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Development of intron targeting (IT) markers for potato and cross-species amplification in *Solanum nigrum* (Solanaceae)[1]

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- *Premise of the study*: Intron Targeting (IT) primers were developed for potato using expressed sequence tags (EST) and NCBI database records to study genetic diversity.
- *Methods and Results*: Twenty-nine polymorphic intron targeting (IT) markers were generated and characterized from 30 samples of potato and 22 samples of *Solanum nigrum* to detect polymorphism. The number of alleles (*A*) per locus ranged from 2 to 7 in the analyzed populations, and the observed heterozygosity (H_O) and expected heterozygosity (H_E) from 0 to 0.833 and 0.750, respectively. All of the primers also amplified in the related species *S. nigrum*.
- *Conclusions*: The developed markers will provide valuable tools for genetic diversity analysis, genetic mapping, and marker-assisted breeding of potato and related *Solanum* species.

Key words: genetic diversity; intron targeting; potato; *Solanum nigrum*; transferability.

The Solanaceae family includes a wide range of important crops, such as potato (*S. tuberosum* L.), eggplant (*S. melongena* L.), and tomato (*S. lycopersicum* L.), and is therefore a valuable source of vegetables as well as ornamental and medicinal plants. Due to their economic value, much effort is currently being invested in developing molecular tools to facilitate the improvement of these crops (Datema et al., 2008). Therefore, the development of more reliable and efficient molecular markers would be valuable in numerous areas of plant genetic analysis and breeding research (Gong et al., 2010). In the case of solanaceous plants, the relatively conserved nature of the gene structures makes it possible to use intron sequences as molecular markers. This high degree of conservation may be due to Solanaceae genomes having undergone relatively few genomic rearrangements and duplications and therefore having similar gene content and order (Mueller et al., 2005).

The close proximity of introns to exons makes them especially well suited for linkage disequilibrium studies that promise to add a powerful new dimension to the understanding and improvement of crop gene pools. One effective strategy for exploiting this information and to generating gene-specific codominant markers is a method called Intron Targeting (IT). This method was first applied by Choi et al. (2004) to construct a linkage map of the legume *Medicago truncatula* Gaertn. The basic principle of IT relies on the fact that intron sequences are generally less conserved than exons, and they display polymorphism due to length and/or nucleotide variation in their alleles. Primers designed to anneal in conserved exons to amplify across introns can reveal length polymorphism in the targeted intron. Such primers can be designed for potato using the available sequences of known genes or by exploiting expressed sequence tag (EST) records from the NCBI database.

In this paper, we report the isolation and characterization of IT primers in potato and demonstrate their potential for breeding studies in different individuals and their transferability to a related species (*Solanum nigrum* L.).

METHODS AND RESULTS

The intron targeting primer design procedure used the blastn search mode of the blastall program from NCBI (http://www.ncbi.nlm.nih.gov/) to find marker candidates with the E-value set to 10^{-20} (up to August 2010). In the first step, sequences of 340 potato genes known to play a role in resistance mechanisms (e.g., catalase) and metabolic pathways (e.g., sucrose-synthesis) were screened to find primer candidates. We selected single- and low-copy genes as primary targets. In these cases, where the exon-intron structures of the genes were known, flanking primers were designed for the exons to amplify across the intercalated introns. In the second step, ~270 *S. tuberosum* EST sequences with unidentified exon-intron structures were analyzed as follows: potato EST sequences were

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Table 1. Characterization of 29 intron targeting primers in potato and *Solanum nigrum*. Shown for each primer are the forward and reverse sequences, size of the original fragment (bp), annealing temperature (T_a), reported putative function acquired by searching the NCBI database with blastx with expected value < 10^{-20}, allele number and size range in potato and cross-species amplification products in *S. nigrum* L., and GenBank accession numbers. All values are based on 30 samples of the F_1 potato population and 22 samples representing natural populations from Hungary (Keszthely and Szolnok), and Croatia (Pula).

