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Evaluation of Variations in Plastid DNA Non-coding Regions in Selected Species of the Genus *Solanum*

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Abstract

Sedláková V., Sedlák P., Zeka D., Domkářová J., Doležal P., Vejl P. (2017): Evaluation of variations in plastid DNA non-coding regions in selected species of the genus *Solanum*. Czech J. Genet. Plant Breed., 53: 127–131.

The diversity of three non-coding plastid DNA loci (*trn*L/*trn*F spacer, *trn*V/16SrRNA spacer, *trn*L/*trn*L intron) was assessed in 16 *Solanum* L. species (135 individuals). Polymorphisms were detected by denaturing gradient gel electrophoresis (DGGE) and verified by direct sequencing. No intraspecific diversity and only poor interspecific diversity was detected. Unique *S. mochiquense* Ochoa specific length polymorphism at the *trn*L/*trn*L locus represented by duplication of an 18 bp segment was discovered. The detected DGGE interspecific *trn*L/*trn*F locus polymorphism did not specifically associate with single point mutations in the sequence confirmed by sequencing. The DGGE method was found to be a simple and cheap pre-exploring tool for mutation detection in compared DNA regions. Some identified polymorphisms can be used in the management of genetic resources.

Keywords: DGGE; DNA sequencing; genetic diversity; point mutations; potato genetic resources

Mutations in non-coding plastid DNA regions are usable to study plant diversity and phylogenetic relationships. Wettstein *et al*. (1978) proved a wide range of diversity in non-coding plastid DNA, whereas Scowcroft (1979) confirmed high conservativeness of plastid genes. The plastome consists of unique large and small single copy regions and two inverted repeats (IRa, IRb) containing cp-rRNA genes (Wakasugi *et al*. 1998).

The genus *Solanum* passed through complex evolution. Especially, the taxonomy of tuber-bearing *Solanum* species is permanently revised. The section *Petota* presently consists of approximately 110 species classified by plastid DNA restriction data into four systematic clades and/or into three clades by nuclear DNA sequencing when it is impossible to distinguish plastid clades 1 and 2 (Spooner *et al*. 2008).

We used denaturing electrophoresis to assess diversity in non-coding loci of plastid DNA because it seemed to be a simple and cheap tool used previously in plant genetics (Liu & Lowes 2013) for detection of mutations. The primary aim was to determine diversity in selected plastid DNA loci to distinguish *Solanum* species in the potato genetic resource collection.

A set of 135 representatives of 16 *Solanum* species maintained in the gene bank of the Potato Research Institute in Havlíčkův Brod was analysed (Table 1). Total DNA was extracted by a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Primers published by Al-Janabi *et al*. (1994) for the *trn*V/16SrRNA locus (297 bp, Tm 63°C) and Taberlet *et al*. (1991)

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for the loci *trn*L/*trn*F (418 bp, Tm 62°C) and *trn*L/*trn*L (557 bp, Tm 62°C) were used in optimised PCR conditions. Amplicons were analysed on the principle of denaturing gradient gel electrophoresis (DGGE) optimised for each locus using the system DCodeTM (Bio-Rad, Hercules, USA). Sequences of all amplicons were determined using the dideoxy method (BigDye Terminator Kit 3.1, Life Technologies, Carlsbad, USA) in an ABI PRISM 310 Genetic Analyser (Life Technologies).

All amplicons were of expected lengths. No intraspecific diversity was found in collections of more numerous species in Table 1 and results of the blastn comparison of sequences with NCBI nucleotide databases are specified in Table 2.

