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cpDNA SSR POLYMORPHISM IN SECONDARY GERMPLASM OF POTATO MAINTAINED IN CZECH GENE BANK

Dukagjin Zeka[1,*], Petr Sedlak[1], Vladimira Sedlakova[1], Jakub Vasek[1], Jaroslava Domkarova[2] and Pavel Vejl[1]

[1]Department of Genetic and Breeding, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, Prague 6-Suchdol 165 21, Czech Republic; [2]Department of Genetic Resources, Potato Research Institute Ltd., Dobrovského 2366, Havlíčkův Brod, Czech Republic
[*]Corresponding author's e-mail: zeka@af.czu.cz

Simple Sequence Repeat (SSR) has been employed successfully in many plant species because of high polymorphism and relatively low cost. Aim of the research was to evaluate cpDNA polymorphisms in germplasm collection of 31 accessions of Czech Gene Bank belonging to 27 *Solanum* species using twenty-three cpSSRs markers. In total, 94 alleles were detected, i.e. 4.09 alleles per each cpSSR locus in average. The highest polymorphism was detected in the locus NTCP9 (16 alleles) and lowest were recorded by two alleles in six of loci. No polymorphism was observed in case of SSR locus NTCP19. Mean value of observed heterozygozity (H_o) was 0.57, whereas mean of Polymorphic Information Contents (PIC) was 0.49. Less variability occurred in exons (H_{ex}=0.41) compared to introns (H_{in}=0.54) and intergenic regions (H_{igr}=0.60) of the chloroplast genome. The most important result was finding a set of four SSR markers (NTCP6, NTCP8, NTCP9 and NTCP12; mean PIC = 0.795) identifying 30 from 31 studied individuals as original haplotypes; only two *S. gourlai* individuals were identical. It is very helpful tool for DNA fingerprinting in collections of potato genetic resources.
Keywords: *Solanum* species, potato genetic resources, molecular description, PIC, cpDNA SSRs.

INTRODUCTION

Genus *Solanum* is one of the largest genera in flowering plants. Cultivated potato (*Solanum tuberosum* ssp. *tuberosum* L.) is the fourth most important food crop for its economic importance. It has been the subject of several phylogenetic and systematic researches in the past. Wild relatives represent very important genetic resources in plant breeding as secondary gene pool. The potato secondary gene pool consists of the broadest range of wild and primitively cultivated relative species compared to other crop plants (Pavek and Corsini, 2001). In the past, about 219 wild species (Hawkes, 1990) and seven cultivated species or subspecies with thousands of Andean farmer varieties were recognized (Hawkes, 1990; Huamám, 1998; Huamám and Spooner, 2002) but the recent molecular research approximately presumed existence of 100 wild and only four cultivated species (Ovchinnikova *et al*., 2011). Especially wild potato species (genus *Solanum*, section *Petota*) represent a tremendously diverse gene pool which is traditionally utilized as a source of various traits (e.g. resistance genes, etc.) for potato breeding (Heřmanová *et al*., 2007).

Hawkes (1990, 1994) concluded the first taxonomy of the genus based on the variability of morphological characters, plants phenology and cytology, but with the development of molecular analysis of nucleic acids, the polymorphism of different nuclear DNA regions i.e., mtDNA and cpDNA introns and non-coding intergenic spacers clarified phylogeny of the genus (Kocyan *et al*., 2007; Miz *et al*., 2008).

Powell *et al*. (1995) presumed the use of PCR-based analysis of mononucleotide repeats as an efficient tool to detect both intraspecific and interspecific variability in the chloroplast genomes of seed plants. The analysis of polymorphic microsatellites thus provides an important experimental tool to examine a range of issues in plant genetics. However, the final *Solanum* genus taxonomy is not acceptably resolved and each new study brings significant exchanges in species classification. Phylogeny based on chloroplast DNA (cpDNA) restriction enzyme sites (Spooner and Sytsma, 1992; Spooner and Castillo, 1997) divide sect. *Petota* into four clades (Table 1): (1) the U.S., Mexican, and Central American diploid species, exclusive of *S. bulbocastanum, S. cardiophyllum*, and *S. verrucosum;* (2) *S. bulbocastanum*, and *S. cardiophyllum;* (3) members of the South American series *Piurana* and some South American species classified to other series and (4) all remaining South American species and the U.S., Mexican, and Central American polyploid species (Spooner and Salas, 2006). Studies of non-coding regions variability resulted into the creation of a set of universal chloroplast PCR markers that are applicable to many plant species (Chiang *et al*. 1998; Bastia *et al*. 2001). At present the SSR markers are considered the best