Primer	Sequences (5′–3′)	Size	T_a	Putative function	No. of alleles and size ranges	GenBank Accession No.
Adk-242	F: TGCTTTTAAAGTCGCACCA R: TTATATCCGGAGCATGTCCAC	242	55	Adenylate kinase gene	4 (112–242)	AJ276864.1
Adk-795	F: GCATGGTTCTTTCCTTCCTG R: TGGGCCAGGAATTTTGCTATC	795	54	Adenylate kinase gene	4 (805–385)	AJ276864.1
Cat-232	F: AGGAGGCGGATCTAGCCTTA R: TGTCAAGAAAGGGGTGTCGT	232	55	Potato catalase gene	2 (210–232)	Z37106.1
Cat-260	F: TGACAACAAATGCTGGTGGT R: AAGGTGGCAAGCTTCTCAAT	260	53	Potato catalase gene	3 (260–310)	Z37106.2
GPSS-275	F: CTTTTGATGGGGGCAGATTA R: CAGCTTCCTGTCAGCATCAG	275	53	ADP-glucose pyrophosphorylase small-subunit gene	3 (275–125)	L36648.1
GPSS-943	F: TCATTGGTGAAGGTTGTGTGA R. ACCACGGAATGGTGAATCTT	943	53	ADP-glucose pyrophosphorylase small-subunit gene	7 (1043–150)	L36648.2
INHWI-509	F: TGAAACTCTCTTGGCACGAA R: TTCTGGCCACCTTTGTTTTC	509	54	Wound-inducible proteinase inhibitor I gene	2 (509–205)	M17108.1
INHWI-545	F: TCAAGTTTGCTCACATCTTGT R: TCGTGCCAAGAGAGTTTCAA	545	54	Wound-inducible proteinase inhibitor I gene	4 (685–425)	M17108.1
InvG-220	F: ACAGGAATCACACCTGCACA R: TCTGCACCCTTAAGTCCACA	220	54	Invertase gene	3 (280–150)	AJ133765.1
InvG-262	F: TTCTCATGTGCTCAGATGCT R: GAGGGCTTGTACATTGACTTCA	262	53	Invertase gene	3 (182–262)	AJ133765.1
InvG-393	F: TGGTTACCATTTTCAGCCAGA R: CCATTGAAATACATTGGTGCTG	393	55	Invertase gene	3 (320–393)	AJ133765.2
LBr-G9	F: TGGATCTGAAGATGGCACTG R: TTGCTCTCAAATCCCACACA	652	55	Transducin family protein	3 (1100–652)	CO267873.1
LBr-4D6	F: GAGTATTCATTCGGGCTTGG R: CTCTACCGACCCGTAGCAAG	196	54	Plastidic ATP/ADP-transporter protein	4 (597–196)	CO267884.1
PatI-433	F: TCAAGCTCGTCATTCACAAAA R: TCAGACGCATCATCCATTTC	433	54	Potato patatin class I. gene	3 (510–285)	M18880
PatI-838	F: CGAACATGGCCCTCATATTT R: TGCACACGAGTTTCTCCAAG	838	53	Potato patatin class I. gene	5 (938–145)	M18880
Pat-In3	F: CAGAAAGTTGCCATCTCAAGC R: GCTGCTGCTGTGGAATAACA	581	53	Potato patatin gene Intron 3	4 (595–220)	X03932.1
Poni1a-718	F: GGTGGTGGTGGTAGCTCAGT R: CCCARRGGCATTAACACTCC	718	55	Potato membrane protein	3 (850–718)	AJ309301.1
Poni1a-442	F: TTTGCCTCGGAACTCTTCAG R: GCCTCAGAAGCAAAGCAAAT	442	54	Potato membrane protein	3 (510–295)	AJ309301.2
Ry1-In3	F: AATGCAGAAGGTGCAACGAT R: TGGGCGAAATTTCATTAACA	199	54	Ry1 resistance gene-like function protein intron 3	2 (450–199)	AJ300266
Ry1-In4	F: TCGAAAAATTCTCAAATGCAAA R: GATTGCTTCGATAGCCTTGG	477	55	Ry1 resistance gene-like function protein intron 4	6 (125–590)	AJ300267
Ry1-In5	F: CCAGCAGAGTTCACTGTTTCA R: GTTGCACAGCTGCTGAGAT	481	54	Ry1 resistance gene-like function protein intron 5	2 (481–501)	AJ300268
Ry1-In6	F: GCTCTCGTCTCCACTTCTGC R: AACTCCTCAGCAACTGCACA	741	56	Ry1 resistance gene-like function protein intron 5	7 (751–110)	AJ300269
S2-317	F: CGGCCAGTTACAATTCTGC R: AATCCAGTGGTGGTCCAGAG	202	55	Self-incompatibility locus linked stylar Rnase gene	6 (455–202)	X62727
STAC1-226	F: GTCTTCCCGCTTTCAAAGAT R: TCAGCAAGGCAAAACATGAG	226	53	1-Aminocyclo- propane-1-carboxylate synthase gene	4 (135–295)	Z27235.1
Suc16-321	F: TCACCGCAATGAGATACTGC R: TATCCCTTTTCCGTGGCTTT	321	54	Sucrose synthase gene	5 (270–385)	U24087.1
Suc16-349	F: TGACGTTGAGAATGACGAACA R: CCAACCTTGCCATTGTGAAT	349	55	Sucrose synthase gene	4 (605–275)	U24087.1
UBQ-627	F: TCTCAATTGCCTTCAATTTCTC R: TCCGGTGAGAGTTTTCACAA	627	53	Polyubiquitin gene	6 (715–520)	U26831.1
Ure-242	F: TGCTTTTAAAGGTCGCACCA R: TTATATCCGGAGCATGTCCAC	242	56	Ure gene for Urease	2 (242–257)	AJ276865.1
Ure-271	F: GAGCAGCCACGAGATTTGA R: CACAAATCAATGCCCAAGC	271	55	Ure gene for Urease	2 (271–451)	AJ276865.2

