) and Spooner et al. (2007) where many these species names were combined under *S. tuberosum*, that in this broader sense contains diploid, triploid, and tetraploid cytotypes. Most Russian taxonomic descriptions were made from living plants of germplasm collections observed in VIR experimental stations. When describing these many potato species, they applied a complex approach based on ploidy, ecogeography, the analysis of morphological and physiological characters (Juzepczuk and Bukasov 1929; Rybin 1929, 1933; Bukasov 1937, 1955, 1978; Lekhnovich 1971).

Currently, The VIR potato collection is categorized into (1) wild species, 2,640 accessions, (2) primitive cultivated species at the 2×, 3×, and 5× ploidy levels, 600 accessions, (3) *S. tuberosum* subsp. *andigenum*, 4×, 2,650 accessions, (4) Chilean landraces of *S. tuberosum* ssp. *tuberosum*, 4×, 120 accessions, (5) breeding cultivars, 2,100 accessions, and (6) interspecific hybrids, 570 accessions (8,680 accessions total in the record books). A separate

curator is responsible for each of these six collections.

The VIR collections continue to be classified under the taxonomic system of Bukasov (1978), in contrast to a later and more generally used (outside of Russia) taxonomic system of Hawkes (1990) that recognizes seven cultivated species. For a comparison of these two systems of cultivated potato species see Supplemental file 1 and Huamán and Spooner (2002). Spooner et al. (2007) used microsatellite data, in combination with morphological data of Huamán and Spooner (2002) to recognize: (1) *S. tuberosum* consisting of two cultivar groups: Chilotanum Group consisting of lowland Chilean landraces, all tetraploid, and Andigenum Group consisting of upland Andean landraces growing from Venezuela south to northern Argentina at the diploid, triploid, and tetraploid levels, (2) *S. ajanhuiri* (diploid), (3) *S. curtilobum* (pentaploid), and (4) *S. juzepczukii* (triploid). The putative parents of these latter three species were wild species and populations at various ploidy levels of the Andigenum Group. The taxonomy of Spooner et al. (2007) came very close to that of Dodds (1962) who grouped cultivated potatoes into three species: two of hybrid origin (*S. curtilobum* and *S. juzepczukii*) and the third, *S. tuberosum*, consisting of five cultivar groups. The herbaria of the Vavilov Institute (herbarium code WIR) and the V. L. Komarov Botanical Institute (herbarium code LE, both in St. Petersburg; herbarium codes follow Index Herbariorum—http://sciweb.nybg.org/science2/IndexHerbariorum.asp) have voucher specimens for most of the germplasm collections filed under the Bukasov (1978) system.

Ecogeography was a major taxonomic character in most Russian crop taxonomic systems, including potato (Juzepczuk and Bukasov 1929; Bukasov 1930; Juzepczuk 1937). This is clearly evident by the nature of the taxonomic categories used in both their wild and cultivated taxa. For example, Lekhnovich (1971), a colleague of Juzepczuk and Bukasov, grouped *S. andigenum* (*S. tuberosum* subsp. *andigena* in system of Hawkes 1990) into subspecies: subsp. *mediamericanum* (from Mexico and Guatemala), subsp. *colombianum* (from Colombia), subsp. *rimbachii* and subsp. *ecuatorianum* (from Ecuador), subsp. *tarmense* and subsp. *centraliperuvianum* (from Central Peru), subsp. *australiperuvianum* (from southern Peru), subsp. *bolivianum* (from Bolivia), and subsp. *argentinicum* and subsp. *runa* (from Argentina). Bukasov (1978) extended this geographic concept even to the higher taxonomic rank of series, for example series *Andigena* Bukasov (from the Andes), series *Chilotana* Bukasov (from Chile), and series *Cisaequatorialia* Bukasov (from the Equator).

The purposes of this study are to assess the current state of the VIR collection of cultivated potato species regarding the state of the collection records, ploidy, and taxonomy. We then use this selected well characterized subset to assess cultivated potato diversity, taxonomy, and phylogenetic relations with closely related wild species.

Materials and methods

Selection of the initial materials

We began by (1) choosing a diverse and quality sample from the entire Russian potato collection regarding representative cultivated and related wild species (Supplemental file 2) and preferentially choosing accessions collected by VIR expeditions (collected from 1926 to 1990). (2) We tried to choose tuber propagated material and in the case of its absence we preferentially selected seeds from the germplasm increases from earlier years. We evaluated these for seed viability. The low levels of seed viability from these early reproductions is not representative of the entire collection, but reflects our choice of older seed reproduction to be related as closely as possible to original material. (3) We assessed quality of the subset of living accessions by adding passport data from supplementary records at VIR to choose accessions spread throughout the range of the cultivated species in South America, and choice of species covering all putative ploidy levels. (4) Then we conducted the first gross evaluation of species identifications of samples in a field plot in Pushkin Russia. (5) The VIR potato collection database lacks complete data for many fields and we filled these in as detailed below. We obtained full locality data from other records and new chromosome counts from this reduced sample. (6) Finally, in 2007, we conducted a morphology-based numerical taxonomic evaluation of this reduced subset in a field plot in Pushkin Russia.

To make this study as comparable as possible to the recent literature, we designed the final experimental subset close to the number of cultivated species accessions in Huamán and Spooner (2002), but with a substitution of accessions from the Russian potato genebank. Although accession-specific chromosome numbers are present in the database, they largely represented expected numbers based on their morphology-based species determination, not actual chromosome counts. The species *S. chaucha*, *S. curtilobum* and *S. juzepczukii* had few accessions in the VIR genebank and for these we obtained 53 accessions from the International Potato Center, Lima Peru. In addition, four accessions of *S. ajanhuiri*, six of *S. chaucha* and five of *S. phureja* (originally collected by VIR expeditions) were ordered from the IPK genebank in Germany. However, we later determined that the accessions of *S. chaucha* (defined as triploid) sent as true seeds from IPK were diploid and tetraploid, showing problems in genebanks in *ex situ* maintenance of large collections of cultivated potato. Our database includes 23 data fields to include basic information such as species name, genebank number, collector, and country of location, locality.