DNA markers not only for their putative influence on transcribed genes but also their relative cheapness (Benemann *et al*., 2012). Microsatellite polymorphism analyses were used to study genetic diversity in numerous crop plant species including potato (Bryan *et al*., 1999), soybean (Powell *et al*., 1995) and sunflower (Wills and Burke, 2006), rape (Li *et al*., 2007) and pepper (Hanáček *et al*., 2009). Although a wide range of germplasm is held in gene banks and evaluated for many economically important traits, yet much has to be done. Many of the wild and cultivated species of potato have not been utilised in potato breeding as expected. The scenario should change in the future since molecular marker assisted selection will be used for faster integration of desirable genes from wild species (Bradshaw *et al*., 2006). In the present study we evaluated cpDNA polymorphism using SSR markers in selected cultivated and non cultivated potato relative species preserved *in vitro* to explore their diversity and to verify future usability of these markers in potato germplasm conservation systems.

MATERIALS AND METHODS

***Plant material and DNA extraction*:** CpDNA SSR polymorphism was assessed in 31 genotypes of cultivated and uncultivated species of *Solanum* genus (Table 1) obtained from a collection of *in vitro* Gene Bank at the Potato Research Institute Ltd. Havlickuv Brod.

Leaf samples (100 mg) from each genotype were collected and total DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Germany).

***cpSSR genotyping and statistical analyses*:** Twenty-three primer pairs amplifying loci in tobacco and potato cpSSRs by Bryan *et al*. (1999) were used. The 12.5 µl reaction mixture contained: 10 ng of total DNA, 1 x buffer KCl, 1.5 mM $MgCl_2$, 0.5 unit of *Taq* polymerase, 0.4 µM forward and reverse primers, and 0.3 mM dNTPs (Fermentas, Lithuania). The temperature conditions of PCR performed in thermocycler C1000 (Bio-Rad, USA) were as follows: the preliminary denaturing for 3 min at 94°C, followed by 27 cycles consisting of 40s denaturing at 94°C, 40s annealing at 60°C and 40s elongation at 72°C and closed by one final extension of 10 min at 72°C. Length polymorphisms of amplicon were analysed by capillary electrophoresis ABI PRISM 310 (Applied Biosystems, USA). The size and number of amplicons were estimated by the program GeneMapper ver. 4.1 (Life Technologies, USA) with help of the GeneScan™ 600 LIZ® size standard (Life Technologies, USA). Population parameters were calculated for each locus. The average heterozygosity per locus was calculated as $H=N/N\text{-}1(1\text{-}\sum p_i^2)$, where N is the number of samples and p_i is frequency of i^{th} allele (Nei, 1987). Polymorphic Information Content (PIC) was calculated using online software available (http://www.genomics.liv.ac.uk /animal/pic.html). A binary matrix was constructed for genotypes. Polymorphic loci were scored as presence (1) and absence (0) of an allele. These values were statistically evaluated using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and dissimilarity matrix was calculated by means Jaccard's coefficient, 1000 replicate

Table 1. List of the analyzed *Solanum* species

Order	Species	EVIGEZ[1] Code	Clade	Order	Species	EVIGEZ Code	Clade
1	*Solanum acaule*	00030	4[cgh]	17	*S. mochiquense*	00050	3[c]
2	*S. andigenum*	00108	4[ag]	18	*S. phureja*	00308	4a
3	*S. berthaultii*	00260	4[cfgh]	19	*S. pinnatisectum*	00051	1[bcdf]
4	*S. bulbocastanum*	00240	2[bcdf]	20	*S. polyadenium*	00290	1[d]
5	*S. bulbocastanum*	PIS 06-17	2[bcdf]	21	*S. polytrichon*	00053	4[d]
6	*S. chacoense*	00037	4[cfh]	22	*S. sparsipillum*	00071	4[cfh]
7	*S. chacoense*	00230	4[cfh]	23	*S. spegazzini*	00060	4[eh]
8	*S. demissum*	00250	4[egh]	24	*S. stenotomum*	00212	4[ag]
9	*S. fendleri*	00275	4[de]	25	*S. stoloniferum*	00295	4[ah]
10	*S. goniocalyx*	00109	4[a]	26	*S. sucrense*	00062	4[c]
11	*S. gourlai*	00045	4[cf]	27	*S. vernei*	00069	4[cgh]
12	*S. gourlai*	00043	4[cfs]	28	*S. vernei*	00234	4[cgh]
13	*S. guerreroense*	00280	4[dh]	29	*S. verrucosum*	00299	4[cegh]
14	*S. incamayoense*	00047	4[ch]	30	*S. x chaucha*	00134	4[a]
15	*S. leptophyes*	00048	4[ch]	31	*S. yungasense*	00070	4[agh]
16	*S. microdontum*	00049	4[ch]				