compared with known genes of tomato or *Arabidopsis thaliana* in TAIR, The Arabidopsis Information Resource, using a set of algorithms implemented in the SPLIGN software tool (Kapustin et al., 2008), was enlisted to find the putative exon and intron sequences. This was achieved by aligning the spliced transcript sequence with its parent genomic sequence to identify correct exon-intron junctions. We preferred a product size within the range of 200–1200 bp and used filtering parameters accordingly to select suitable exons. After locating the precise positions of the introns, the joined sequences of the flanking

exons were passed to the primer designer program PRIMER 3 (http://frodo.wi.mit.edu/primer3/input.htm). Finally, a total of 56 oligonucleotide primer pairs were designed.

Thirty individuals of a potato F_1 segregating population originating from a cross between the cultivar White Lady and breeding line S440 were used to demonstrate the utility of the designed primers for breeding studies. In addition,

TABLE 2. Results of initial primer screening in potato (*S. tuberosum*) and black nightshade (*S. nigrum*). Number of alleles (*A*), observed heterozygosity (H_o), and expected heterozygosity (H_E) for each population are shown.

	F1 potato ($N = 30$)			Keszthely ($N = 10$)		
Locus	A	H_o	H_E	A	H_o	H_E
Adk-242	4	0.364	0.723	4	0.500	0.708
Adk-795	4	0.545	0.636	4	0.667	0.583
Cat-232	2	0.500	0.375	2	0.833	0.486
Cat-260	3	0.273	0.455	3	0.273	0.247
GPSS-275	3	0.636	0.498	3	0.636	0.749
GPSS-943	4	0.667	0.597	3	0.455	0.567
INHWI-509	2	0.333	0.444	2	0.167	0.153
INHWI-545	4	0.667	0.597	4	0.545	0.636
InvG-220	3	0.455	0.567	3	0.428	0.562
InvG-262	3	0.451	0.684	3	0.500	0.640
InvG-393	3	0.429	0.571	3	0.400	0.480
LBr-G9	3	0.636	0.749	3	0.273	0.455
LBr-4D6	4	0.667	0.583	4	0.455	0.567
PatI-433	3	0.818	0.775	3	0.714	0.670
PatI-838	3	0.833	0.569	2	0.833	0.486
Pat-In3	4	0.667	0.583	4	0.714	0.626
Poni1a-718	3	0.727	0.589	3	0.364	0.450
Poni1a-442	3	0.714	0.670	3	0.455	0.526
Ry1-In3	2	0.091	0.091	2	0.182	0.314
Ry1-In4	3	0.273	0.247	3	0.636	0.498
Ry1-In5	2	0.455	0.567	2	0.285	0.498
Ry1-In6	3	0.364	0.671	4	0.667	0.750
S2-317	4	0.667	0.625	2	0.285	0.244
STAC1-226	4	0.667	0.625	4	0.500	0.708
Suc16-321	2	0.143	0.337	3	0.333	0.653
Suc16-349	4	0.500	0.708	4	0.667	0.583
UBQ-627	3	0.285	0.255	3	0.500	0.568
Ure-242	2	0.200	0.500	2	0.200	0.180
Ure-271	2	0.300	0.420	2	0.285	0.244