Our initial selection included 438 accessions of cultivated species (385 from the VIR collection and 53 from CIP, Supplemental file 2) of seven cultivated species and three subspecies according to the taxonomic system of Hawkes (1990) (Supplemental file 1). In making this selection we maximized the diversity of the names of Bukasov (1978) as maintained in the VIR collection: *S. ajanhuiri*, *S. chaucha* (including *S. tenuifilamentum* Juz. et Bukasov), *S. curtilobum*, *S. juzepczukii*, *S. phureja* (including *S. canarense* Buk. and *S. rybinii* Juz. et Bukasov), *S. stenotomum*, (including *S. stenotomum* Juz. et Bukasov and *S. goniocalyx* Juz. et Bukasov), *S. tuberosum* L. subsp. *andigenum* (including *S. andigenum* Juz. and Bukasov), and *S. tuberosum* subsp. *tuberosum* (including *S. chilotanum* Hawkes, *S. molinae* Juz.). In addition, 198 accessions of 16 wild taxa close relatives of cultivated potato in the *S. brevicaule* complex (Van den Berg et al. 1998; Spooner et al. 2005) were selected: *S. brevicaule, S. bukasovii, S. canasense*, *S. candolleanum, S. gourlayi* subsp. *gourlayi*, *S. gourlayi* subsp. *vidaurrei*, *S. hondelmannii, S. leptophyes*, *S. multidissectum, S. oplocense, S. sparsipilum* (including *S. catarthrum*, *S. ruiz-zeballosii*), *S. spegazzinii* (including *S. famatinae*), and *S. vernei*. For the SSR analysis we also included species outside of the *S. brevicaule* complex that are putative progenitors of *S. ajanhuiri* (*S. megistacrolobum* and *S. toralapanum*), *S. curtilobum* (*S. acaule*), and added *S. albicans* that is closely related to *S. acaule* (Hawkes 1990). With these 198 accessions of wild species accessions we initially selected a total of 636 accessions (438 cultivated and 198 wild) (Supplemental file 2). This subset was composed of: 172 (142 cultivated + 30 wild) accessions reproduced by tubers, 411 (243 cultivated + 168 wild) accessions reproduced by seeds, and 53 accessions by in vitro plants obtained from CIP (Supplemental file 2).

Selection of the initial materials: seed viability testing

At VIR, most accessions of diploid cultivated species and wild species are stored as true seeds with seed increases conducted over various years. Most accessions of tetraploid potatoes, and all triploid and pentaploid accessions are stored as tubers. Unfortunately, only 172 (142 cultivated and 30 wild) accessions in our subset were maintained as tubers, and the seed collections could have been the result of cross-pollinations with other accessions (the field increases at VIR did not use controlled pollinations), and the integrity of the original collection has been lost. Hence, we preferentially selected seeds from the reproductions of earlier years (20–30-year-old seeds) to be as close as possible to the original seeds. Seed storage was in suboptimal conditions at room temperature and inviable seeds were found in these reproductions. We tried to germinate such seeds by two methods: in vitro (for 99 accessions) and treated seeds with GA_3 solution (Supplemental file 2). The first method (in vitro culture with a nutrient medium MS + GA_3 (2 mg/l) + indolyl-3-acetic acid (0.1 mg/l) + kinetin (0.5 mg/l) was used for 99 accessions (77 accessions of diploid and tetraploid cultivated species and 22 accessions of wild species). Out of 99 accessions we were able to produce seedlings for 35 accessions, but seeds of 64 accessions were inviable (Supplemental file 2). The second method (treatment of seeds with the GA_3 solution, 15,000 ppm) was used for 166 samples of cultivated diploid and tetraploid species and for 146 samples of wild species (20–30-year-old reproductions). This produced seedlings for 89 accessions (53.6%) of

cultivated species and 63 accessions (43.1%) of wild species. Thus, our laboratory tests of seed viability of the 411 accessions in 2006 led to a reduction by the end of 2006 to 187 accessions (118 cultivated and 69 wild) due to inviable seeds. Due to this reduction our total subset by the end of 2006 consisted of 412 accessions (313 cultivated, 99 wild) (Supplemental file 2). These 412 accessions were derived from VIR seeds (187), VIR tubers (172) and CIP in vitro plants (53).

Selection of the initial materials: filling the passport database

The VIR potato germplasm database had geographical information for country for most accessions but only for 40% of the accessions had information for administrative subdivisions within countries. We supplemented these data with additional locality data from (1) unpublished reports from VIR expeditions, (2) unpublished journal entries of the Introduction Department of VIR, (3) unpublished journals of scientific research of the Potato Quarantine Greenhouse at the Pavlowsk Experimental Station of VIR, (4) unpublished journals of the VIR Potato Department. These journals cover the period from 1933 to 1996 and represent many volumes of handwritten books. Determining locality data from these notebooks was often difficult because of the history of record keeping in the VIR system. All samples introduced to VIR from original expeditions or through donations from other genebanks first came through the Introduction Department and an Introduction Number was assigned. The accessions were then transferred to Potato Quarantine at the Pavlowsk Experimental Station where it received a separate quarantine number, and a separate set of records was made. Then, the sample was transferred to the Potato Department, where yet another number, the VIR catalogue number, was assigned. Our checking of all these three sets of records uncovered mistakes and increased the accuracy of our database.

