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ALLELE DIVERSITY FOR ACID VACUOLAR INVERTASE GENE *Pain-1* FRAGMENT IN POTATO (*Solanum tuberosum* L.) VARIETIES AND LINES

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Abstract

The economic efficiency of potato production depends not only on the yield quantity and quality, but also on the tuber storage conditions. The tuber nutritional and technical value is determined by the starch content. Under the cold stress, the starch degrades into reducing sugars (cold-sweetening), which significantly reduces the tuber quality. Plant invertases catalyze irreversible hydrolysis of sucrose into glucose and fructose. Nowadays it is definitely known that vacuolar acid invertase (*Pain-1*) plays a major role in the cold induced sweetening of potato tubers. In the present work genetic diversity and allelic polymorphism of *Pain-1* gene associated with important agronomical traits of potato tubers is characterized. Gene fragment (exon 5—exon 7) polymorphism was analyzed in 69 cultivars and lines of Russian and foreign breeding origin. In the *Pain-1* nucleotide sequences, 66 SNPs were identified, of which 25 SNPs (SNP_{1628}, SNP_{1648}, SNP_{1700}, SNP_{1709}, SNP_{1717}, SNP_{1724}, SNP_{1726}, SNP_{1738}, SNP_{1788}, SNP_{1794}, SNP_{1797}, SNP_{1808}, SNP_{1815}, SNP_{1818}, SNP_{1831}, SNP_{1837}, SNP_{1847}, SNP_{1861}, SNP_{1865}, SNP_{1872}, SNP_{1885}, SNP_{1886}, SNP_{1890}, SNP_{1907}, SNP_{1909}) were described for the first time. The studied fragment contains a significant replacement for SNP1544 (C/A) which correlates with an increased starch content in the tubers and is homozygous in the Kazakhstani varieties Ulan and Astana. In the exons, 27 out of 42 SNPs led to amino acid substitutions. Most accessions had single amino acid substitutions. The maximum substitution number (seven to eight) characterized the Zhukovskii ranii variety and the lines 165 and 162. No substitutions were observed in the Frittella variety and the line 84. Therefore, the common level of gene fragment polymorphism in the analyzed potato accessions was shown to be rather high. Among the analyzed sequences, 78 allelic variants were described, including 64 specific variants and 14 variants common for several accessions. The obtained data may be helpful in potato breeding for an increase in starch content.

Keywords: acid vacuolar invertase, *Pain-1*, exons, polymorphism, SNPs, amino acid substitution, potato breeding, starch content in tubers, cold-induced sweetening

Alongside with wheat, maize and rice, potato (*Solanum tuberosum* L.) is one of the most widely spread and most economically significant global food, forage and industrial crops. The potato production efficiency depends on not only the amount and quality of the harvest, but also on the subsequent conditions of its storage, which is usually effected at low temperatures so that to prevent loss of moisture, molding, sprouting and disease transfer.

The nutritional and technological value of potato tubers is determined by content of starch [1]. The plants' starch performs a storage function, because it does not produce any increased osmotic pressure, being chemically inert (as distinct from glucose and sucrose) [2, 3]. However, it is starch that is "a weak link"

in storage of tubers, because under the influence of low temperatures, significant changes occur in carbohydrate metabolism [4], namely, sucrolytic enzymes. It results in decomposition of starch into simple sugars (glucose and fructose), which take part in maintenance of intracellular osmotic pressure, thus increasing resistivity of plants to low temperatures. At temperature below +3 °C the protective reactions of tubers to overcooling activate, and starch starts decomposing intensively, which is accompanies by accumulation of reducing sugars (cold-induced sweetening) [5, 6]. As a result, due to worsening of palatability traits of potato tubers their commercial qualities deteriorate as well [7, 8]. In addition, during heat treatment (cooking boiled potatoes or potato chips) the reducing sugars interact with free amino acids, causing change of colour of the product and accumulation therein of a carcinogen, the acrylamide [9-11].

The search of nucleotide and amino acid replacements associated with accumulation of starch and sugars in tubers and absence of cold-induced sweetening is very significant for selection.

A small family of vegetable invertases, which include cell wall, vacuolar and cytoplasmic invertases, catalyzes not-reversible sucrose splitting to glucose and fructose. It has been clearly shown that acidic vacuolar invertase coded by gene *Pain-1* plays a leading part in cold-induced sweetening of potato tubers. Inhibition of gene *Pain-1* decreases accumulation of reducing sugars at low temperatures [12-16]. Potato (*Solanum tuberosum*) gene of acidic vacuolar invertase is identified, its structure and expression are studied, and single nucleotide substitutions (SNPs) are found, which can exert the decisive influence on the enzyme activity [17, 18].