[1]Plant Genetic Resources Documentation in the Czech Republic

[a]Ovchinnikova *et al*. (2011); [b]Rodríguez and Spooner (1997); [c]Spooner and Castillo (1997); [d]Spooner and Sytsma (1992); [e]Spooner *et al.* (1991); [f]Spooner *et al.* (2007a); [g]Spooner *et al.* (2008); [h]Spooner (2009)

bootstrapping by software DARwin v. 5.0 (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

A total of 94 alleles of 23 cpSSR loci were detected in the potato relatives' collection. Range of alleles per locus was very variable. The number of alleles ranged from two to 16 per locus. Results of SSR analysis are summarised in Table 2. Observed PIC values varied from 0.16 to 0.91 (mean=0.49) and average observed heterozygosity values varied from 0.18 to 0.95 (mean=0.57). Variability of SSR in coding and non-coding regions was estimated based on their average heterozygosity. Less variability in exons (H_{ex}=0.41, n=2) was observed in comparison to introns (H_{int}=0.54, n=7) and intergenic regions (H_{igr}=0.60, n=14). This is very well explainable because the exons are highly conserved in general due to their genetic activity and higher selectiveness of potentially lethal mutation in these regions. The genetic distance (D) among different genotypes was reproduced in a UPGMA radial dendrogram (Fig. 1) based on Jaccard dissimilarity coefficient. The highest range of polymorphism was found in the locus NTCP9. Average heterozygosity value of 0.95 for a total of 16 alleles was observed. The locus was more polymorphic; the range of alleles was broader for eight and also level of heterozygosity was higher for 0.25 as compared to Bryan *et al*. (1999). Martyrosyan *et al*. (2007) also reported that the NTCP9 cpSSR displayed the highest variability in the examined cultivars. Allele size in locus NTCP9 ranged from 238 to 308bp. The shortest amplicon was detected in *S. polyadenium* (*00290*) and the longest in the *S. goniocalyx* (*00109*). The lowest level of polymorphism was recorded in a couple of loci showing 2 alleles (NTCP24, NTCP26, NTCP28, NTCP29, NTCP33 and NTCP37) and less (NTCP19). The heterozygosity values in the couple varied from 0.18 (NTCP37) to 0.50 (NTCP24). Marker NTCP19 detected no polymorphism; this is in line with the previous report of Bryan *et al.* (1999).

Cluster analysis comprising distance of 30 different haplotypes (Figure 1). Clustering shows slight distance between genotypes *S. chacoense 00037* and *S. chacoense 00230* due to the presence of single nucleotide length polymorphisms in loci NTCP6, NTCP8, NTCP14 and NTCP27 and, 12bp difference in locus NTCP9. The specie *S. yungasense* was also sorted to the same clade. It is worth mentioning that these three genotypes manifested very similar habitus in the greenhouse culture and thus the molecular analysis confirmed morphological similarity of these two species. The loci in NTCP6, NTCP9, NTCP18, NTCP24, NTCP27, NTCP33 and NTCP 39 revealed 1bp difference between *S. bulbocastanum PIS06-17* and *S. bulbocastanum 00240*. Whereas, SSR differences in length