We then filled data fields in the passport database that consist of 23 data fields. In total, about 5,000 new data fields were added for most of the 412 living accessions. After rechecking passport data, 35 accessions were excluded from our subset because of missing or ambiguous data. We then pared down our accessions further. Our first assessment of plant morphology in 2006 led to an elimination of 27 accessions due to gross taxonomic misidentifications. Sixteen accessions of wild species did not produce tubers under the conditions at St. Petersburg and were also eliminated. Finally, in the beginning of 2008 our experimental subset included 292 accessions (238 cultivated [Fig. 1], and 54 wild [Supplemental files 1, 2]). In summary, reducing the subset of living samples of 412 (313 cultivated, 99 wild) accessions was done due to incorrect passport data (35 accessions), incorrect chromosome numbers (42 accessions), gross taxonomic misidentifications (27 accessions) and inability to produce tubers at Pushkin, located in northwestern Russia (16 accessions) (Supplemental file 2).

Fig. 1 Map showing the areas distribution of 238 accessions of 7 cultivated species used in our study

Chromosome counts

We obtained chromosome counts from mitotic cells of root tips from 1 to 2 clones per accession, using standard root squash and stain techniques. Root tips

were incubated for 24 h at 0–2°C and fixed in 3:1 ethanol acetic acid solution. The root tips were stained in 2% acetoorsein and then squashed. Only metaphase plates showing clearly separated chromosomes were counted. Chromosome numbers were determined from 1 to 3 separate roots of each plant and from a minimum of five cells per one root.

Data measurement

In May 2007 we planted 238 clonal accessions (704 plants) of landrace populations of seven cultivated species (Supplemental files 1, 2). We measured the first three of five plants in a row or the three that survived (a few accessions had only two plants for measurement). The database contains information from 78 characters (30 qualitative and 48 quantitative). To the 75 characters in Huamán and Spooner (2002) we added three new characters: presence of flowers, fruits, and tubers (Supplemental file 3). Corolla color, tuber skin color, and tuber flesh color were measured with the R.H.S. Colour Charts (Royal Horticultural Society 1986), based on recommendations of Tucker et al. (1991). In accordance with the CIP Descriptor List, the color of corollas and tubers were entered as integers.

We analyzed qualitative characters, such as plant habit, shapes, and colors, through decomposition to binary "dummy variables" (Sokolova and Razorenova 1996; Fil and Novikova 2000; Dougherty 2006). This was accomplished by adding new variables in accordance with the number of degrees of every qualitative character, and the presence or absence of a particular degree was denoted by 1 or 0, respectively. This technique was used, rather than averaging quantitative measures of these characters (as in Huamán and Spooner 2002) to avoid averaging characters that would give assessments of these traits (e.g., a shapes or colors) that did not exist in nature. For example, according to the CIP descriptor of tubers, tuber shape (character 70) is divided into seven states: globose, ovate, obovate, elliptic, oblong, elongate-oblong, elongate, and we assessed these states. To avoid shape averaging, seven new variables were added: globose shape, ovate shape, etc. (Supplemental file 3). Mean values for these new variables are clearly meaningful, as they indicate frequency of occurrence of this shape in the population. For example, for species we calculated the frequency of occurrence every shape; in the case of *S. ajanhurii* the values were 70_1 = 0.75, 70_4 = 0.25, other gradations = 0. This scoring method indicates that 75% of samples have globose shape of tubers and 25% are elliptic. The variables that lacked variance were excluded. For example, "rosette plant habit" was excluded when considering cultivated species only, which lack this trait.

To avoid the multicollinearity and singularity of correlation matrix in factor analysis one column was excluded in accordance with rules of work with "dummy variables" (Dougherty 2006); for example we eliminated "elongated shape" (70_7) because it had the minimal frequency (3.1% for cultivated species). From pairs of highly linearly correlated characters (colors of abaxial and adaxial interpetolar tissues ($r = 0.98$), color of abaxial and adaxial corolla rays ($r = 0.90$), only one character from each pair, that is, color of abaxial interpetolar tissue and color of adaxial corolla ray was used. In total, the database with binary transformations contains information for 138 characters for 238 accessions of cultivated species, considering the qualitative characters that were linearly decomposed.

The module Factor Analysis of the program StatSoft Statistica 6.0 was used to analyze all characters for their means, ranges, standard deviations, and confidence limits for mean values. Factor analysis used the method of extraction of principal components.

SSR analysis

In total, 19 nuclear microsatellite (nSSR) primer pairs were studied. They were chosen from four sources: six primer pairs from the previously identified potato genetic identification (PGI) kit (Ghislain et al. 2004), three pairs from Milbourne et al. (1998), seven pairs from Feingold et al. (2005), and three pairs from the Institute for Genomic Research (http://www.tigr.org/). They were recommended to us by Marc Ghislain at CIP, based on his choice of primers with maximum quality of bands, spread throughout the genome, and high polymorphism in a cultivated potato germplasm base (Ghislain et al. 2009).

PCR reactions were performed in a 10 μl volume containing 1× reaction buffer with 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 15 pM M13-tailed forward SSR primer, 15 pM reverse SSR primer, 25 pM of

700 or 800 IRDye-labeled M13 forward primer, 1 unit of Taq polymerase, and 40 ng of genomic DNA. PCR was carried out using the following cycling profiles: 4 min at 94°C; 31 cycles of 45 s at 94°C, 1 min at annealing temperature (Ta), and 1 min at 72°C, with a final extension step of 5 min at 72°C. PCR products were separated by electrophoresis on a LI-COR 4300S DNA Analyzer system. Size calibration was performed with the molecular weight ladder (LI-COR IRDye 50–350). SSR alleles were detected and scored by using SAGA Generation 2 software as present (1) or absent (0). Missing data were scored as "9". Number of alleles, allele size, and polymorphic index content (PIC) were calculated for 286 accessions (230 cultivated, 56 wild) (Supplemental file 2). The polymorphic information content (PIC) was calculated as $\mathrm{PIC} = 1 - \sum \left(p_i^2\right)$, where p_i is the frequency of the *i*th allele detected in all accessions (Nei 1973). Genetic diversity analysis was performed with the program DARwin5 (http://darwin.cirad.fr/darwin). A dissimilarity matrix was calculated by using the Dice coefficient, 60% of minimal proportion of valid data required for each unit pair. The dendrogram was built by using the Weighted Neighbor-Joining method (NJ). Bootstrap analyses used 1,000 replicates.

