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PLANT GENETICS

Sorbitol-6-Phosphate Dehydrogenase (*S6PDH*) Gene Polymorphism in *Malus* Mill. (Rosaceae)

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Abstract—The sorbitol-6-phosphate dehydrogenase gene (*S6PDH*) sequences of six *Malus* accessions, from five different taxonomic sections, were studied for the first time. The exon-intron structure and polymorphism of the nucleotide and amino acid sequences of these genes were characterized. The intraspecific polymorphism of the *S6PDH* gene was assessed for the first time in 40 Russian and foreign apple (*Malus domestica*) cultivars. It was demonstrated that the interspecific polymorphism level of the *S6PDH* coding sequences in the studied *Malus* species was 4%, and the intraspecific polymorphism level in *M. domestica* cultivars was very low (0.96%).

Keywords: apple, carbohydrate metabolism genes, sorbitol-6-phosphate dehydrogenase, genetic polymorphism

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INTRODUCTION

According to various taxonomic studies, genus *Malus* Mill. (Rosaceae) includes 25–47 cultivated and wild species [1], the most important of which is the domesticated apple (*Malus domestica* Borkh.), the world's fourth largest fruit crop in terms of production (http://faostat.fao.org/).

The unique feature of *Malus* species, along with other Rosaceae fruit tree crops (genera *Pyrus*, *Prunus*, and others), is that, in contrast to other families of higher plants, the end product of photosynthesis, in addition to sucrose and starch, is sorbitol.

It was shown that sorbitol is the main carbohydrate synthesized in leaves and transported to apple fruits [2]. As carbon source, sorbitol plays a key role in vegetative growth of Rosaceae pome and stone fruits [3]. The supply and distribution of sorbitol is the key factor in carbohydrate metabolism of fruits, that affects such fruit quality traits as sugar–acid balance and starch accumulation [4].

Furthermore, it was demonstrated that sorbitol is involved in plant responses to abiotic stress, including osmotic stress and exposure to low temperatures [5–7].

The key enzyme in sorbitol synthesis is sorbitol-6-phosphate dehydrogenase (S6PDH), which converts glucose-6-phosphate into sorbitol-6-phosphate. This enzyme is localized in the cytosol and chloroplasts of apple leaf cells [8].

At present, the full-length sequence of the *S6PDH* gene, which encodes sorbitol-6-phosphate dehydrogenase, is known for only one representative of the genus *Malus*: *Malus domestica* Gala (*S6PDH1*, JF764598, 3595 bp; *S6PDH2*, JF64599, 3402 bp) [8]. These sequences were mapped to chromosome X of the *Malus domestica* genome and represented allelic variants of the gene [8, 9]. The two sequences consist of six exons and five introns and encode the proteins of 310 amino acid residues. The main differences between them are localised in introns III, IV, and V [8].

In studies of transgenic apple trees with both increased and decreased *S6PDH* expression, it was demonstrated that changes in the enzyme activity affected the sorbitol/sucrose balance and their accumulation in leaves and altered the carbohydrate composition of fruits [3, 4, 10, 11].

These data confirm the importance of sorbitol-6-phosphate dehydrogenase for growth and formation of apple fruits.

Despite the key role of sorbitol-6-phosphate dehydrogenase in fruit growth and development in Rosaceae species, currently there are no data on the S6PDH gene polymorphism in *Malus* species. The study of the *S6PDH* intraspecific polymorphism in *M. domestica* is of special interest, as, in addition to a more complete assessment of the genetic variability of the members of the genus, it may also have practical applications in breeding programs.

Table 1. *Malus* species selected for cloning of the sorbitol-6-phosphate dehydrogenase gene

Species	Section	Accession no. in the collection of Vavilov Institute of the Plant Industry	Accession no. NCBI
M. sikkimensis (Wenz.) Koehne.	*Docyniopsis* Schneid.	2412	KR824941
M. florentina (Zucc.) Schneid.	*Sorbomalus* Zab.	2345	KR824940
M. toringoides (Rehd.) Hughes		14959A	KR824943
M. baccata var. *coerulescens* (L.) Borkh.	*Gymnomeles* Koehne.	2333	KR824942
M. coronaria (L.) Mill.	*Chloromeles* (Decne.) Rehd.	14986	KR824944
M. orientalis Uglitzk.	*Malus* Langenf.	29484	KR824939
M. domestica Borkh. Skala		–	KR824938

Thus, the present study was focused on the identification and analysis of sortbitol-6-phosphate dehydrogenase gene polymorphism in wild and cultivated *Malus* species, including a wide range of apple (*M. domestica*) cultivars.

