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LEAFY intron 2-based markers of wild Solanum genomes for introgression breeding

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SUMMARY
SCAR (sequence characterized amplified region) markers developed from polymorphic sequences of FLORICAULA/LEAFY intron 2 (FLint2) discern genomes A, B and D and subgenomes A1-A3 in tuber-bearing *Solanum* species. Screening 125 *Solanum* accessions representing 26 species from six series of section Petota produced the evidence mostly consistent with the established genome classification in wild *Solanum* species (Hawkes, 1990; Matsubayashi, 1991). Potato hybrids with several *Solanum* species listed in their pedigrees mostly retained FLint2 markers of introgressed wild germplasms.

KEYWORDS
FLORICAULA/LEAFY, *Solanum* genomes, taxonomy of section Petota, FLint2-derived markers

INTRODUCTION

Many wild tuber-bearing *Solanum* species (section Petota) are important sources of late blight resistance transferred to the cultivated potato by sexual and somatic hybridization or by genetic engineering, and DNA markers considerably facilitate introgression of resistance genes (Simko *et al.*, 2007). In particular, these markers would assist monitoring the transfer of alien germplasm and its further loss from the hybrids as the latter go through backcrosses and are finally maintained as registered potato varieties.

FLORICAULA/LEAFY is a nuclear-encoded homeotic gene in control of several key morphogenetic processes, including floral transition (Moyroud *et al.*, 2010). The gene contains two introns, and the polymorphisms of intron 2 (FLint2) are widely employed by plant molecular taxonomists (for recent references see Peng *et al.*, 2010; Zheng *et al.*, 2011). Smith and Baum (2006) were first to use FLint2 for the systematics of Solanaceae (Physaleae). Earlier we described manifest inter- and intraspecific polymorphisms of FLint2 in *S. demissum* and *S. tuberosum ssp. tuberosum* (Drobyazina and Khavkin, 2007). Here we report a set of FLint2-derived SCAR markers, which discriminate between genomes A, B and D in the section Petota.

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MATERIALS AND METHODS

Seeds and microtubers of wild *Solanum* species were obtained from the Vavilov Institute of Plant Industry, Russia (VIR), The Centre for Genetic Resources, the Netherlands (CGN), and NRSP-6 Potato Genebank, USA (PI), and potato tubers, from VIR and the Institute of Potato Husbandry, Russia. Genomic DNA was isolated from green leaves of individual plants by a modified STAB method (Doyle and Doyle, 1987) or with AxyPrep™ Multisource Genomic DNA Miniprep kit (www.axygenbio.com/products). Conservative primers for gene regions within exons 2 and 3 (Fig. 1) described previously (Drobyazina and Khavkin, 2007) were used to amplify FLint2 sequences. Standard protocols were employed for PCR amplification and cloning gene fragments. The programs BLAST 2.2.26 (blast.ncbi.nlm.nih.gov/Blast.cgi) and WU-BLAST (www.ebi.ac.uk/Tools/sss/wublast/nucleotide.html) were used for mining databases. For phylogenetic analysis, we used the matrix of pair distances and the Neighbor Joining clustering algorithm with the MEGA5 software (Tamura *et al.*, 2011).

RESULTS AND DISCUSSION

First, we obtained 32 FLint2 sequences from nine *Solanum* species representing five series of the section Petota (Table 1). The intraspecific homology varied from 79 to 100 %, with most variable FLint2 sequences found in polyploid *S. demissum* and *S. stoloniferum* and practically identical sequences, in such diploid species as *S. verrucosum* and *S. bulbocastanum*. The phylogenetic analysis separated the cloned FLint2 sequences into several discernable clusters presumably corresponding to different *Solanum* genomes and subgenomes.

Figure 1. Primers (LFYex2-F/LFYex3-R) flanking FLORICAULA/LEAFY intron 2 (FLint2) against the prototype gene sequence (Genbank accession number EU371047).