Comparison of morphological and SSR results

For comparison of morphological and SSR results we used a subset of 222 identical accessions of only cultivated species (Supplemental file 4). Morphological mean characters for every accession were standardized (STAND), dissimilarity matrices were generated using Euclidean distances (in SymInt) in NTSYS-pc. For the SSR database, dissimilarity matrices were generated using the Dice coefficient (in SymQual). Clustering was performed using the unweighted pair-group method (UPGMA) for morphological data and NJ for SSR data. Correspondence between the morphological and SSR trees was compared using the Mantel matrix-correspondence test in NTSYS-pc.

Mapping the accessions

We used the program MapInfo, version 9.5 to visualize the geographic data and to produce the distribution map (Fig. 1) of the areas where the seven cultivated potato species used in this study were collected.

Results

Morphological results

Principal Components Analysis (PCA) of 78 characters for 238 accessions of seven cultivated species (Fig. 2) mainly distinguishes three species: (1) *S. curtilobum*, (2) *S. juzepczukii*, and (3) all other cultivated species as a group to include *S. ajanhuiri* (except 2 accessions), *S. chaucha*, *S. phureja*, *S. stenotomum*, *S. tuberosum* subsp. *andigenum,* and *S. tuberosum* subsp. *tuberosum*. The 18 most significant factor loadings of the PCA are listed in Supplemental file 5 and the statistical distributions of these 18 characters are graphed in Supplemental file 6 (means, ranges, and standard deviations) and Supplemental file 7 (means, standard errors, and 95% confidence intervals of the means). The first factor explains 10.5% of the total variation, and contains dimensions of plant and leaf size; the second factor explains 7.1% of the total variation and contains corolla characters such as the frequency of intense purple color of corolla parts and the shape of the corolla. Supplemental file 6 documents extensive overlap of the distribution of these characters, but if viewed on the basis of significance of means (Supplemental file 7) there is greater separation. *Solanum chaucha* (Fig. 2, green) and *S. phureja* (Fig. 2, red) are largely separated from each other but entirely intermixed within the main cluster of the other cultivated species (Fig. 2).

SSR results, polymorphic index content (PIC), allele distribution statistics

Most accessions in the SSR analysis (98%) are present in the morphological analysis, except for two accessions of *S. phureja* and one of *S. stenotomum* (Supplemental file 2). In addition, eight accessions (seven *S. acaule* and one *S. albicans*) are present in the SSR analysis and absent in the morphological analysis (Supplemental file 2). SSR analysis of the subset of seven cultivated and 14 closely related wild species (containing 297 accessions) detected 223 alleles in 19 SSR loci (Supplemental file 8).

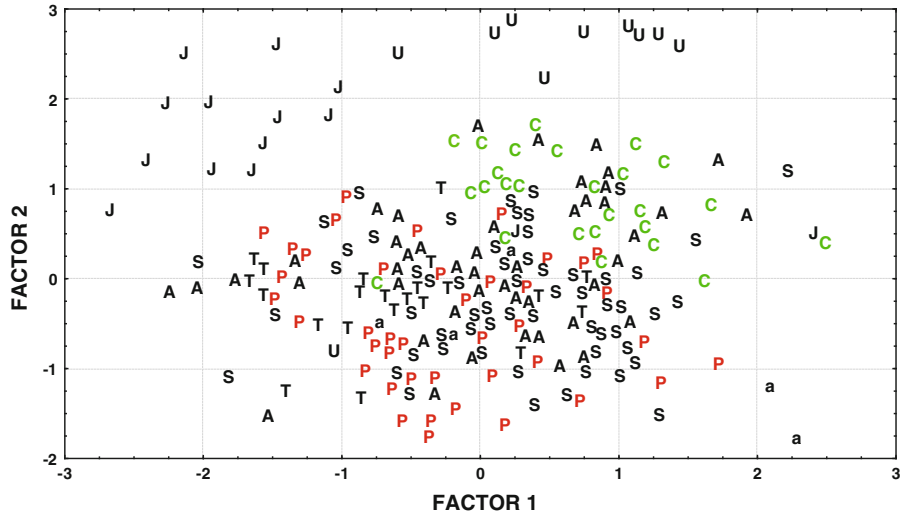

Fig. 2 Principal component analysis of all 232 accessions of cultivated species with the reduced morphological data set, without tuber and fruit characters, and with mean substitution of missing data. *Solanum ajanhuiri*—a, *S. chaucha*—C, *S. curtilobum*—U, *S. juzepczukii*—J, *S. phureja*—P, *S. stenotomum*—S, *S. tuberosum* subsp. *andigenum*—A, *S. tuberosum* subsp. *tuberosum*—T. *Solanum phureja* and *S. chaucha* are colored *red* and *green*, respectively, to highlight their separation within the cluster of other taxa, as discussed in the text

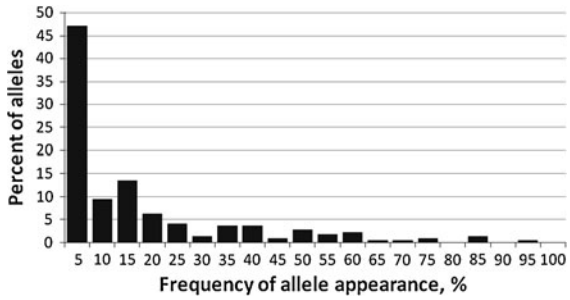

Fig. 3 Bar chart of frequency of allele appearance for the subset of 297 (235 cultivated and 62 wild) accessions