MATERIALS AND METHODS

A total of 46 samples, including *Malus* representatives belonging to five different sections (*Docyniopsis*, *Sorbomalus*, *Chloromeles*, *Gymnomeles*, *Malus*), and 40 Russian and foreign *M. domestica* cultivars were analyzed. All plant material was provided by the Michurin All-Russia Research and Development Institute of Fruit Crop Genetics and Breeding and the Maikop Experimental Station of the Vavilov Research Institute of Plant Industry (Tables 1 and 2). The total DNA from all selected samples was extracted by the standard technique [12].

Based on the known sequences of apple sorbitol-6-phosphate dehydrogenase, a combination of primers (S6e1F 5'ATGTCCACCGTCACCCTGA3' and S6e6R 5'AGTCTTGGAAGGTAGACTGGTAC3') was designed to amplify the coding sequences of sorbitol-6-phosphate dehydrogenase genes in all analyzed samples. In addition, combinations of intragenic primers were designed for amplification and sequencing of the *S6PDH* genes (S6i1F 5'ATATTCTTACTCFTAGCTGTC3' and S6e2R 5'GCTTCACTCTTGTAATGAGCT3', S6e2F 5'CTCATTACAARAGTGAAGCAG3' and S6e5R 5'AGCCAAGCAATCTCTAGTTAG3') (figure).

Amplification of the *S6PDH* sequences was carried out with the Dialat Ltd. (Russia) reagent kit according to a standard protocol.

To clone the sorbitol-6-phosphate dehydrogenase genes, six *Malus* species from different sections were chosen, as well as a sample of *M. domestica* cv. Skala (Table 1). Cloning of the *S6PDH* sequences was carried out with the Qiagen PCR Cloning Kit according to the instructions of the manufacturer.

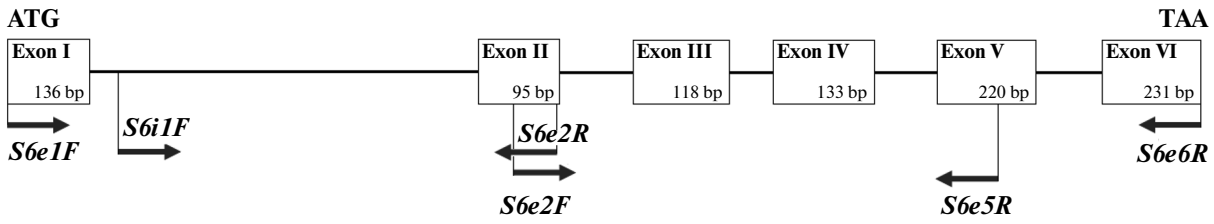
The *S6PDH* sequences of *M. domestica* cultivars were determined by direct sequencing with the above mentioned primers.

Sequencing of the sorbitol-6-phosphate dehydrogenase gene fragments was performed by standard method with the BigDye system (Applied Biosystems), on ABI PRISM 310 Genetic Analyzer, using the corresponding primers.

Sequences were aligned and analyzed using MEGA 5 software [13].

RESULTS AND DISCUSSION

The *S6PDH* full-length coding sequences from seven *Malus* species chosen for the analysis were amplified, cloned, and sequenced using the designed primers. The sequences obtained were deposited in the NCBI database (Table 1).