Ploidy	Species	Genbank accession numbers	Homology, %
Diploid	*S. bulbocastanum*	JQ617270, JQ617271	94
	S. cardiophyllum	JQ617268, JQ617269, JQ617276	89-100
	S. ehrenbergii	JQ617264, JQ617265, JQ617272, JQ617273	95-98
	S. microdontum	JQ617256- JQ617261	100
	S. pinnatisectum	*	99
	S. verrucosum	JQ617266, JQ617267, JQ617274, JQ617275	97-100
Polyploid	*S. stoloniferum* (4n)	JQ617262, JQ617263, JQ617277-JQ617279	80-97
	S. tuberosum ssp. *tuberosum* (4n)	DQ266895, DQ256076, DQ285574-DQ285576,	82-93
	S. demissum (6n)	DQ256075, DQ266893, DQ266894	79-89

* Two sequences under registration

Table 1. The intraspecific homology of FLint2 sequences.

To verify FLint2 markers of *Solanum* genomes, we screened 125 accessions representing 26 species from six series of section Petota (Table 2). The data thus obtained are mostly consistent with the established genome classification in wild *Solanum* species section Petota (Hawkes, 1990; Matsubayashi, 1991).

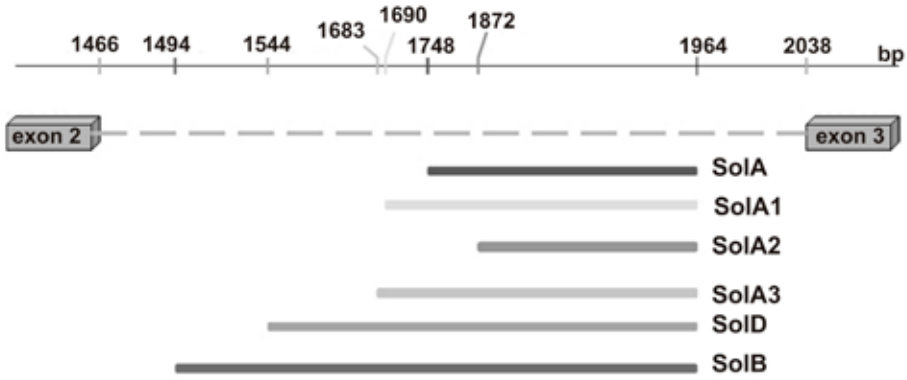

Figure 2. Markers of Solanum genomes and subgenomes derived from FLint2 sequences. Marker positions are shown regarding the prototype gene EU371047.

The genome A cluster included FLint2 sequences from *S. verrucosum* (A), *S. tuberosum ssp. tuberosum* (AA), and *S. microdontum* (A). One of two FLint2 variants from *S. stoloniferum* and one of three FLint2 sequences cloned from *S. demissum* also belonged to genome A cluster. In addition, our analysis distinctly discriminated between genomes A in *S. verrucosum* and *S. microdontum*, the species corresponding to putative subseries tuberosa1 and tuberosa3 (Hawkes, 1990). *S. stoloniferum* contained genome A variant found in *S. verrucosum*, whereas *S. tuberosum* apparently comprised both these variants. The genome B cluster embraced FLint2 sequences from *S. bulbocastanum*, *S. cardiophyllum* and *S. ehrenbergii* and one of two FLint2 variants found in *S. stoloniferum*. Genomes of *S. cardiophyllum*, *S. ehrenbergii* and *S. pinnatisectum* (the latter was designated by Matsubayashi, 1991, as ApiApi) were in fact closer to genome B than to genome A. FLint2 polymorphisms definitely separated the pinnatisectum genome from two former genomes B. This evidence seems to support the existence of the characteristic genome Bpi. Finally, two of three FLint2 variants from *S. demissum* clustered separately from all other species; presumably, they correspond to genome D. FLint2 sequences from *S. bulbocastanum* were evidently discriminated from *S. cardiophyllum* and *S. ehrenbergii*, and FLint2 sequences from *S. stoloniferum* clustered with two latter rather than with the former species. These observations support the conclusions by Spooner and his associates (Pendinen *et al.*, 2008; Spooner *et al.*, 2008; Rodriguez and Spooner, 2009) made previously on the basis of other polymorphic fragments of wild *Solanum* genomes. They are contrary to the suggestion that *S. bulbocastanum* provided genome B germplasm to *S. stoloniferum*; the latter presumption is based on the identity of the RB/R*pi-blb*1 gene sequences in two species (Wang *et al.*, 2008).

