BIOLOGIA PLANTARUM 54 (3): 543-546, 2010

BRIEF COMMUNICATION

Study of the origin of the rarely cultivated edible *Solanum* species: morphological and molecular data

P. POCZAI*, K. MÁTYÁS, J. TALLER and I. SZABÓ

Department of Plant Science and Biotechnology, Georgikon Faculty, University of Pannonia, Festetics 7, Keszthely, H-8360, Hungary

Abstract

The present study applies RAPD technique and morphometric analysis to study the diversity of some accessions belonging to section *Solanum*. A total of 252 products were amplified with 23 12-mer arbitrary primer pairs, among which 210 were found to be polymorphic. Sixteen morphological characters were measured and used to compile a dendrogram. Both the morphological and RAPD marker analysis clearly separated the different accessions into similar groups. The results indicate that the analyzed cultivars with unknown origin could be derived from *S. retroflexum*. We found morphological differences among the *S. scabrum* subsp. *scabrum* accession which were not reflected in the molecular data. Presumably these accessions represent cultivated forms selected for their habit, fruit quantity and/or quality and leaf size, respectively.

Additional key words: genetic diversity, RAPD, *Solanum retroflexum, Solanum scabrum.*

The *Solanum nigrum* L. complex, commonly known as black nightshades, is one of the largest and most variable species group of the genus *Solanum*. It consists of about 30 species, most of which originate from the New World tropics, particularly South America (Edmonds 1972). Although the majority of species belonging to this section are common weeds especially in Europe and North America, there are minor crop plants serving food sources in many developing countries mostly in the African continent. There is a widespread confusion over the precise identification of the taxa involved (Edmonds and Chweya 1997). For example, the morphological distinction of *S. scabrum* Mill., which is native in West and Central Africa, seems to be clear, but it was not obvious through the historical examination of the species. Another notable species, *S. retroflexum* Dun. (syn. *S. burbankii* Mill.), has been used since the beginning of the 20th century in North America. Nowadays several popular cultivars from different black nightshades are available in commercial trade in Mexico, although, the origin of these cultivars is still not clear.

The usefulness of the random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland 1990, Williams *et al*. 1990) to investigate genetic diversity between different plant groups has been demonstrated by several studies (*e.g.* Cordeiro *et al*. 2008, Ray Choudhury *et al*. 2008, Refoufi and Esnault 2008, Yang *et al*. 2008).

In our previous study (Poczai *et al*. 2008), we have analysed the subgeneric relationships of section *Solanum*. In the present study, we wanted to investigate the genetic relationships of different infraspecific taxa belonging to section *Solanum* by using morphological and molecular markers and to reveal considerable confusion around the taxonomy and origin of some cultivated species.

From section *Solanum* twelve accessions were used in the analysis and *S. tuberosum* L. was included as an out-group. All plant material was provided by the Georgikon Botanical Garden, University of Pannonia, Hungary. These were: three accessions of *S. nigrum* (Nig 1,2,3), four accessions of *S. scabrum* (Sca 1,2,3, Ssp), one accession of *S. retroflexum* (Ret), *S. villosum* (Vil) and *S. tuberosum* (Tub), and three accessions of edible cultivated cultivars Hei Tien Tsai (Hei), Mrs Bee's nonbitter (Mrs), and Chiquelite (Chi). These cultivars were proposed to be derived from *S. nigrum*.

The following morphological characters were

Received 3 November 2008, *accepted* 2 June 2009.

Abbreviations: RAPD - random amplified polymorphic DNA, ME - mean error, MAE - mean absolute error, MaxAE - maximum absolute error, MSE - mean square error, NJ - Neighbour-Joining.