Previously it has been shown that the maximum number of polymorphic sites is detected in C-terminal region (exon 5—stop codon), which has been studied well enough for foreign varieties; moreover, in the said region SNPs associated with the amount of starch in tubers have been discovered [17, 19]. Analysis of polymorphism of this element for a set of 19 potato varieties identified nine allelic variants [20].

The search of new allelic variants of gene *Pain-1* determining commercially significant attributes of potato and the assessment of the occurrence frequency of such variants is deemed important for optimization of the selection process [21-23].

In the presented research, based on the analysis of nucleotide and amino acid polymorphism, 78 allelic variants were identified for fragments of gene of acidic vacuolar invertase *Pain-1* for varieties and lines of potatoes, which have not been characterized for this attribute previously, and the presence of 42 SNPs is demonstrated; 34 of them have been described for the first time.

The research objective was analysis of C-terminal region of acidic vacuolar invertase *Pain-1* for 69 varieties and lines of potatoes of different origin, which are now used in selection programs.

Techniques. The allelic polymorphism of *Pain-1* acidic vacuolar invertase gene fragment was studied for 69 potato varieties, including 55 varieties of domestic and foreign selection, as well as 14 lines used now in selection programs (Lorch Potato Research Institute). The tubers were sprouted in a greenhouse in light and temperature regime 8/16 hours and 16/22 °C (night/day) with illumination intensity 10-12 klx. The selected specimens were characterized by different starch content and unequal resistance to abiotic factors.

The nuclear DNA was extracted by the protocol suggested by K. Edwards et al. (1991) from fresh leaves according to description [24]. For amplification of *Pain-1* gene fragment a pair of primers IV5exF (GAAGCCTCATTTGAAGTGGAC)—IVendR (AATGTATGGGTTCCTGGAAACCG) was used.

PCR was performed in 15 μl reaction mixture. It contained 1× buffer solution with 50 mM Trisc-HCl (pH 8.6), 50 mM KCl, 0.1% Tween 20 (Dialet Ltd, Russia), 1.5 M $MgCl_2$, 20 mM dNTP, 10 μM of the respective primer, 0.25 U Taq DNA-polymerase and ~ 100 ng of genomic DNA. The amplification was carried out with a Mastercycler gradient device (Eppendorf, Germany) using reagents kit produced by Dialat Ltd. (Russia). Temperature/time profile of PCR: 5 min at 94 °C; 35 cycles: 30 s at 94 °C, 40 s at 57.5 °C and 1 min at 72 °C. Final elongation: 1 min at 72°C. The PCR products were analyzed by electrophoresis in 1% agarose gel (LE 2 Agarose, Helicon, Russia) in 1× TBE-buffer solution with ethydium bromide at the field intensity increasing from 70 to 100 V/cm. The results were documented in BioDoc II system (Biometra GmbH, Germany). Commercial 1 Kb and 100 bp DNA markers were used as fragment size standard (Thermo Fisher Scientific, USA). The amplified fragments were sequenced with the same primers (Applied Biosystems 3730 DNA Analyzer, Applied Biosystems, USA).

Balancing, translating and analysis of polymorphism of nucleotide and amino acid sequences were performed with software MEGA 7.0 (25). The sequence from NCBI database (GeneID:102577489) was used as a reference.

Results. Nucleotide polymorphism of *Pain-1* gene fragment. A fragment of C-terminal region containing the sequence from exons 5 to 7 was amplified and sequenced in the studied specimens. The length of the obtained sequences of *Pain-1* in all analyzed specimens was the same and amounted 707 bps. Exon 7 was the longest one (199 bps), and exon 6 was the shortest one (91 bps). No indels have been found in the sequence of analyzed specimens, while in previous studies for Bryanskii rannii variety we have demonstrated the presence of 11-nucleotide deletion (CAAGCTTATAT) and mononucleotide insertion (T) in intron 6 [20].

The polymorphism level of the analyzed sequences of acidic vacuolar invertase in 69 potato varieties and lines amounted 9.33% on average. Totally 66 SNPs were identified (42 SNPs in exons and 24 SNPs in introns). Among the discovered individual nucleotide replacements 24 SNPs were localized in intron sequences, the intron polymorphism being greater than the exon one on the whole amounting 10.16%.