Table 2. Genomes and subgenomes in Solanum species section Petota tentatively discerned with FLint2 markers

Series*	Species	Ploidy	Genomes after**		Genomes and subgenomes***
			1	2	
TUB	*S. berthaultii* Hawkes	2n		A1A1	A3
	S. brevicaule Bitter	2n-4n			A1A2A3D
	S. immite Dunal	2n		A1A1	A2
	S. kurtzianum Bitter&Wittm.	2n			A1
	S. marinasense Vargas	2n			A2
	S. microdontum Bitter	2n		A1A1	A3
	S. phureja Juz.&Bukasov	2n			A1 A2
	S. spegazzinii Bitter	2n			A1 A2
	S. vernei Bitter&Wittm.	2n			A1 A2
	S. verrucosum Schltdl.	2n	AA	A1A1	A1
	S. tuberosum ssp. *andigenum* (Juz.&Bukasov)Hawkes	4n			A1A3
	S. tuberosum ssp. *tuberosum* L.	4n			A1A2 / A1A3
LON	*S. fendleri* A. Gray	4n			A1B
	S. hjertingii Hawkes	4n			A1B
	S. stoloniferum Schltdl.	4n	AABB	A4A4BB	A1B
DEM	*S. brachycarpum* Correll	6n			A1A3
	S. demissum Lindl.	6n	AADDDdDd	A1A4[B,C,D]	A1D
	S. hougasii Correll	6n			A1B
	S. iopetalum (Bitter)Hawkes	6n			A1
BUL	*S. bulbocastanum* Dunal	2n	AbAb	BB	B
PIN	*S. cardiophyllum* Lindl./*S. ehrenbergii* (Bitter)Rydb.	2n	ApiApi		B
	S. jamesii Torr.	2n	ApiApi		B
	S. pinnatisectum Dunal	2n	ApiApi		B (Bpi?)
	S. tarnii Hawkes&Hjert.	2n			B

*Series in the section *Petota*: TUB – *Tuberosa* (Rydb.)Hawkes; LON – *Longipedicellata* Buk.; DEM – *Demissa* Buk.; BUL – *Bulbocastana* (Rydb.)Hawkes; PIN – *Pinnatisecta/Cardiophylla* (Rydb.)Hawkes; **As classified by (1) Matsubayashi, 1991, and (2) Hawkes, 1990: ***Molecular evidence from FLint2 polymorphisms.

FLint2 polymorphic sequences were further employed to develop SCAR markers discerning different *Solanum* genomes A, B and D. Positions of these markers are schematically presented in Fig. 2. We also managed to discriminate between three variants of genome A (tentative subgenomes A1, A2 and A3) corresponding to tuberosa 1, tuberosa 2 and tuberosa 3 subseries as suggested by Hawkes (1990). Subgenome A2 was detected in the species belonging both to the tuberosa 2 subseries (*S. brevicaule*, *S. immite*, *S. marinasense*) and to the tuberosa 3 subseries (*S. phureja*, *S. spegazzinii* and *S. vernei*). Two subspecies of tetraploid *S. tuberosum* include subgenomes A1A2 or A1A3. All analyzed species from the series Longipedicellata comprised subgenome A1. This evidence supports the cytogenetic observations by Pendinen *et al.* (2008) who presumed that *S. verrucosum* was the donor of genome A for *S. stoloniferum*. A separate subcluster characteristic for *S. demissum* seems to correspond to genome D; meanwhile other Demissa species (*S. brachycarpum*, *S. iopetalum* and *S. hougassii*) lacked the putative FLint2 marker of genome D. At the same time, *S. hougassii* contained the genome B marker.

Are FLint2 markers of wild *Solanum* genomes maintained in the registered potato cultivars following

several crosses and backcrosses? When the genome-specific FLint2 markers described above were used to screen potato hybrids highly resistant to late blight and combining several wild *Solanum* germplasms (strictly speaking, with several *Solanum* species listed in their pedigrees), most hybrids retained FLint2 markers of genomes A1, A3 and D, and some, genome B.

CONCLUSIONS

SCAR markers for *Solanum* genomes were developed from the polymorphic sequences of FLORICAULA/LEAFY intron 2 (FLint2). Screening 26 species representing six series of the tuber-bearing section Petota demonstrated that these markers presumably discerned genomes A, B and D and in particular subgenomes A1-A3. Potato hybrids with several *Solanum* species listed in their pedigrees mostly retained FLint2 markers of introgressed genomes.

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