* Corresponding author; e-mail: guanine@ex1.georgikon.hu

measured: leaf length, leaf width/leaf length ratio, anther length, plant height, number of flowers per inflorescence, length of peduncle, width of calyx lobe, corolla radius, stylus length, number of seeds per berry, total mass of fruit harvested, number of fruits per bunch, seed length, calyces length, diameter of berries, *etc.* (Table 1). Genomic DNA was extracted from approximately 50 mg of young fresh leaves using the procedure of Walbot and Warren (1988). Ten plants of each accession were bulked for DNA extraction as described by Spooner *et al*. (1997), as bulk extractions are able to sample more diversity within a population (Miller and Spooner 1999). Primer pairs were used to maximize the number of unique bands obtained per primer, as suggested by Williams *et al*. (1993). Each reaction was performed twice to verify reproducibility. After a screening procedure 23 primer pairs were selected for the analysis. Amplification reactions were performed in 0.01 cm^3 volumes in 384-well plates containing: 0.005 cm^3 *NFW* (nuclease free water, *Promega,* Madison, USA), approx. 20 ng template DNA, 0.5 μM of each primer, 0.2 mM dNTP (*Fermentas*, Burlington, Canada), 0.001 cm^3 10× PCR buffer (1 mM Tris-HCl, pH 8.8 at 25˚C, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1 % *Triton X-100*) and 0.5 U of *DyNazyme II* (*Finnzymes*, Espoo, Finland) polymerase. All reactions were done in a *MasterCycler ep384* (*Eppendorf*, Hamburg, Germany) with the following conditions: 2 min at 94 °C for initial denaturation, 35 cycles of 30 s denaturation at 94 °C, 1 min annealing at 50 °C (both in the SCoT and ISSR assay), and 2 min extension at 72 °C, followed by a final extension for 5 min at 72 °C. Amplification products were separated on 1.5 % agarose gels (*Promega*, USA) in 0.5× TBE buffer (300 V, 1.5 h) and post-stained with ethidium-bromide. The gels were documented using the *GeneGenius Bio Imaging System* (*Syngene*, Cambridge, UK). Only well-resolved and unambiguous bands were scored, discarding faint bands. It was assumed that fragments of equal length had been amplified from corresponding loci. The amplified fragments were scored as (1) at the presence and (0) at the absence of homologous bands. A distance matrix has been computed, from this binary matrix according to Nei and Li (1979) based on Dice's similarity coefficient (Dice 1945). A dendrogram was constructed by the Neighbour-Joining (NJ) method; the original matrix was bootstrapped 1000 times (in the case of the RAPD data) and 100 times (in the case of morphological data) in order to measure the reliability of the branching patterns, and the quality of the resulting phylogenetic groups. These bootstrap values are shown at the nodes of the dendrograms as percentages. The program *TREECON* (*version 1.3b*, Van de Peer and Wachter 1994) was used for the calculation of Nei and Li (1979) distant matrixes, bootstrap analysis and inferring tree topologies with NJ methods. The fit between the initial dissimilarities, and tree distances were tested calculating the mean error (ME), mean absolute error (MAE), maximum absolute error (MaxAE), mean square error (MSE) values and cophenetic correlation coefficient (*r*) (methods by Perrier *et al*. 2003) for both the morphological and molecular data using *DARwin* (Perrier and Jacquemoud-Collet 2006).

All morphological parameters were measured for each individual per accession in both two years. For coding the quantitative data into discrete character states, the gap weighting method of Thiele (1993) was applied, considering the order and distribution of the means for a certain character and converting them to ordered, multistate characters, where the distance between the means is represented by the distance between the ordered character states in the matrix based on the following formula: $X_{new} = n \times [(X - min)/(max - min)]$. Max and min are the maximum and minimum mean values of the character across all species and X is the mean value of the

Table 1. Measured values of the morphological traits for the accessions analysed (d1, d2 - longer and shorter diameters).

Morphological trait	Sca1	Sca2	Sca3	Ssp	Ret	Hei	Mrs	Chi	Nig1	Nig2	Nig3	Vil
Plant height [cm]	183	192	86	85	68	72	75	78	65	63	63	48
Leaf length [cm]	13	14	15	14	4.5	4.8	4.3	4.5	5	6	6.2	4.5
Ratio: leaf width/leaf length	1.3	1.4	1.4	1.3	1.5	1.6	1.5	1.5	1.53	1.8	1.9	1.6
Number of flowers per infloresence	8	8	10	10	7	7	6	7	6	6	6	6
Length of peduncule [mm]	4.5	5	5	4.8	12	13	12.8	13	16	18	18	8
Width of calyx lobe [mm]	3	3.5	3	3.2	2.1	2.2	2.1	2.1	1.3	1.4	1.4	2
Calyces length [mm]	3	3	4	4	3	2.5	2.5	2.5	2.3	2.4	2.35	2
Corolla radius [mm]	7	7	9	9	5	5.5	5	6	6	6	6	5
Anther length [mm]	3	2.9	3	2.8	1.5	1.6	1.6	1.7	2.5	2.3	2.2	2
Stylus length [mm]	4	3.9	3.8	3.7	3.4	3.3	3.2	3.4	3	3.1	3	3
Total mass of fruit harvested [kg]	1.85	1.75	6.69	7.35	0.12	0.42	0.33	0.21	0.13	0.17	0.18	0.11
Fruit per bunch	6	6	8	8	4	5	4	6	5	5	5	4
Diameter of berries [mm] d1	10.9	10.5	10.6	10.4	8	8.3	7.8	8.1	8.4	8.3	8.3	7.3
d2	9.8	9.8	9.1	9.8	7.8	8.3	8	7.8	8.4	8.2	8.2	7.3
Number of seeds per berry	120	130	110	120	25	20	20	23	65	57	50	35
Seeds length [mm]	2.1	2	2.2	2.2	1.65	1.5	1.5	1.4	1.8	1.8	1.8	1.8