The exon sequences are especially interesting for study of allelic variants, because they code amino acids, and the changes in exons may eventually result in functional changes of protein. In the exon sequences being analyzed (exons 5 to 7) in 69 potato varieties and lines we have discovered 42 SNPs, which is 8.91% of the total length of studied exons. Such a degree of polymorphism in exons is unexpectedly high as compared with that detected in previous studies of vacuolar invertase gene. For example, during study of *Pain-1* homologue genes DNA in 219 potato specimens 28 individual point replacements have been described, 24 SNPs being detected in the analyzed fragment (exon 5–exon 7) [17]. In addition, we have previously studied polymorphism of this gene in a set of 19 Russian selection varieties and 25 SNPs have been identified on the said gene fragment of acidic vacuolar invertase at the total polymorphism degree of 3.53% [20].

The sequences of exons 5 to 7 of *Pain-1* were analyzed for presence of known [17, 20] and new nucleotide replacements, which have not been detected in other varieties yet. As a result, it appeared that among SNPs found in 69 studied potato varieties and lines specimens, SNP_{1544}, SNP_{1574}, SNP_{1596}, SNP_{1629}, SNP_{1661}, SNP_{1843}, SNP_{1857}, SNP_{1895}, SNP_{1896} (designated according to the sequential number of nucleotide on cDNA) have been described previously [17, 20]. In coding sequences of the specimens 34 nucleotide replacements

were identified; 25 of them have never been described previously: SNP_{1628}, SNP_{1648}, SNP_{1700}, SNP_{1709}, SNP_{1717}, SNP_{1724}, SNP_{1726},SNP_{1738}, SNP_{1788}, SNP_{1794}, SNP_{1797}, SNP_{1808}, SNP_{1815}, SNP_{1818}, SNP_{1831}, SNP_{1837}, SNP_{1847}, SNP_{1861}, SNP_{1865}, SNP_{1872}, SNP_{1885}, SNP_{1886}, SNP_{1890}, SNP_{1907}, SNP_{1909}.

It was reported that specific nucleotide replacements in the primary sequence of *Pain-1* can be associated with commercially valuable traits, such as increased starch content [17]. A significant replacement was found within the site in question, SNP_{1544} (C/A). It correlates with the increase in starch quantity in tubers and was detected in homozygous state in Ulan and Astana varieties. In Courtney, Courage, Elizabeth, Zhukovskii rannii, Ryabinushka, Favourite, Vector, Innovator, Kuznechanka varieties the SNP_{1544} replacement was also present, but in heterozygous state. Previously the presence of SNP_{1544} was demonstrated for one more variety of Russian selection —Bryanskii rannii [20].

The allelic variants of *Pain-1* gene fragments in potato varieties and lines. Totally in the analyzed set of 69 specimens of potato varieties and lines we have found 78 allelic variants of *Pain-1* acidic vacuolar invertase gene fragment (the data is presented in online version of the article on web site http://www.agrobiology.ru, Table 1). Herewith, 64 allelic variants appeared to be unique, 14 — shared by several specimens. The most widely spread allelic variants were Pain-1_A62 (in 13 specimens), Pain-1_A63, Pain-1_A64 (in 6 specimens), Pain-1_A12 (in 5 specimens). Each of the allelic variants Pain-1_A1, Pain-1_A28, Pain-1_A34, Pain-1_A36, Pain-1_A37, Pain-1_A38, Pain-1_A46, Pain-1_A65 was repeated in two specimens. For 15 varieties (Astana, Didar, Karassiyskii, Miras, Ushkonir, Ognivo, Lileya, Krepysh, Khozyayushka, Valentino, Nayada, Kamenskii, Lady Clair, Aroza, Spiridon) variety-specific allelic variants were identified, which are unique for the studied set.

The majority of 69 analyzed potato varieties and lines appeared to be homozygous, and several allelic variants corresponded to them. Varieties Ulan, Astana, Fritella, Ognivo, Lileya, Kholmogorskiyi, Krepysh, Khozyayushka, Valentino, Nayada, Kamenskii, Bryanskii delikatesny, Clair, Aroza, Didar, Karassiyskiy, Miras, Ushkonir, Spiridon and line 84 were homozygous in terms of the gene in question. The allelic variant Pain-1_A12 identified in varieties Fritella, Chernskii/1 and lines 84 and 111/1 appeared to be common with sequence *S. tuberosum* represented in database NCBI (GeneID:102577489) (see http://www.agrobiology.ru, Table 1).

Thus, in the fragment, being analyzed exon 5— exon 7 of *Pain-1* gene in 69 specimens of potato varieties and lines 78 allelic variants were identified (see http://www.agrobiology.ru, Table 1), which indicates high polymorphism degree. Previously, for full-size coding sequence of acidic vacuolar invertase only 11 allelic variants were described [17], and 9 allelic variants were found in 19 varieties of Russian, Byelorussian and Kazakhstan selection [20].