	Szolnok ($N = 7$)			Pula ($N = 5$)		
Locus	A	H_o	H_E	A	H_o	H_E
Adk-242	4	0.545	0.636	4	0.545	0.636
Adk-795	4	0.667	0.583	4	0.545	0.636
Cat-232	2	0.500	0.375	2	0.833	0.486
Cat-260	3	0.200	0.500	3	0.200	0.500
GPSS-275	3	0.429	0.571	3	0.636	0.749
GPSS-943	3	0.451	0.684	3	0.429	0.571
INHWI-509	2	0.333	0.444	2	0.333	0.444
INHWI-545	4	0.667	0.625	4	0.500	0.708
InvG-220	3	0.400	0.340	3	0.100	0.255
InvG-262	3	0.428	0.540	3	0.200	0.180
InvG-393	3	0.285	0.255	3	0.425	0.532
LBr-G9	3	0.455	0.526	3	0.167	0.292
LBr-4D6	4	0.667	0.750	4	0.333	0.681
PatI-433	3	0.833	0.569	3	0.167	0.486
PatI-838	2	0.833	0.486	2	0.833	0.486
Pat-In3	4	0.545	0.636	4	0.500	0.542
Poni1a-718	3	0.636	0.749	3	0.116	0.278
Poni1a-442	3	0.451	0.684	3	0	0
Ry1-In3	2	0.100	0.255	2	0	0
Ry1-In4	3	0.451	0.684	3	0.333	0.500
Ry1-In5	2	0.273	0.247	2	0.500	0.611
Ry1-In6	4	0.667	0.583	4	0.284	0.791
S2-317	2	0.200	0.180	2	0.273	0.247
STAC1-226	4	0.636	0.749	4	0.364	0.723
Suc16-321	3	0.273	0.247	3	0.182	0.274
Suc16-349	4	0.667	0.583	4	0.667	0.583
UBQ-627	3	0.285	0.255	3	0.182	0.512
Ure-242	2	0.273	0.247	2	0.091	0.091
Ure-271	2	0.285	0.255	2	0	0

to evaluate the transferability of the IT primers 22 individuals of *Solanum nigrum* were employed to perform cross-species amplification. These individuals represented three natural populations located in Hungary (Keszthely, Szolnok) and Croatia (Pula). Voucher specimens were deposited in the University of Pannonia, Keszthely, Hungary (Appendix 1). Genomic DNA from each individual was extracted from 50 mg of young fresh leaves according to the protocol of Walbot and Warren (1988). PCR was carried out in 20-μL volume containing 20 ng of genomic DNA, 0.2 mM of dNTPs, 2 μL 1×PCR buffer, 20 pmol of each primer, and 0.4 Unit DynaZyme DNA polymerase (Finnzymes, Espoo, Finland). PCR was performed on a RoboCycler 96 (Stratagene, La Jolla, California, USA) with an initial 3 min of denaturation at 94°C, followed by 35 cycles of 94°C for 1 min, appropriate annealing temperature (Table 1) for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min. The PCR products were separated on 1.5% agarose gel (Promega, Madison, Wisconsin, USA) in 0.5×TBE (Tris-HCl-Boric acid-EDTA) buffer (220 V; 1.5 h) and stained with ethidium bromide. In many cases where the polymorphisms manifested as small size difference, the PCR products were separated on 2.5% Metaphor agarose gels (Cambrex, East Rutherford, New Jersey, USA). The genetic statistics based on the potato F_1 population of 30 individuals and on the three *Solanum nigrum* populations of 22 individuals were calculated using the program POPGENE (version 1.31; Yeh et al., 1999) including the number of alleles (*A*), observed heterozygosity (H_O) and expected heterozygosity (H_E). The results indicated that 29 loci showed clear polymorphic patterns (Table 1) while others could not amplified. The number of alleles ranged from 2 to 7 in the analyzed populations. The observed heterozygosity ranged from 0 to 0.833, and the expected heterozygosity ranged from 0 to 0.750 (Table 2). The results from cross-species amplification in *Solanum nigrum* populations revealed that all 29 primers generated specific and polymorphic products. These results demonstrate the potential of these newly developed IT markers for further genetic analysis in potato and related *Solanum* species.