current taxon and n is the number of allowed character states. For the calculation of gap weights the program *MorphoCode* (Schols *et al*. 2004) was used. The gap weights were transformed into 1, 0 character matrix, analyzed with distance methods, based on the formula of Nei and Li (1979), then clustering was preformed using NJ method (Fig. 1*A*).

The 23 RAPD primer pairs used in the analysis produced 252 reliable amplified products, from which 210 were polymorphic and used to construct the dendrogram (Fig. 1*B*). We found morphological differences between the accessions of *S. scabrum* analyzed here but the clustering according to these differences can not be detected in the molecular analysis. The accession Sca1 and Sca2 were 1.2 - 1.5 m tall and produced less berries than the accession Sca3, which was 0.5 - 0.7 m tall and produced much more berries than the others. The *Solanum* sp. accession was identified as *S. scabrum* but it resembled more to Sca3 in habit and in berry yield. Olet *et al*. (2005, 2006) also found morphological differences between accessions of *S. scabrum*. These studies concluded that two subspecies of *S. scabrum* exist, the one subsp. *scabrum* with 1.5 - 2.0 m tall, characterized by a large number of leaves and small number of fruits, and subsp. *laevis* < 1 m tall with lower number of leaves and large number of fruits. *S. scabrum* subsp. *laevis* represents the wild form of *S. scabrum*, previously incorrectly thought to be *S. nigrum* in Uganda (Olet *et al*. 2006). Although, these subspecies exist in the case of *S. scabrum* there are shorter cultivated forms (Sca3 and Ssp) which can be identified as subsp. *scabrum* too. However, the short accessions analyzed here are more similar to subsp. *laevis* in their habit; they differ from this described wild form in a number of characteristics: angled, winged stems with prominent ridge teeth, posture peduncles at right angle of the stem, broader range in flower and anther colour, persistent fruits. According to Olet *et al*. (2006) Sca1 and Sca2 can be identified as subsp. *scabrum*, but they lack of some characters (small fruit, reflexed pedicel). These accessions represent different cultivars of *S. scabrum.* These differences could be attributed to breeding selection for different plant types. *S. scabrum* is the most intensively cultivated leafy vegetable in section *Solanum*, and as such has undergone selection by farmers (Manoko *et al*. 2008). Presumably in this case, leaf size, stem wing and stem branching characters were the basis for selection. In the field experiments, the short forms produced more fruits than the taller ones. Most likely, the short types were selected for the number of fruits rather then leaf production, because the fruits are used as a source of dye, ink and food, respectively. Cultivars Hei Tien Tsai, Mrs. Bee's Nonbitter and Chiquelite can be bought in commercial trade, and believed to be cultivars of *S. nigrum* and in other studies of *S. retroflexum*. These cultivars are similar to wild *S. nigrum*, but the berries had opaque cuticles and on the white flowers, in the petals outer surface, a distinct purple vein can be observed. These parameters (missing in all other related species) refer to that they could be originated from *S. retroflexum*. Chiquelite and the other two cultivars are much like *S. retroflexum*, but the berries are larger. The main distinction is in the quality and the quantity of the berries produced by these cultivars. However, the RAPD analysis places them together in a cluster with *S. retroflexum* denoting that they must be cultivated forms, hypothetically selected from this species for their better quality and quantity of berries.