Table 2. Results of cpSSR analysis

Locus	Location in cp chromosome	Het	PIC	No. of alleles	Allele size range (bp)
NTCP 3	*trn*K intron	0.66	0.57	4	190-193
NTCP 4	*trn*K/*rps* 16 intergenic region	0.63	0.56	4	156-162
NTCP 6	*rps*16/*trn*Q intergenic region	0.84	0.79	7	122-171
NTCP 7	ORF98/*trn*S intergenic region	0.53	0.41	3	150-169
NTCP 8	*trn*G intron	0.81	0.75	5	247-251
NTCP 9	*trn*G/*trn*R intergenic region	0.95	0.91	16	238-308
NTCP 10	*atp*F intron	0.62	0.55	4	110-113
NTCP 12	*rps*2/RF862 intergenic region	0.79	0.73	6	117-122
NTCP 14	*psb*M/*trn*D intergenic region	0.79	0.73	5	143-148
NTCP 18	*psb*C/*trn*S intergenic region	0.73	0.65	4	185-188
NTCP 19	*ycf*3 intron	0.00	0.00	1	152
NTCP 20	*ycf*3 intron	0.66	0.58	4	112-116
NTCP 23	*rps*4/*trn*T intergenic region	0.64	0.55	4	107-110
NTCP 24	*atp*B exon	0.50	0.37	2	148-149
NTCP 26	*psa*I/ORF184 intergenic region	0.32	0.26	2	165-166
NTCP 27	*trn*P/*psa*J intergenic region	0.67	0.59	4	159-162
NTCP 28	*rpl*20/*rps*12 intergenic region	0.28	0.23	2	153-154
NTCP 29	*clp*P intron	0.49	0.36	2	150-151
NTCP 30	*clp*P intron	0.53	0.41	3	149-151
NTCP 33	*rpo*A exon	0.32	0.26	2	145-146
NTCP 37	*rrn*5/*trn*R intergenic region	0.18	0.16	2	137-138
NTCP 39	*trn*R/*rrn*5 intergenic region	0.52	0.44	3	149-151
NTCP 40	*rp*12/*trn*H intergenic region	0.54	0.52	5	263-287
Mean		0.57	0.49	4.09	NA

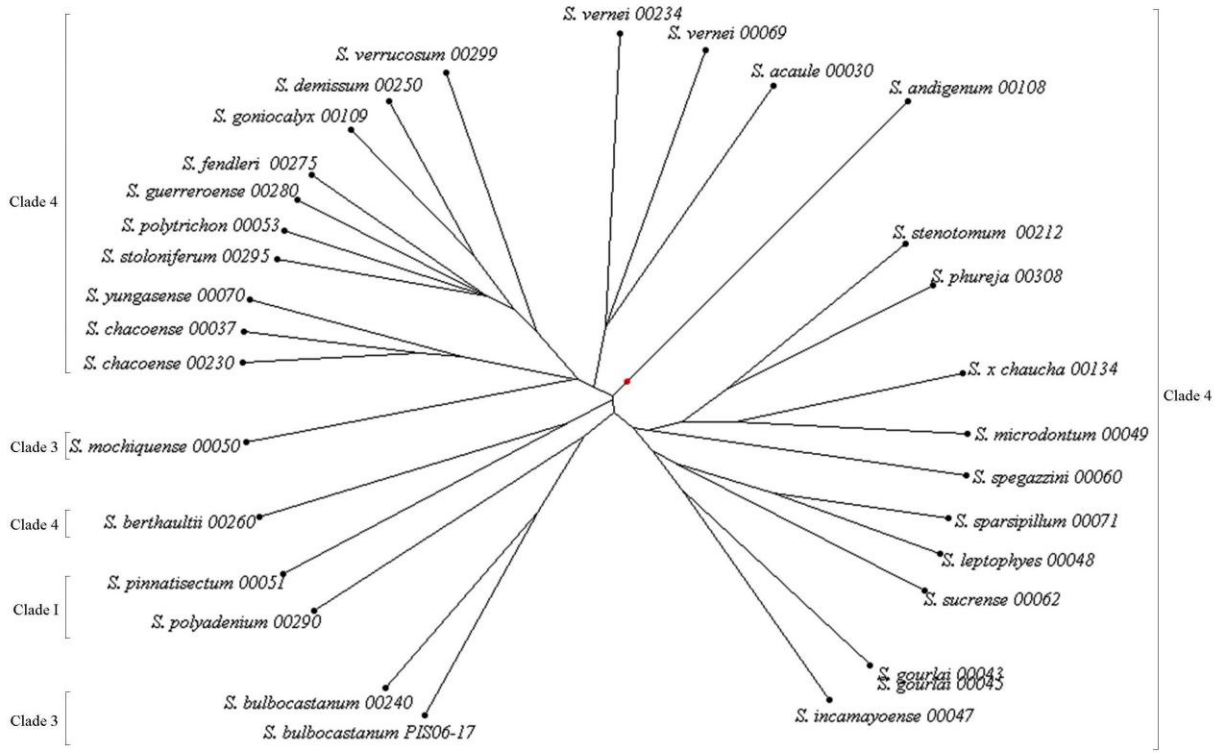

Figure 1. Radial scheme of clustering detected haplotypes based on Jaccard dissimilarity coefficient and UPGMA clustering method using 23 SSR markers.

from 1-3bp between genotypes *S. vernei 00037* and *S. vernei 00234* were found in loci NTCP8, NTCP10, NTCP14, NTCP27, NTCP28, NTCP29 and NTCP39, an obvious contrast was observed in locus NTCP40 (21 bp) and in locus NTCP9 (30 bp). Finally genotypes *S. gourlai 00043* and *S. gourlai 00045* were of the same haplotype.