Amino acid sequences polymorphism analysis. Nucleotide sequences of exons were translated, and the supposed protein amino acid sequence was analyzed. Acidic vacuolar invertase consists of 639 aa. The length of the sequence available for analysis coded by fragment exon 5—stop codon was 156 aa, which corresponds to protein amino acid positions from 484 to 639. We selected the sequence of β-fructosidase of potato *S. tuberosum* from database NCBI GenBank (NP_001274993.1) as a reference sequence.

Totally 27 sites of amino acid replacements were identified in the sequences in question (the data is presented in online version of the article on web site http://www.agrobiology.ru, Table 2). Herewith, a characteristic of most specimens was the presence of singular amino acid replacements. However, in some specimens we found identical sets of amino acid replacements — uniform

amino acid patterns. For example, the common amino acid pattern was characteristic of eight potato varieties (Manifest, Arsena, Meteor, League, Breeze, Vesna belaya, Kolobok and Zolskii). One more common amino acid pattern was characteristic of varieties Aroza, Krepysh, Didar, Karasayskii, Miras and Kamenskii (see http://www.agrobiology.ru, Table 2).

The maximum quantity of amino acid replacements were discovered in Zhukovskii rannii variety (8 replacements) and lines 165 (8 replacements) and 162 (7 replacements). The overwhelming majority of specimens had 3 to 5 replacements. The absence of amino acid replacements as compared to the reference sequence was shown for Fritella variety and line 84 (see http://www.agrobiology.ru, Table 2).

The analyzed potato specimens were divided into three types: the first type is homozygous and, consequently, having one amino acid sequence variant (Ulan, Astana, Fritella, Ognivo, Lileya, Kholmogorskii, Krepysh, Khozyayushka, Valentino, Nayada, Kamenskiy, Bryanskii delikatesny, Lady Clair, Aroza, Didar, Karasayskii, Miras, Ushkonir, Spiridon, line 84); the second type is the specimens with heterozygous state of nucleotide sequences, in which the identified SNPs do not result in replacements of amino acid residues and change of amino acid sequence (varieties Bashkirskii and Ocharovaniye, lines 27 and 58); and finally, the third type — varieties and lines heterozygous in terms of nucleotide sequences, in which different gene alleles code non-identical amino acid sequences (remaining 34 varieties and 11 lines of potatoes).

Previously, during analysis of replacements for specific amino acid sites it was shown that only two variants of amino acid residues are absent [17]. In this paper, for the most part of identified polymorph sites the presence of only two amino acid variants is also characteristic. For example, for position 515 all studied varieties and lines have either residue of threonine (T), or residue of lysine (K) (see http://www.agrobiology.ru, Table 2). However, for two sites in amino acid protein sequence (amino acid residues 629 and 639) we found three replacement variants. For example, glycine (G) was in position 629 in varieties Red Scarlet, Yantar, Bashkirskii, Spiridon, Elizabeth, Zhukovskii rannii and line 9, serine (S) in Valentino variety, and alanine (A) in all other varieties and lines. In varieties Chernskii, Red Scarlet, Krasavchik, Kholmogorskii, Newton, Aladdin, Nadezhda, Favourite, Astana and lines 84, 58, 111, 152, 141, 7, 46, 162 the amino acid residue 632 in protein Pain-1 was arginine (R), in varieties Lileya, Ognivo, Yantar, Aroza, Krepysh, Didar, Karasayskii, Miras, Kamenskiy, Khozyayushka, Ushkonir, Courtney, Elizabeth, Courage, Ryabinushka, Vector — proline (P), in varieties Bryanskii delikatesnii, Ocharovaniye, Clair, Nayada, Bashkirskii, Spiridon, Lady Roseta and line 27 — glutamine (Q). In other specimens, the amino acid residues varied, depending on the allele (see http://www.agrobiology.ru, Table 2).

As noted above, SNP_{1544} replacement is the most important; it results in replacement of threonine with lysine (T515K). Herewith, the presence of lysine residue correlates with high starch content [17]. Among the analyzed specimens such replacement was discovered in varieties of Kazakhstan selection, Ulan and Astana, and in heterozygous state in varieties Kuznechanka, Sirenevy tuman, Courtney, Elizabeth, Courage, Innovator, Ryabinushka, Zhukovskii rannii, Vector and Favourite.

Thus, we have carried out the analysis of nucleotide and amino acid polymorphism of a gene fragment of acidic vacuolar invertase in 69 potato varieties and lines, which have never been studied on this attribute before. The presence of 42 SNPs has been demonstrated, including 34 sites described for the first time. The presented data witness a high degree of polymorphism of the gene

fragment in the studied specimens. A total of 78 allelic variants of *Pain-1* gene fragment have been discovered, which can further be used in selection for creation of varieties with increased starch content.

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