CONCLUSIONS

The sequence-based IT markers developed here may have wider application within different populations of potato and related species in the genus *Solanum.* These primers may potentially useful in applied research fields such as solanaceous crop breeding programs, as further primers could easily be designed based on the methods described here. Due to their polymorphism and close association with functional genes, these markers will also be useful in studies of genetic diversity in natural populations and germplasm collections, as well as for genetic mapping of potato and related species. In conclusion, the IT method is a simple and efficient marker system that can be adapted for different purposes in plant molecular genetics. It is simple to use due to being agarose-based and produces codominant markers for potato research and breeding as well as for genetic diversity analysis in the genus *Solanum.*

LITERATURE CITED

Choi, H. K., D. Kim, T. Uhm, E. Limpens, H. Lim, J.-H. Mun, P. Kalo, et al. 2004. A sequence-based genetic map of *Medicago trunculata* and comparison of marker colinearity with *M. sativa. Genetics* 166: 1463–1502.

Datema, E., L. A. Mueller, R. Buels, J. J. Giovannoni, R. G. F. Visser, W. J. Stiekema, and R. C. H. J. van Ham. 2008. Comparative BAC end sequence analysis of tomato and potato reveals overrepresentation of specific gene families in potato. *BMC Plant Biology* 8: 34.

Gong, Y. M., S. C. Xu, W. H. Mao, Q. Z. Hu, G. W. Zhang, J. Ding, and Z. Y. Li. 2010. Generation and characterization of 11 novel EST derived microsatellites from *Vicia faba* (Fabaceae). *American Journal of Botany* 97: e69–e71.

Kapustin, Y., A. Souvorov, T. Tatusova, and D. Lipman. 2008. SPLIGN: Algorithms for computing spliced alignments with identification of paralogs. *Biology Direct* 3: 20.

Mueller, L. A., T. H. Solo, N. Taylor, B. Skwarecki, R. Buels, J. Binns, C. Lin, et al. 2005. The SOL genomics network: A comparative resource for Solanaceae biology and beyond. *Plant Physiology* 138: 1310–1317.

Walbot, V., and C. Warren. 1988. Regulation of Mu element copy number in maize lines with an active or inactive mutator transposable element system. *Molecular & General Genetics* 211: 27–34.

Yeh, F. C., R. C. Yand, and T. Boyle. 1999. POPGENE (Version 1.31): Microsoft Window-bases freeware for population genetic analysis, University of Alberta and the Centre for International Forestry Research. http://www.ualberta.ca/~fyeh/index.htm.

Appendix 1. Representative voucher specimens deposited at the Georgikon Faculty, University of Pannonia (GF-UP).

Taxon — Voucher specimens and locality.

Solanum nigrum — Hungary, Keszthely — UPG0013.1,UPG0013.2, UPG0013.3, UPG0013.4, UPG0013.5, UPG0013.6, UPG0013.6, UPG0013.7, UPG0013.8, UPG0013.9, UPG0013.10;
Hungary, Szolnok — UPG0015.1, UPG0015.2, UPG0015.3, UPG0015.4, UPG0015.5, UPG0015.6, UPG0015.7;
Croatia, Pula — UPG0016.1, UPG0016.2, UPG0016.3, UPG0016.4, UPG0016.5

Solanum tuberosum — PRC0WL, PRC0S440, PRC0B13, PRC00F6, PRC00H9, PRC0453, PRC0086, PRC0464, PRC0460, PRC0458, PRC0461, PRC0446, PRC0079, PRC0070, PRC0085, PRC0054, PRC00A4, PRC0465, PRC0454, PRC0G11, PRC0456, PRC0001, PRC0457, PRC0455, PRC0450, PRC0008, PRC0447, PRC0445, PRC0021, PRC0451