The results of cluster analysis in our work are more or less in concordance with "Spooner's" taxonomy dividing section *Petota* into four clades (Spooner and Sytsma, 1992; Spooner and Castillo, 1997). However, it is necessary to analyze more genotypes using more cpSSR markers for acceptance or elimination of our conclusions. But, in terms of partial results, it's interesting to emphasize, that the clustering data patterns are in relatively good concordance with results obtained from previous phylogenetic studies of potato and relative species by Bryan *et al*. (1999) and Sukhotu and Hosaka (2006). As expected, the closely related species *S. yungasense* and *S. chacoense* shared one common cluster. Similar situation was in case of analysis of several genotypes from the *Solanum brevicaule* BITTER complex first defined by Ugent (1970) as a taxonomically confusing group of putative ancestors of the cultivated potato species endemic to central Peru, Bolivia, and northern Argentina where wild members of the complex are difficult to distinguish from the cultivated members. In our work the complex represented *S. gourlai 00043* and *00045, S. incamayoense* 00047, *S. leptophyes* 00048, *S. spegazzini* 00060, *S. sucrense* 00062 and *S. sparsipillum* 00071. All these genotypes were clustered side by side as documented in Fig. 1. Conversely *Solanum fendleri* and *S. verrucosum* appeared to be possible morphological candidates for inclusion into the complex (Van den Berg *et al*., 1998) in this study plastid DNA data separated these two genotypes from the members of the *S. brevicaule* complex. It corresponds to data of Miller and Spooner (1999) and Spooner and Castillo (1997). Similarly, here we classified all analyzed species of series *Longipedicellata* (*S. fendleri, S. polytrichon, S. stoloniferum*) to one group together with *S. guerreroense* (series *Demissa*). *Solanum demissum* (serie *Demissa*) was also classified nearly this group which corresponds with previous phenotic analysis of morphological and molecular data by Spooner *et al*. (2001) and Van den Berg *et al*. (2002), respectively. Our results also can partially support the theory of near phylogenic distance of *S. x chaucha* with Andigena group and *S. stenotomum* formulated by Jackson *et al*. (1977). They presume *S. x chaucha* as a triploid result of interspecific hybridisation between Andigena group and *S. stenotomum* grown with considerable frequency by native farmers in a region of the hybrid origin. In our diagram in (Figure 1) members of these three species are on relatively

near distance. Similarly, *S. phureja 00308* was classified as very similar to *S. stenotomum 00212*. This result corresponds to Hawkes's (1994) hypothesis according to which the species *S. phureja* was selected by Andean farmers from *S. stenotomum*. Spooner *et al*. (2007b) found many expectations of clustering cultivated species e.g., *S goniocalyx* landraces were invariably intermixed with those of *S. stenotomum*, whereas *S. phureja, S. stenotomum* and *S. x chaucha* shared common cluster like in our research.

The main aim of the work was to find the most effective set of SSR markers for next use in system of fast and detection of duplicities. Therefore, different marker sets scenarios were also analyzed to find the suitability and critical number of markers to distinguish in evaluated species for practical use of cpSSR markers in gene bank collection management. Data set of seven NTCP markers used by Provan *et al*. (1999) and Sukhotu and Hosaka (2006) was tested and with slights exceptions gave results in line with our findings. Nevertheless, we tested particularly limited number of SSR markers with possibility to find significant polymorphisms. Finally we selected a subset of four markers (NTCP6, NTCP8, NTCP9 and NTCP12) sufficient to distinguish 30 haplotypes. However, information generated here for phylogeny is not sufficient.