*Trn*L/*trn*L intron was of poor diversity. Distinctive 18 bp polymorphism specific of *S. mochiquense* (*S.moch*) was found in positions from 448 to 466 as documented in Figure 1. The sequence was loaded to the nucleotide database NCBI (ID KM516167) for its uniqueness. Sequencing of the *trn*L/*trn*L locus also found *S. bulbocastanum* Dun. (*S.blb*) specific C/G substitution in position 230 undetectable by DGGE or restriction assay, because it does not change the number of hydrogen bonds and no restriction site was

Table 1. Selected characteristics of analysed species of the genus *Solanum*

Solanum species	GRIN code	No. of genotypes	DGGE *trn*L/*trn*F allele	Plastid clade (Spooner's taxonomy)	Chromosome number 2*n*[a]	EBN	Area of occurrence[a]
S. tuberosum subsp. *andigenum* Juz. & Bukasov	07S0300235	1	A	4[e]	48	4	Peru
S. × *sucrense* Hawkes	07S0300062	1	A	4[c]	48	4	Bolivia
S. tuberosum subsp. *tuberosum* 2*n* (DH 165, DH 322)	07S0500005 07S0500101	2	A	4[c]	24	2	Peru, Bolivia, Chile
S. polytrichon Rydb.	07S0300053	1	A	4[d]	48	2	Mexico
S. stenotomum subsp. *goniocalyx* Juz. & Bukasov	07S0300109	1	A	4[c]	24	2	Peru
S. verrucosum	07S0300296, 298-300	4	A	4[ce]	24	2	Mexico
S. pinnatisectum	07S0300051	1	A	1[bcd]	24	1	Mexico
S. phureja Juz. & Bukasov	07S0300308	1	B	4[c]	24	2	Colombia
S. bulbocastanum	PI 243510	80					
	07S0300034, 035, 262, 263, 265-270, 309-313	15	B	2[bcd]	24	1	Mexico
S. polyadenium Greenm.	07S0300052, 281, 284-290	9	B	1[d]	24	1	Mexico
S. berthaultii	07S0300031, 033, 233, 251, 252, 254-257, 259-261	12	C	4[bc]	24	2	Bolivia
S. chacoense	07S0300037	1	C	4[b]	24	2	Bolivia
S. microdontum	07S0300049	1	C	4[c]	24	2	Argentina
S. vernei Bitt. & Wittm.	07S0300068, 069, 234	3	C	4[c]	24	2	Argentina
S. yungasense	07S0300070	1	C	4[c]	24	2	Argentina
S. mochiquense	07S0300050	1	C	3[c]	24	1	Peru

[a]Bradshaw and Mackay (1994); [b]Spooner *et al.* (2007); [c]Spooner and Castillo (1997); [d]Spooner and Sytsma (1992); [e]Spooner *et al.* (2008); EBN – endosperm balance number

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Table 2. Significant matching of plastid DNA loci sequences with NCBI nucleotide databases

Locus	Matched sequences	Sequence identity (%)	NCBI accession (*Solanum* genus)
*trn*L/*trn*L	all species in Table 2 showed 100% identity (excluding *S. bulbocastanum* and *S. mochiquense*)	100	*S. tuberosum* subsp. *tuberosum* (HM006842.1, DQ386163.2, DQ131549.1) *S. pinnatisectum* (DQ180453.1) *S. brevicaule* Bitter (DQ180443.1) *S. laciniatum* (HM006852.1, HM006851.1, HM006835.1, DQ180467.1) *S. aviculare* (HM006854.1, HM006853.1) *S. aggregatum* Jacq.(DQ180460.1) *S. triflorum* Nutt. (DQ180457.1) *S. nitidum* Ruiz & Pav.(DQ180451.1) *S. caesium* Griseb. (HM006843.1, DQ180445.1) *S. aligerum* Schltdl. (DQ180441.1) *S. symonii* H.Eichler (HM006858.1, HM006857.1, HM006838.1) *S. linearifolium* Geras. (HM006850.1, HM006849.1, HM006833.1) *S. vescum* F.Muell (HM006848.1, HM006847.1, HM006832.1)
*trn*L/*trn*F	*S. berthaultii*	100	*S. tuberosum* subsp. *tuberosum* (HM006842.1, DQ386163.2, FJ490824.1, DQ231562.1)
	S. tuberosum subsp. *tuberosum* 2n DH165 and *S. vernei* (100% mutual identity)	99	across *Solanum* (mismatching and indels)
	S. chacoense *S. yungasense* *S. microdontum* (100% mutual identity)	100	*S. brevicaule* (DQ180443.1)
	S. bulbocastanum	100	*S. bulbocastanum* (DQ180444.1, DQ347958.1)
	S. pinnatisectum	99	*S. pinnatisectum* (DQ180453.1) (mismatching)
*trn*V/16SrRNA	Partial sequence of *S. berthaultii*	100	*S. lycopersicum* (AC239738.5, AM087200.3, AY216521.1, DQ347959.1) *S. tuberosum* subsp. *tuberosum* (DQ386163.2, DQ231562.1) *S. bulbocastanum* (DQ347958.1)
		99	*Nicotiana tomentosiformis* L. (AB240139.1) *N. sylvestris* Speg. & Comes (AB237912.1) *N. tabacum* L. (Z00044.2) *N. plumbaginifolia* Viv. (X70938.1) *Atropa belladonna* L. (AJ316582.1) *Datura innoxia* Mill. (FJ971407.1)
		95–99	57 species out from *Solanaceae*