Schematic representation of primer positions used for *S6DPH* cloning and sequencing

Table 2. Nucleotide substitutions in the *S6PDH* sequences in *Malus domestica* cultivars taken into analysis

No.	Cultivar*	Nucleotide substitution**	No.	Cultivar*	Nucleotide substitution**
1	Skala	C/T(260) G/A(443) G/C(492)	21	Rozhdestvenskoe	
2	Berkutovskoe		22	Bylina	
3	Charodeika		23	Iyul'skoe Chernenko	
4	Stroevskoe		24	Streifling	
5	Bogatyr'	C/T(746)	25	Papirovka	
6	Martovskoe	C/T(228) C/T(746)	26	Arkad Letniy	
7	Blagovest	G/C(492)	27	Korichnoe Polosatoe	G/C(492)
8	Flagman	G/C(492)	28	Anis	
9	Spartan		29	Bessemyanka Michurinskaya	G/C(492)
10	Zhigulevskoe		30	Pepin shafraniy	
11	Svezhest		31	Letnee Polosatoe	
12	Uspenskoe	A/G(405) G/C(492)	32	Bashkirsky Izumrud	
13	Valyuta		33	Avgustovskoe	
14	Moskovskoe		34	Aborigen	
15	Sinap Orlovsky		35	Valentina	
16	Kandil Orlovsky		36	Bagration	G/C(492)
17	Renet Simirenko	T/G(792)	37	Primorskoe	T/C(99)
18	Fregat		38	Bashkirsky Krasavets	G/C(492)
19	Topaz		39	Antonovka Zheltaya	C/T(228) C/T(348) G/C(492)
20	Kurnakovskoe	A/G(405)	40	Antonovka Bezlepestnaya	

* Provided by N.I. Savelyev, Michurin All-Russia Research and Development Institute of Fruit Crop Genetics and Breeding.
** Number from the first nucleotide of the coding sequence.

Table 3. Exon and intron length and polymorphism of cloned *S6PDH* sequences in *Malus* accessions

Exons	Length, bp	SNPs	Introns	Length, bp	SNPs
Exon I	136	4	Intron I	1752–1833	171
Exon II	95	10	Intron II	264–269	25
Exon III	118	5	Intron III	138–156	8
Exon IV	133	6	Intron IV	113–114	15
Exon V	220	10	Intron V	98–121	11
Exon VI	231	3			
All exons	933	38	All introns	2372–2474	230

Table 4. Amino acid substitutions in S6PDH sequences of *Malus* samples

No.	Exon	Species	Amino acid substitution	Substitution type [18]
1	I	*M. sikkimensis, M. baccata*	I/L	C
2	II	*M. sikkimensis*	A/T	C
3		*M. sikkimensis*	L/F	R
4		*M. coronaria*	E/K	R
5		*M. baccata*	P/T	C
6	III	*M. domestica* Skala	A/V	R
7		*M. florentina*	K/R	C
8	IV	*M. florentina*	P/L	R
9		*M. baccata*	L/S	R
10		*M. domestica* Skala	K/R	C
11		*M. florentina*	A/T	C
12	V	*M. domestica* Skala, Blagovest, Uspenskoe, Flagman, Korichnoe Polosatoe, Bagration, Bashkirsky Krasavets, Antonovka Zheltaya, Bessemyanka Michurinskaya	D/E	C
13		*M. toringoides*	G/D	R
14		*M. sikkimensis*	C/R	R
15		*M. toringoides*	A/T	C
16		*M. baccata*	P/S	C
17	VI	*M. domestica* Martovskoe, Bogatyr'	P/L	R

S6PDH Sequence Polymorphism in Malus Species

Comparison of *S6PDH* sequences from seven *Malus* species with the known *S6PDH* sequences of *M. domestica* cv. Gala (JF764598, JF764599) showed that all of them were homologous to *S6PDH2* JF764599. The sequences contained six exons and five introns and differed from another *S6PDH1* JF764598 variant in introns III, IV, and V.

The length of cloned sorbitol-6-phosphate dehydrogenase genes varied from 3305 bp in *M. baccata* to 3407 bp in *M. orientalis* (Table 3). The total level of interspecific variability was 8%. The polymorphism of exon sequences varied from 1.3% in exon VI to 10.5% in exon II, which appeared to be the most polymorphic (Table 3). In exons, several species-specific substitutions, as well as SNPs shared by a number of species, were identified. The sorbitol-6-phosphate dehydrogenase gene sequence from *M. florentina* accession, section *Sorbomalus*, was found to be the most different from the others.