***Conclusion*:** Plastid DNA analysis using cpSSRs markers exhibited a high level of genetic polymorphism within researched *Solanum* genotypes except for two genotypes *S. gourlai 00043* and *S. gourlai 00045*. Moreover, it was suitable to detect significant molecular variation within studied potato genetic resources. Our data revealed the presence of 25 distinct chloroplast haplotypes of uncultivated genotypes and five in cultivated genotypes with the evidence that 22 of 23 pairs of primers detect variability among *Solanum* species. The set of four markers with high genetic information content can be used to distinguish large number of haplotypes. The current methodical approach is useful tool for characterization of potato secondary gene-pool and for optimization of *in vitro* genetic resources collections; for example searching of duplicities or genetic resources stability check.

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REFERENCES

Bastia, T., N. Scotti and T. Cardi. 2001. Organelle DNA analysis of *Solanum* and *Brassica* somatic hybrids by PCR with "universal primers". Theor. Appl. Genet. 102:1265-1272.

Benemann, D.P., L.N. Machado, L.W.P. Argel, V.J. Bianchi, A.C. Oliveira, L.C. Maia and J.A. Peters. 2012. Identification, characterization and validation of SSR markers from the gerbera EST database. Plant Omics J. 5:156-159.

Bradshaw, J.E., G.J. Bryan and G. Ramsay. 2006. Genetic resources (Including wild and cultivated *Solanum* species) and progress in their utilisation in potato breeding. Potato Res. 49:49-65.

Bryan, G.J., J. Mcnicoll, G. Ramsay and R.C. Meyer. 1999. Polymorphic simple sequence repeat markers in chloroplast genomes of *Solanaceous* plants. Theor. Appl. Genet. 99:859-867.

Chiang, T.Y., B.A. Schaal and CH. I. Peng. 1998. Universal primers for amplification and sequencing a noncoding spacer between the atpB and rbcL genes of chloroplast DNA. Bot. Bull. Acad. Sin. 39:245-250.

Hanáček, P., T. Vyhnánek, M. Rohrer, J. Cieslarová, and H. Stavělíková. 2009. DNA polymorphism in genetic resources of red pepper using microsatellite markers. Hort. Sci. (Prague) 36:127-132.

Hawkes, J.G. 1990. The potato, evolution, biodiversity, and genetic resources. In: T.A. Gavrilenko, O.Y. Antonova, and L.A. Kostina (eds.). Study of genetic diversity in potato cultivars using PCR analysis of organelle DNA. Russ. J. Genet. 43:1301-1305.

Hawkes, J.G. 1994. Origins of cultivated potatoes and species relationships. In: J.E. Bradshaw and G.R Mackay (eds.). Potato genetics. CAB International, Cambridge.

Heřmanová, V., J. Bárta and V. Čurn. 2007. Wild potato species: Characterization and biological potential for potato breeding. Czech J. Genet. Plant Breed. 43:73-81.

Huamám, Z. 1998. Collection, maintenance, and evaluation of potato genetic resources. Plant Var. Seeds 11:29-38.

Huamám, Z. and D.M Spooner. 2002. Reclassification of landrace populations of cultivated potatoes (*Solanum* Sect. *Petota*). Am. J. Bot. 89:947-965.

Jackson, M.T., J.G. Hawkes and P.R. Rowe. 1977. The nature of *Solanum* × *chaucha* Juz. Et Buk., a triploid cultivated potato of the South American Andes. In: T. Sukhotu and K. Hosaka (eds.). Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers. Genome 49:636-647.

Kocyan, A., L.B. Zhang, H. Schaefer and S.S Renner. 2007. A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. Mol. Phyl. Evol. 44:553-577.

Li M., C. Zhang, W. Qian and J. Meng. 2007. Genetic diversity of *Brassica* species revealed by amplified fragment length polymorphism and simple sequence repeat markers. Hort. Environ. Biotechnol. 48:9-15.

Martyrosyan, E.V., N.N. Ryzhova and E.Z. Kochieva. 2007. Polymorphism of chloroplast microsatellite DNA loci in Russian potato cultivars. Russ. J. Genet. 43:1325-1327.

Miller, J.T. and D.M Spooner. 1999. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (*Solanaceae, S*. sect. *Petota*): molecular data. Plant Syst. Evol. 214:103-130.

Miz, R.B., L.A. Mentz and T.T Souza-Chies. 2008. Overview of the phylogenetic relationships of some southern Brazilian species from section *Torva* and related sections of "spiny *Solanum*" (*Solanum* subgenus *Leptostemonum*, Solanaceae). Genetica 132:143-158.