The most widespread allele (STG0025-198) was shared by 93% of the accessions. However, the vast majority of the alleles (173 out of 223) occurred with a frequency of less than 25%, and about half of the alleles (104 out of 223) were rare (occurring with a frequency of <5%) (Fig. 3). There were no species-specific alleles (that are present in all accessions of only one species and are absent in all other analyzed taxa). A few alleles are nearly specific in some groups of species. For example, two alleles, STM5127-230 and STI004-64, are specific in high frequency for a group of related species to include *S. acaule* and its hybrid derivatives *S. curtilobum* and *S. juzepczukii*, and are absent in the other taxa (Fig. 4). These two "group-specific" alleles (STM5127-230 and

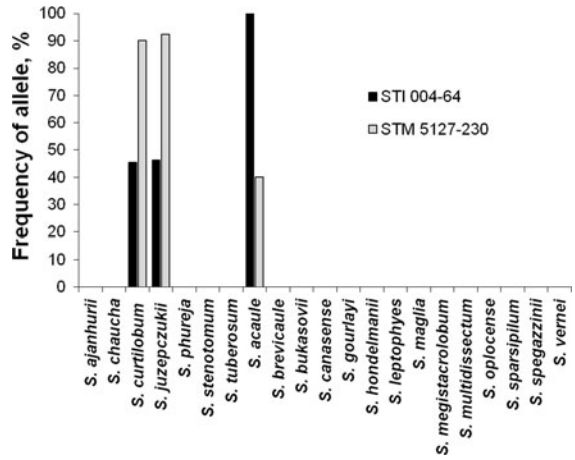

Fig. 4 Frequency distribution of alleles STI 004-64 (*black*), STM 5127-230 (*white*) which are specific for the most accessions of a group of related species: *S. acaule, S. curtilobum, S. juzepczukii*

STI004-64) simultaneously were absent in two out of 24 accessions of *S. curtilobum* (13384) and *S. juzepczukii* (25011) and these two accessions were situated separately in PCA from the other 22 accessions of *S. curtilobum* and *S. juzepczukii* (Fig. 2). Also, two alleles, STM 5121-280 and STI 014-127, which were widely shared by the other cultivated species were absent in *S. acaule*, *S. curtilobum,* and *S. juzepczukii*.

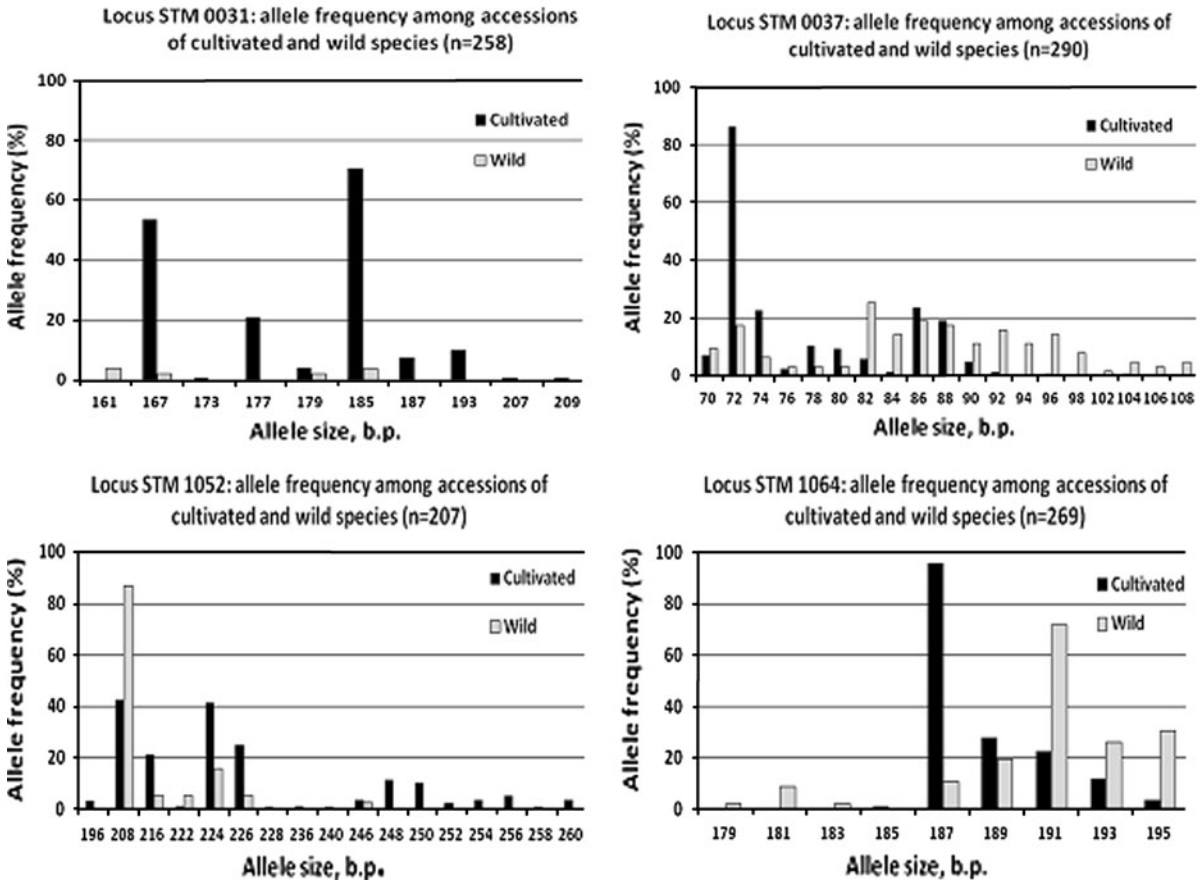

Fig. 5 Frequency of alleles STM0031, STM0037, STM1052, STM1064 in 297 accessions

Most of the loci were characterized by high PIC values and a high degree of heterozygosity (Supplemental file 8), especially for polyploid species, where tri- and tetrazygote variants occurred with significant frequency. The group of cultivated species was not homogenous, and was highly polymorphic at individual loci (Supplemental file 9). For the diploid cultivated species *S. phureja* and *S. stenotomum,* and the triploid species *S. chaucha*, the mean PIC value was smaller 0.56–0.57 (Supplemental file 9), which was influenced by a low degree of polymorphism in the loci STI033, where only one allele STI033-112 was found for all accessions of these three species.