generated. Interestingly, the comparison of the most frequent haplotype showed high homology not only to species originated in South America, but also to phylogenetically distinct species *S. laciniatum* Aiton and *S. aviculare* G.Forst. (subgenus *Archaesolanum* endemic in the Australian region unique for the chromosomal number $n = 23$). Our results support a hypothesis of their origin in a common progenitor with recent South American *Solanaceae* and subsequent diversification within the Pangea disruption presented by Olmstead and Palmer (1997).

The DGGE analysis of *trn*L/*trn*F locus detected three alleles varying in mobility (named A, B and C). Sequencing of alleles obtained from *S. tuberosum* ssp. *tuberosum* L. (*S.tub* ssp. *tub*) DH165 (A), *S. verrucosum* Schltdl. 299 (A), *S. pinnatisectum* Dun. (*S.pinn*) 051 (A), *S.blb* (B), *S. chacoense* Bitter (C), *S. yungasense* Hawkes (C), *S. microdontum* Bitter 049 (C), *S.moch* (C) and *S. berthaultii* Hawkes (*S.ber*) 260 (C) revealed a wide range of single nucleotide substitutions and deletions. Unfortunately, no DGGE allele specifically associated with any nucleotide

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                          430        440        450        460        470        480
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
S.moch      420 GGACGAGAATAAAGATAGAGTCCCGTTCTACATGTCAATACCGGCCTACATGTCAATACCGGCAA
S.ber       420 GGACGAGAATAAAGATAGAGTCCCGTT------------------CTACATGTCAATACCGGCAA
S.tubDH165  420 GGACGAGAATAAAGATAGAGTCCCGTT------------------CTACATGTCAATACCGGCAA
S.bulb      420 GGACGAGAATAAAGATAGAGTCCCGTT------------------CTACATGTCAATACCGGCAA
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Figure 1. Alignment of the nucleotide sequences of the *trn*L/*trn*L locus of *S. mochiquense*, *S. berthaultii*, 2*n S. tuberosum* DH165 and *S. bulbocastanum* (BioEdit Sequence Alignment Editor Ver. 5.0.9)