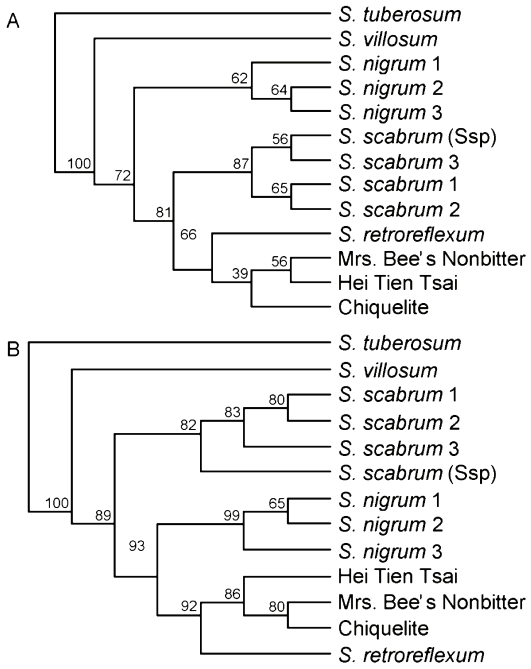

Fig. 1. *A* - The dendrogram created from the morphological characters based on the Nei-Li (1979) index using the Neighbour-Joining method with 100 bootstrap replicates. Fit criteria: Cophenetic r: 0.8765, ME: -0.0121, MAE: 0.0201, MaxAE: 0.0422, MSE: 0.0008. *B* - dendrogram from the RAPD data. Fit criteria: cophenetic r: 0.8524, ME: -0.0107, MAE: 0.0182, MAE: 0.0182, MaxAE: 0.0577, MSE: 0.0005.

During the field experiments the three accessions of *S. nigrum* showed some morphological distinction. Two *S. nigrum* accessions (Nig2, Nig3) were moderately pubescent, with glandular-headed multicellular hairs; they were identified as subsp. *nigrum*. One *S. nigrum* accession (Nig1) was villous, with occasionally appressed, glandular-headed multicellular hairs identified as subsp. *schultesii*. The clustering of *S. nigrum* accessions revealed from the molecular data did not support the separation of these subspecies. It is very difficult to detect molecular diversity within different subspecies and among cultivated forms. Although, the morphology of cultivated species is different from its wild ancestor and displays much greater diversity, in isozyme, DNA or other molecular markers the diversity remains almost the same (Lester and Daunay 2003). It can be concluded that hair characteristics have low taxonomic value at the subspecies level in section *Solanum*, confirming the results of Manoko *et al*. (2008),

Dehmer (2001) and Dehmer and Hammer (2004). The present study shows that *S. nigrum*, *S. scabrum* are closely related species, although this is supported by only moderate bootstrap value, it confirms our previous conclusions (Poczai *et al*. 2008). We also found that there is close relationship between *S. retroflexum* and *S. nigrum*, which can be affirmed by the successful crosses by Ganapathi and Rao (1986) made between *S. nigrum* (2n = 6x = 72) and *S. retroflexum* (2n = 4x = 48) as female parent. In summary, these results suggest genomic homology between these two species. Because, this relationship is weekly supported from bootstrap value it need to be confirmed by further studies.

References

Cordeiro, A.I., Sanchez-Sevilla, J.F., Alvarez-Tinaut, M.C., Gomez-Jimenez, M.C.: Genetic diversity assessment in Portugal accessions of *Olea europea* by RAPD markers. - Biol. Plant. **52**: 642-647, 2008.

Dehmer, K.J.: Conclusions on the taxonomy of the *Solanum nigrum* complex by molecular analysis of IPK germplasm accessions. - In: Van de Berg, R.G., Barendse, G.W.M., Van der Weerden, G.M., Mariani, C. (ed.): *Solanaceae* V: Advances in Taxonomy and Utilization. Pp. 85-96. Nijmegen University Press, Nijmegen, 2001.

Dehmer, K.J., Hammer, K.: Taxonomic status and geographical provenance of germplasm accessions in the *Solanum nigrum* L. complex: AFLP data. - Genet. Res. Crop Evol. **51**: 551-558, 2004.

Dice, L.R.: Measuring of amount of ecological association between species. - Ecology **26**: 297-302, 1945.

Edmonds, J.M.: A synopsis of the taxonomy of *Solanum* sect. *Solanum* (*Maurella*) in South America. - Kew. Bull. **27**: 79-114, 1972.

Edmonds, J.M., Chweya, J.A. (ed.): Black Nightshades *Solanum nigrum* L. and Related Species. Promoting the Conservation and Use of Underutilized and Neglected Crops. - Institute of Plant Genetics and Crop Plant Research, Gatersleben and International Plant Genetic Resources Institute, Rome 1997.