Comparison of cultivated and wild species

The wild and cultivated species analyzed as two groups differed in the number (Supplemental file 10) and in frequency (Fig. 5) of alleles. The best loci to distinguish the cultivated from the wild species were STM0031, STM1052, STM0037, and STM1064 (Fig. 5).

SSR phenetic results

Multivariate statistical analyses were performed on 19 nuclear SSR (nSSR) loci for 281 accessions (226 cultivated, 55 wild) (Supplemental files 2, 4), with a total of 6.5% missing data (Supplemental file 8). The SSR NJ tree (Fig. 6) divided the 14 wild and 7 cultivated species into four clusters that we so designate based on (1) the high bootstrap value of cluster 1 supporting *S. acaule, S. curtilobum,* and *S. juzepczukii*, (2) the predominance of diploid and triploid landraces in Cluster 2, (3) the predominance of tetraploid landraces in cluster 3, and (4) wild versus cultivated for cluster 4.

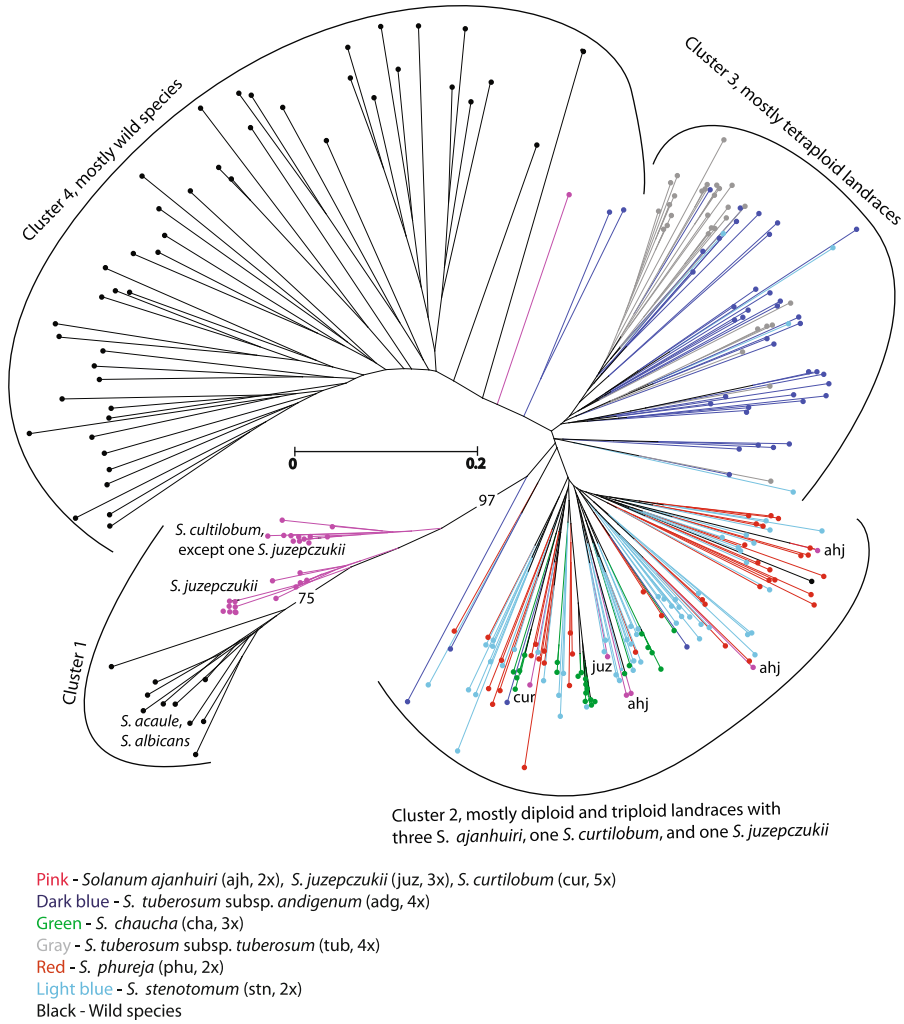

Fig. 6 Neighbor joining analysis of 19 SSR primer combinations of 281 accessions (226 cultivated and 55 wild) from the VIR collection, with bootstrap support at the base of the tree or supporting major species groups over 70% indicated; many such support values internal to the tree without species groups were deleted for clarity of presentation. Species are color coded as: *pink*—*Solanum ajanhuiri* (ajh, 2×), *S. juzepczukii* (juz, 3×), *S. curtilobum* (cur, 5×); *dark blue*—*S. tuberosum* subsp. *andigenum* (adg, 4×); *green*—*S. chaucha* (cha, 3×); *gray*—*S. tuberosum* subsp. *tuberosum* (tub, 4×); *red*—*S. phureja* (phu, 2×); *light blue*—*S. stenotomum* (stn, 2×); *black*—the remaining wild species

Cluster 1

The wild species *S. acaule* (tetraploid) and *S. albicans* (hexaploid) and the cultivated hybrid derivatives of *S. acaule*, the cultivated species *S. curtilobum* and *S. juzepczukii.* High bootstrap values (<50%) for the main inner branches of the entire tree were absent except for Cluster 1 formed by *S. acaule*, *S. albicans*, *S. curtilobum*, and *S. juzepczukii* (bootstrap value 97%), and at the base of the cluster containing *S. acaule* and *S. albicans* (bootstrap value 75%). All other high bootstrap values (not shown) were found in outer branches of the tree, joining groups of few accessions without any clear patterns regarding geography or taxonomy.