The introns were more polymorphic, and, in addition to single substitutions, contained indels. The most variable in length was the longest, intron I (1752–1833 bp), where a number of insertions and deletions were identified.

It should be noted that the presence of the polymorphic microsatellite $(CT)_{17-24}$ in intron III of all the studied sequences was also characteristic of the *S6PDH* sequences of the genus *Prunus* (Rosaceae) [14].

Specific indels were found in the introns of *M. baccata* (intron V, deletion of 23 bp) and *M. orientalis* (intron I, insertion of 23 bp). In addition to the species-specific, indels typical of species groups were identified. For instance, the studied sequences of *M. baccata* and *M. sikkimensis* had the 15-bp deletion in intron I.

The variability of intronic sequences is of particular interest, since they may be more informative in addressing the systematics and evolution of *Malus* species. For instance, the *S6PDH* fragments were successfully used for *Prunus* (Rosaceae) phylogeny reconstruction [14].

Polymorphism of the S6PDH Amino Acid Sequence in Malus Species

The obtained nucleotide sequences were translated and amino acid substitutions were detected. The length of all the seven sequences was 310 amino acid residues. In *Malus* species, 34 polymorphic nucleotide positions were identified with the 13 of these, leading to seven conservative and six radical amino acid substitutions (Table 4). The highest number of substitutions (four) were identified in *M. sikkimensis* and *M. bac-*

cata. Interestingly, three amino acid substitutions were also detected in *M. domestica* cv. Skala, while no substitutions were detected in another studied representative of the section *Malus*, *M. orientalis*.

It is known that the sorbitol-6-phosphate dehydrogenase protein of *Malus domestica* belongs to the family of plant aldo-ketoreductases (AKR) [15]. At present, the main structural elements of amino acid sequences have been described for sorbitol-6-phosphate dehydrogenase of *Malus domestica*, mannose-6-phosphate reductase of *Apium graveolens*, and sorbitol-6-phosphate dehydrogenase of *Oryza sativa*, which belong to this family [16]. Based on these data, all three conserved motifs, which are typical of aldo-ketoreductases, were identified in the studied *Malus* S6PDH sequences. It is interesting that two conservative amino acid substitutions were identified in motif 2 (amino acid residues 146–163) of S6PDH from *M. florentina* and *M. domestica* cv. Skala (Table 4).

Intraspecific Polymorphism of the S6PDH Sequence in Malus domestica

Since the studied *S6PDH* sequences in *Malus* representatives proved to be polymorphic, it was interesting to study the variability of this gene in Russian and foreign *M. domestica* cultivars. The coding regions and possible changes in them, which lead to the changes in the protein sequence, were of particular interest.

We selected 40 Russian and foreign *M. domestica* cultivars with different, economically important traits (Table 2).

The length of the sequenced *S6PDH* fragment in all of the studied cultivars constituted 930 bp (exons I to VI). However, the polymorphism level of these sequences was extremely low. There were nine identified SNPs (0.96%) (Table 2), and heterozygotes were detected at some positions. Four of the identified SNPs led to the amino acid substitutions in the corresponding protein sequence (Table 4).

Thus, sorbitol-6-phosphate dehydrogenase genes were for the first time identified and analyzed in representatives of the genus *Malus* belonging to five different taxonomic sections, and *S6PDH* intraspecific polymorphism was examined in 40 Russian and foreign apple cultivars for the first time.

The examined sorbitol-6-phosphate dehydrogenase sequences were quite conservative. This possibly results from the fact that this gene is highly functional and is essential for the normal growth and formation of apple fruits. For instance, the interspecific polymorphism level of *S6PDH* coding sequences in the studied *Malus* species was 4%. The low intraspecific polymorphism level of *S6PDH* coding sequences (0.96%) in *M. domestica* cultivars can also indicate a narrow genetic basis of modern apple cultivars.

It it interesting that the interspecific polymorphism of previously studied fragments of another carbohydrate metabolism gene, the sucrose synthase gene (*Sus*) in *Malus* species, was comparable with the interspecific polymorphism of the *S6PDH* coding sequences and constituted 2.8% [17].

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