change or specific combination of changes was detected. Since the method indicated polymorphisms, the only way to interpret them was direct sequencing. Mainly the blastn comparison of sequences with databases revealed wide intraspecific diversity at this locus. Compared with the 2*n S.tub* ssp. *tub* DH322 *trn*L/*trn*F locus sequence, five substitutions in *S.pinn*, three substitutions and one deletion in position 110 (microsatellite region T_7) of *S.moch* and also the most frequent substitution C/A in position 126 (almost in all wild 2*n* = 2*x* species) were identified. One hundred percent identity of *S.tub* ssp. *tub* DH165 sequence was observed in comparison with *S. verrucosum* 299 but lesser compared to other *S.tub* ssp. *tub* accessions in the database. In contrast, *S.ber* 260 showed 100% homology to four *S.tub* ssp. *tub* accessions in the database. This could demonstrate plastid diversity in cultivated potato as a consequence of potato evolution and breeding. For instance the Apta variety (selected in Germany in 1951, parent of 2n DH165) originated from the mating of a maternal interspecific hybrid with the Hindenburg variety (European Cultivated Potato Database). Although detailed pedigree data are missing, our results indicate the origin of Apta's plastid DNA in some wild species or primitive potato cultivar in its pedigree. One hundred percent *trn*L/*trn*F locus sequence identity of *S. chacoense*, *S. yungasense* and *S. microdontum* 049 indicates genetic relations (Figure 2) of these species. It is also supported by other indicators in Table 1 such as common geographical origin of the identical karyotype EBN (Endosperm Balance Number) and presence in potato clade 4. Matching data for *S.blb* and *S.pin* 051 are shown in Table 2.

DGGE of the *trn*V/16SrRNA locus indicated two sequentially different DNA fragments in amplicons. This apparently affected sequencing and only partial sequences for *S.blb* and *S.ber* were obtained. The longest repeatedly sequenced *S.ber* fragment (247 bp) was completely homologous to *Solanum lycopersicum* L.,

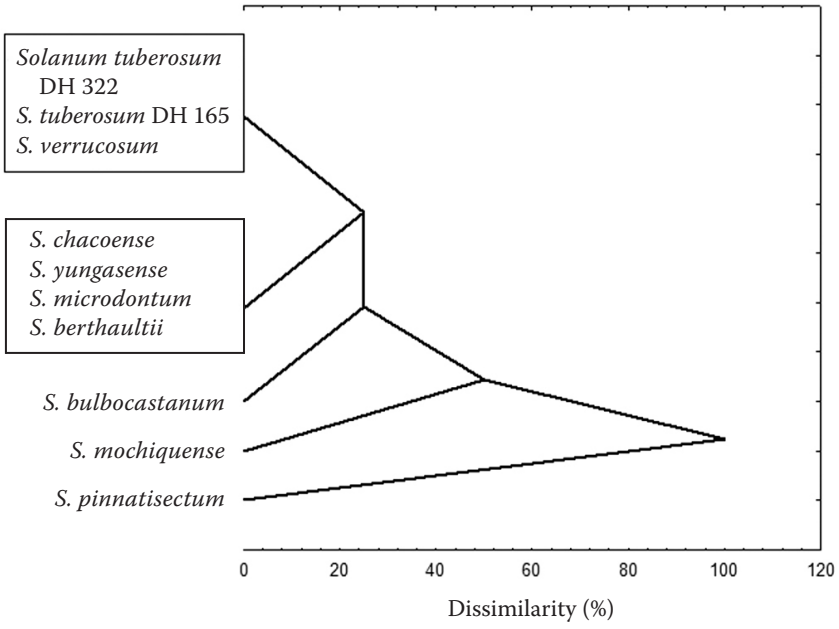

Figure 2. Comparison of neighbour joining of dissimilarity coefficients of the *trn*L/*trn*F locus sequences using Statistica CZ 9.1 (Statsoft, Tulsa, USA)

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S.tub ssp. *tub* and *S.blb*. High identity was observed in other *Solanaceae* genera and species of different families (Table 2). This documents higher locus conservativeness in contrast to the variable *trn*L/*trn*F locus, but the troubles with sequencing could indicate diverse indels in spacer copies present in IRa and IRb as described by Wakasugi *et al*. (1998).

Very low diversity across the wild *Solanum* species in non-coding plastid DNA loci confirms a hypothesis of conservativeness of *Solanum* plastome. The methodology and polymorphisms detected can be used in the management of plant genetic resources in gene banks.

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