Cluster 2

A diploid cultivated species cluster and the triploid cultivated species *S. chaucha*. This cluster also contained one accession of the wild species, *S. canasense* (k-19054), two accessions of the tetraploid species *S. tuberosum* subsp. *andigenum* (8264, 12643), one accession of *S. curtilobum* (13384) and

one accession of *S. juzepczukii* (25011). Notably, accessions 13384 and 25011 stood separately from their main species clusters with morphological data as well, although they have chromosome numbers (60 and 36, respectively) characteristic of *S. curtilobum* and *S. juzepzcukii*. Perhaps accession 25011 is *S. chaucha*, not *S. juzepczukii*, which is supported by the position of the medial pedicel articulation of accession 25011; whereas *S. juzepczukii* accessions have high pedicel articulation. Similar to the morphological results (Fig. 2), there is a rough tendency for *S. chaucha* (green) to group separate from *S. phureja* (red), but with many exceptions.

Cluster 3

A tetraploid cultivated species cluster formed mainly by accessions of both subspecies of *S. tuberosum*. Some accessions of subsp. *tuberosum* are in a subcluster in a part of cluster 3, but intermixed with accessions of subsp. *andigenum*.

Cluster 4

Most of the wild species of series *Tuberosa* (system of Hawkes 1990) only sometimes formed species-specific clusters. Also included here were two accessions of the cultivated species *S. tuberosum* subsp. *andigenum* (1717, 1690), and one of *S. ajanhurii* (2311).

Comparison of morphological and SSR results

The correlation of morphological and SSR results as determined by MXCOMP in NTSYS-pc is 0.16. As explained by Rohlf (1993), correlations below 0.7 are extremely low. Low correlations between morphological and molecular datasets are often typical (Ghislain et al. 2006). Despite this low correlation, there are similarities of these results, most notably, the separation of most accessions of *S. curtilobum* and *S. juzepczukii* from the other cultivated species, and the difficulty to separate the other cultivated species from each other.

Chromosome counts

We obtained chromosome counts for 390 accessions (285 cultivated and 105 wild), 253 accessions of cultivated species, and 95 accessions of wild species with the expected chromosome number. In contrast, 32 accessions of cultivated species and 10 accessions of wild species (42 accessions total, 10.8%) had unexpected chromosome numbers (Supplemental file 2).

Discussion

As detailed in the Introduction, the Russian National potato collection has been extremely significant due to a diverse range of innovations regarding the first potato germplasm collections, the first potato cytotaxonomic studies, and critical initial studies of taxonomy, especially of the cultivated species. The collection, however, was in great need of the studies presented here to determine its viability, ploidy, allelic diversity, passport data, and taxonomy. We found many of the old accessions to be inviable, ploidy to be incorrect, allelic diversity to be considerable (Fig. 3), passport data to be present but not summarized in a single database, and taxonomy to be outdated. Because of the history of the collection and scattered record keeping, considerable effort was needed regarding choice of accessions, determination of reliable passport data, filling in the database with data from different sources, choice of a geographically diverse subset, and ploidy determinations. We are currently ensuring that our now well-documented and diverse subset will be maintained in an in vitro collection to make it useful for future studies.

The morphological taxonomic results are very similar to those of Huamán and Spooner (2002), despite using a different germplasm base, a different evaluation environment (upland central Peru vs. lowland northern Russia), different scoring methods for some of the traits, and replications of measurements (one plant per accession in Huamán and Spooner (2002), and three plants per accessions in the present study). This similarity includes phenetic distinctiveness of *S. curtilobum* and *S. juzepczukii* and a rough separation of *S. chaucha* and *S. phureja*. The main difference between the two studies is that the present study failed to distinguish *S. ajanhuiri* from the majority of the other accessions.

Our use of means, standard errors, and 95% confidence intervals of the means to display the data was not used in Huamán and Spooner (2002) and it shows the significantly different means of some traits

(e.g., trait 3, plant height; trait 39, peduncle length; trait 56, frequency of intense purple adaxial interpetiolar tissue) despite the great overlap of overall measurements shown in the ranges and standard deviations of Supplemental files 6 and 7. The overlap in such traits, however, (Supplemental file 6), combined with the lack of phenetic structure (Fig. 2), and lack of SSR support (Fig. 6), precludes the recognition of the many species of cultivated potatoes of prior taxonomic treatments. Neither the morphological nor the SSR results support the 17 cultivated species of Bukasov's system (1978), or the seven cultivated species of Hawkes's system (1990). However, it is important to note that Russian taxonomists tried to group cultivated potatoes into "superspecies complexes". For example, Bukasov (1978) separated cultivated species into three series based on differences in their morphology, geography and origin: (1) series *Chilotana* (*S. chilotanum* = Chilean subsp. *tuberosum*), (2) series *Andigena* (di-, tri- and tetraploid Andean potatoes exclusive of *S. juzepczukii*) and (3) series *Subacaulia* (*S. curtilobum* and *S. juzepczukii*). However, in the earliest treatments, Russian taxonomists (Bukasov 1937, 1955; Lekhnovich 1971) separated *S. curtilobum* and *S. juzepczukii* from the other cultivated species and placed them into the series *Acaulia* with wild species *S. acaule*. Therefore, our results of differentiation of cultivated species are similar, if we equate the Russian superspecies complexes as our species or vice versa.

The SSR data of the present study also support the distinctive nature of *S. curtilobum* and *S. juzepczukii* within cultivated potatoes. The SSR results support these two cultivated species, and their wild ancestor, *S. acaule*, to be related, in agreement with prior hypotheses (Bukasov 1933; Juzepczuk 1937; Hawkes 1962; Schmiediche et al. 1980, 1982), AFLP results of Kardolus (1998), and SSR results of Spooner et al. (2007).