Ganapathi, A., Rao, G.R.: The crossability and genetic relationship between *Solanum retroflexum* Dun. and *S. nigrum* L. - Cytologia **51**: 757-62, 1986.

Lester, R.N. Daunay, M.-C.: Diversity of African vegetable *Solanum* and its implications for a better understanding of plant domestication. - In: Knüpffer, H., Ochsmann, J. (ed.): Rudolf Mansfeld and Plant Genetic Resources. Proceedings of a Symposium Dedicated to the 100th Birthday of Rudolf Mansfeld. Pp. 137-152, ZADI, Bonn 2003.

Manoko, M.L.K., Van den Berg, R.G., Feron, R.M.C., Van der Weerden, G.M., Mariani, C.: Genetic diversity of the African hexaploid species *Solanum scabrum* Mill. and *Solanum nigrum* L. (Solanaceae). - Genet. Res. Crop Evol. **55**: 409-418, 2008.

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

Nei, M., Li, W.H.: Mathematical model for studying genetic variation in terms of restriction endonucleases. - Proc. nat. Acad. Sci. USA **76**: 5269-5273, 1979.

Olet, E.A., Huen, M., Lye, K.A.: African crop or poisonous nightshade; the enigma of poisonous or edible black nightshades solved. - Afr. J. Ecol. **43**: 158-161, 2005.

Olet, E.A., Heun, M, Lye, K.A.: A new subspecies of *Solanum scabrum* Miller Found in Uganda. - Novon **16**: 508-511, 2006.

Perrier, X., Flori, A., Bonnot, F.: Data analysis methods. - In: Hamon, P., Seguin, M., Perrier, X., Glaszmann, J.C. (ed.): Genetic Diversity of Cultivated Tropical Plants. Pp. 43 - 76. Enfield, Science Publishers, Montpellier 2003.

Perrier, X., Jacquemoud-Collet, J.P.: DARwin software http://darwin.cirad.fr/darwin, 2006.

Poczai, P., Taller, J., Szabó, I.: Analysis of phylogenetic relationships in the genus *Solanum* (Solanaceae) as revaled by RAPD markers. - Plant. Syst. Evol. **275**: 59-67, 2008.

Ray Choudhury, P., Singh, I.P., George, B., Verma, A.K., Singh, N.P.: Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. - Biol. Plant. **52**: 648-653, 2008.

Refoufi, A., Esnault, M.A.: Population genetic diversity in the polyploid complex of the wheatgrasses using isoenzyme and RAPD data. - Biol. Plant. **52**: 543-547, 2008.

Schols, P., D'hondt, C., Geuten, K., Merckx, V., Janssens, S., Smets, E.: MorphoCode: coding quantitative data for phylogenetic analysis. - Phyloinformatics **4**:1-4, 2004.

Spooner, D.M., Ugarte, M.L., Skroch, P.W.: Species boundaries and interrelationships of two closely related sympatric diploid wild potato species, *Solanum astleyi* and *S. boliviense*, based on RAPDs. - Theor. appl. Genet. **95**: 764-771, 1997.

Thiele, K.: The holy grail of the perfect character: the cladistic treatment of morphometric data. - Cladistics **9**: 275-304, 1993.

Van de Peer, Y., De Wachter, Y.: TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environments. - Commun. appl. Biosci. **10**:569-570, 1994.

Walbot, V., Warren, C.: Regulation of Mu element copy number in maize lines with an active or inactive Mutator transposable element system. - Mol. gen. Genet. **211**: 27-34, 1988.

Welsh, J., McClelland, M.: Fingerprinting genomes using PCR with arbitrary primers. - Nucl. Acids Res. **18**: 7213-7218, 1990.

Williams, J.G.K, Hanafey, M.K, Rafalski, J.A., Tingey, S.V.: Genetic analysis using random amplified polymorphic DNA markers. - Methods Enzymol. **218**: 704-740, 1993.

Willams, J.K.F., Kubelik, A.R., Livak, K.G., Rafalski, J.A., Tingey, S.V.: DNA polymorphism amplified by arbitrary primers are useful as genetic markers. - Nucleic. Acids. Res. **18**, 6531-6535, 1990.

Yang, R.W., Zhou, Y.H., Ding, C.B., Zheng, Y.L., Zhang, L.: Relationships among *Leymus* species assessed by RAPD markers. - Biol. Plant. **52**: 237-241, 2008.