Nei, M. 1987. Molecular Evolutionary Genetics, Columbia University Press, New York.

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

Pavek, J.J. and D.L. Corsini. 2001. Utilization of potato genetic resources in variety development. Am. J. Potato Res. 78:433-441.

Perrier, X. and J.P. Jacquemoud-Collet. 2006. DARwin (http://darwin.cirad.fr/darwin), Version 5.0.

Powell, W., M. Morgante, C. Andre, J.W. Mcnicol, G.C. Machray, J.J Doyle, S.V. Tingey and J.A Rafalski. 1995. Hypervariable microsatellites provide a general source of polymorphic DNA markers for the chloroplast genome. Curr. Biol. 5:1023-1029.

Provan, J., W. Powell, H. Dewar, G. Bryan, G.C. Machray and R. Waugh. 1999. An extreme cytoplasmic bottleneck in the modern European cultivated potato (*Solanum tuberosum*) is not reflected in decreased levels of nuclear diversity. Proc. R. Soc. Lond. Ser. B. Biol. Sci. 266:633-639.

Rodríguez, A. and D.M Spooner. 1997. Chloroplast DNA analysis of *Solanum bulbocastanum* and *S. cardiophyllum*, and evidence for the distinctiveness of *S. cardiophyllum* subsp. *Ehrenbergii* (sect. *Petota*). Syst. Bot. 22:31-43.

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

Spooner, D.M., K.J. Sytsma and E. Conti. 1991. Chloroplast DNA evidence for genome differentiation in wild potatoes (*Solanum* sect. *Petota*: Solanaceae). Am. J. Bot. 78:1354-1366.

Spooner, D.M. and K.J. Sytsma. 1992. Reexamination of series relationships of Mexican and Central American wild potatoes (*Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Syst. Bot. 17:432-448.

Spooner, D.M. and R.T. Castillo. 1997. Reexamination of series relationships of South American wild potatoes (*Solanaceae*: *Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Am. J. Bot. 84:671-685.

Spooner, D.M., R.G. Van den Berg and J.T. Miller. 2001. Species and series boundaries of *Solanum* series *Longipedicellata* (Solanaceae) and phenetically similar species in ser. *Demissa* and ser. *Tuberosa*: implications for a practical taxonomy of sect. *Petota*. Am. J. Bot. 88:113-130.

Spooner, D.M. and A. Salas. 2006. Structure, biosystematics, and genetic resources. In: J. Gopal and S.M. P. Khurana (eds.) Handbook of potato production, improvement, and post-harvest management. Haworth's Press, Inc., Binghampton, New York.

Spooner, D.M., D. Fajardo and G.J Bryan. 2007a. Species limits of *Solanum berthaultii* Hawkes and *S. tarijense* Hawkes and the implications for species boundaries in *Solanum* sect. *Petota*. Taxon 56:987-999.

Spooner, D.M., J. Núñez, G. Trujillo, M. del Rosario Herrera, F. Guzmán and M. Ghislain. 2007b. Extensive simple sequence repeat genotyping of potato landraces supports a major re-evaluation of their gene pool structure and classification. Proc. Natl. Acad. Sci. USA. 104:19398-19403.

Spooner, D.M., F. Rodríguez, Z. Polgár, H.E. Jr. Ballard and S.H. Jansky. 2008. Genomic origins of potato polyploids: GBSSI gene sequencing data. Pl. Genome, suppl. Crop Sci. 48:27-36.

Spooner, D.M. 2009. DNA barcoding will frequently fail in complicated groups: An example in wild potatoes. Am. J. Bot. 96:1177-1189.

Ugent, D. 1970. The potato. Science 170:1161-1166.

Van den Berg, R.G., G.J. Bryan, A. Del Rio and D.M. Spooner. 2002. Reduction of species in the wild potato Solanum section *Petota* series *Longipedicellata*: AFLP, RAPD and chloroplast SSR data. Theor. Appl. Genet. 105:1109-1114.

Van den Berg, R.G., J.T Miller, M.L. Ugarte, J.P. Kardolus, J. Villand, J. Nienhuis and D.M. Spooner. 1998. Collapse of morphological species in the wild potato *Solanum brevicaule* complex (sect. *Petota*). Am. J. Bot. 85:92-109.

Wills, D.M. and J.M. Burke. 2006. Chloroplast DNA variation confirms a single origin of domesticated sunflower (*Helianthus annus* L.). J. Hered. 97:403-408.