Of much greater taxonomic controversy and ambiguity regards the taxonomy and phylogeny of the other Andean cultivated potatoes and the Chilean tetraploids. For example, the classification of the cultivated triploid species (exclusive of *S. juzepczukii*) provides a clear example of differing approaches to cultivated potato taxonomy and evolution. Theoretically, we can expect a high genetic diversity within triploids taking into account the great ability of potato species to form unreduced gametes that could result in the formation of both autotriploid and allotriploid cytotypes. Indeed, the wide morphological and distributional diversity of the triploids (exclusive of *S. juzepczukii*) suggests both a high level of unreduced gamete formation in diploid species and intensive gene flow among them, as well as between 4× and 2× cultivated potatoes. Russian taxonomists indicated the existence of autotriploid forms (cytotypes) in the diploid species *S. stenotomum* (Bukasov 1978). Besides *S. juzepczukii*, Russian taxonomists (Juzepczuk and Bukasov 1929; Lekhnovich 1971; Bukasov 1978) recognized several cultivated allotriploid species (*S. chocclo*, *S. mamilliferum*, *S. tenuifilamentum*) that differed in morphology and eco-geography and originated from natural crosses between their concept of two closely related and highly polymorphic species *S. andigenum* and *S. stenotomum* (Bukasov 1978). *Solanum chaucha* was recognized by Bukasov (1978) and Lekhnovich (1971) as an autotriploid species (evolved from *S. phureja*) whose tubers lacked dormancy similar to *S. phureja*, in contrast to their other recognized species that possessed tuber dormancy. Hawkes (1963) joined all these triploid species (*S. chaucha*, *S. chocclo*, *S. mamilliferum*, *S. tenuifilamentum*) together under *S. chaucha* and considered it to be formed by hybridization between *S. tuberosum* subsp. *andigenum* and *S. stenotomum*. Hawkes (1990) did not propose the existence of autotriploid forms in diploid cultivated potatoes. After his 1963 taxonomic revision (Hawkes 1963) the common approach in most genebanks was to identify all cultivated potatoes having 36 chromosomes (and being morphologically different from *S. juzepczukii*) as *S. chaucha* without consideration of their auto- or allotriploid origin. The absence of tuber dormancy was indicated by Hawkes (1963) only for one cultivated diploid species, *S. phureja.*

Lack of understanding of the nature of cultivated triploids led to further taxonomic confusion. For example, Ghislain et al. (2006) revealed about 30% of *S. phureja* accessions in the CIP collection to be triploid, and Spooner et al. (2007) showed clustering of the triploid and diploid *S. phureja* accessions with SSR data. These authors used these data to show the many exceptions to a definition of *S. phureja* by using a combination of diploid clones lacking tuber dormancy. Based on Russian classifications, these autotriploid forms of *S. phureja* lacking dormancy

tubers were the original Russian concept of *S. chaucha*. Differentiation of *S. chaucha* accessions on several subclusters observed in our present study and in Spooner et al. (2007) may reflect a different origin. Triploids identified in gene banks as *S. chaucha* could be autotriploids of *S. phureja*, *S. stenotomum*, or they could derived from 4× × 2× crosses (S. *tuberosum* subsp. *andigenum* × *S. stenotomum*), or from 2× × 2× crosses (*S. phureja* × *S. stenotomum*) with participation of unreduced gametes of one of the diploid species; although such groups could be poorly morphologically distinguished. In such cases grouping of diploid and triploid (exclusive of *S. juzepczukii*) potato landraces would be expected. Separation of *S. chaucha* and *S. phureja* observed in present study might be connected with predominance of allotriploid accessions of *S. chaucha* derived from hybridization of *S. tuberosum* subsp. *andigenum* and *S. stenotomum*.

The present SSR study, like of Spooner et al. (2007), groups both subspecies of *S. tuberosum* (Hawkes system) together, and groups many accessions of Chilean subsp. *tuberosum* within this broad grouping. A slight microsatellite differentiation of Chilean and Andean tetraploid landraces are in concordance with their nuclear-cytoplasmic, day-length, and slight morphological differentiation (Raker and Spooner 2002; Huamán and Spooner 2002). Also, the present SSR results of the other cultivated species are very similar to those of Spooner et al. (2007), despite using a different germplasm base, and greater reliability in the present study of correct ploidy determinations. However, like the present morphological results, the main difference is the failure of the present study to distinguish *S. ajanhuiri*, grouping it with the majority of cultivated species, not with *S. curtilobum* and *S. juzepczukii* as in Spooner et al. (2007). A few accessions of *S. ajanhuiri* in Spooner et al. (2007), like in present study, were grouped with 4× and 2× cultivated potatoes. We cannot explain this result. Based on other data we still consider *S. ajanhuiri* a valid species, and consider possible misidentification of some of the accessions used in both studies. Another difference between studies is that the present study placed the wild species of series *Tuberosa* (system of Hawkes 1990) in a separate cluster, unlike Spooner et al. (2007) where the few examined wild species grouped with *S. curtilobum* and *S. juzepczukii*. Both morphological and SSR data obtained in the present study, like prior recent studies, document the need to reevaluate cultivated potato taxonomy, and we support the four cultivated species taxonomy of Spooner et al. (2007)

In summary, two SSR studies (the present study and Spooner et al. 2007) using a group of SSR's specifically selected for maximum utility in cultivated potato (Ghislain et al. 2009), and with different germplasm collections, come to nearly the same conclusions that many cultivated potato species could not be differentiated based not on their geography or ploidy levels, but they could be clearly grouped based on their different phylogeny, with *S. curtilobum* and S. *juzepczukii* supported as clear allopolyploids, *S. tuberosum* Andigenum Group to be best recognized as a complex of very poorly distinguished 2×, 3×, and 4× populations held together by extensive gene flow, slight and incomplete differentiation of the Chilean tetraploids, and *S. ajanhuiri* in need of further study. Maintenance of traditional species of prior treatments is only sometimes possible by only using chromosome counts (Ghislain et al. 2006); a reliance on computer-assisted multivariate analysis, and will never produce stable identifications. While prior taxonomic treatments have done an excellent job of highlighting the tremendous variation in cultivated potatoes, we consider these taxonomic systems to not reflect evolution or to be repeatable and practical in an applied sense.

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