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## EXPRESSION OF THE α-AMYLASE GENE *StAmy23* IN PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC TISSUES OF POTATO (*Solanum tuberosum* L.) CULTIVARS

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## Abstract

Potato (Solanum tuberosum L.) is the fourth most important agricultural crop after cereals. Almost every tissue of a potato plant contains starch, the regulation of metabolism and the physiological role of which depends on the type of tissue, the stage of plant development and external factors. Starch hydrolysis is catalyzed by  $\alpha$ - (AMY) and  $\beta$ - (BAM) amylases. By degradation of cytosolic phytoglycogen, StAmy23 amylase regulates tuber cold-induced sweetening and physiological dormancy. Few available studies on StAmy23 have focused on gene activity in potato tubers, including in response to cold stress. In this study, StAmy23 expression pattern in photosynthetic and non-photosynthetic tissues of potato plants of three cultivars, differing in starch content in tubers, was determined for the first time. Structural and phylogenetic analyses revealed that the closest homologs of StAmy23 are the  $\alpha$ amylases of various potato and tomato cultivars. Analysis of the carbohydrate content in freshly harvested tubers of the studied potato cultivars showed a similar high starch content for cv. Gala and cv. Saturna and almost 2 times lower for cv. Barin (6.3 vs. 11.34 mg/g of tissue). The largest amount of reducing sugars was found in tubers of cv. Saturna; cv. Gala tubers contained 4.5 and 24.5 times less of glucose/fructose than cv. Barin and cv. Saturna tubers, respectively (0.016/0.000 vs. 0.056/0.016 and 0.217/0.175 mg/g of tissue). For the first time, the expression profile of StAmy23 was determined not only in tubers, leaves and stems, but also in other organs and tissues of the potato plant. A high level of gene expression in stems and fruits was shown. In non-photosynthetic roots and stolons, StAmy23 transcription level either corresponded (cv. Saturna) or significantly exceeded (cv. Barin, cv. Gala) that in tubers. In stems, the highest and lowest StAmy23 transcription levels were observed in cv. Gala and cv. Saturna, respectively (0.58 and 0.13). Leaves and tuber peels showed similar, relatively low levels of StAmy23 expression. In fruits, the highest StAmy23 expression was found in cv. Barin (0.29), in the roots and tubers - in cv. Gala (0.55 and 0.17), and in the stolons - in cv. Barin and cv. Gala (0.31 and 0.33). A positive association was proposed between the level of StAmy23 transcription and the starch content (but not the content of reducing sugars) in tubers. The transcriptional activity of the StAmy23 gene in photosynthetic tissues of potato plants suggests the participation of encoded  $\alpha$ -amylase in starch hydrolysis not only in storage organs, but also in vegetative organs to maintain physiological growth processes and plant stress response.

Keywords: Solanum tuberosum, potato,  $\alpha$ -amylase StAmy23, starch content, reducing sugars, gene expression

Potato (*Solanum tuberosum* L.) is the fourth most important agricultural crop after cereals (rice, wheat, and corn) to ensure food security and economic development in the world. The main nutritional properties of potatoes are determined primarily by the quality and quantity of proteins, minerals and starch in the tubers.

The formation of plant storage organs depends on the import of carbon compounds from the initial photosynthetic tissues to provide substrates for the biosynthesis of all metabolites, including starch. After harvesting and during a certain period of storage, potato tubers are in a state of physiological dormancy, the violation of which negatively affects the consumer properties and technological characteristics of tubers [1, 2]. Tubers are stored at low temperatures (below + 4 °C) in order to slow down sprouting, moisture loss and pathogenesis. Such storage often leads to so-called cold induced sweetening (CIS), which manifests itself in the accumulation of reducing sugars (glucose, fructose) and, as a consequence, leads to browning of potatoes during frying with the formation of acrylamide [3, 4]. Understanding the mechanisms of regulation of tuber dormancy and their response to abiotic stress (for example, low temperatures) is very important both for potato seed production and for its subsequent processing.

Starch is found not only in storage organs. Starch granules can be found in almost every tissue of a plant at some stage in its life cycle. Starch metabolism is universal, and its regulation and physiological role vary depending on the tissue, the stage of plant development, and external factors [5-7]. In photosynthetic tissues, starch is subject to rapid degradation, which occurs at night, under stressful conditions, or during aging [7, 8]. Starch accumulates in plastids: spare - in amyloplasts of heterotrophic organs, transient - in chloroplasts of photosynthetic organs [9, 10].

Thus, starch can be a source of sugars when carbon is needed, or can serve as a kind of depot when sugars are present in excess, which allows optimal use of carbon stores [11, 12]. Decomposition of starch occurs hydrolytically or phosphorolytically. The hydrolytic pathway involves  $\alpha$ -amylases (AMY, EC 3.2.1.1) and  $\beta$ -amylases (BAM, EC 3.2.1.2) [13, 14].

AMY is an endoamylolytic enzyme that specifically hydrolyzes  $\alpha$ -1,4glucan bonds to form various linear and branched maltooligosaccharides. Multiple  $\alpha$ -amylase genes encode their different isoforms, which can play different roles depending on tissue localization and plant species. For example, suppression of  $\alpha$ -amylase I-1 in rice leads to an increase in starch accumulation in young leaves [15, 16]. In contrast, in *Arabidopsis*, all single, double, and triple AtAMY knockout mutants exhibit normal starch degradation [17, 18].

Five AMY genes have been identified in the potato genome, the activity of the products of which is specific with respect to various substrates and in different cellular structures, for example, in chloroplasts and amyloplasts [4, 19]. Two of them, StAmy1 and StAmy23, are expressed in tubers, but only StAmy23 is induced by low temperatures [20]. The homologue of this gene in the apple tree, the Amy $\delta$  gene, is also sensitive to cold; its expression is activated in berries at 0.5 °C [21]. Amylase StAmy23 is localized in the cytoplasm and regulates cold saccharification of tubers through the degradation of cytosolic phytoglycogen: silencing of StAmy23 leads to an increased starch content and a decrease in the amount of reducing sugars in tubers stored at low positive temperatures [4]. In addition, StAmy23 is involved in the regulation of potato tuber dormancy, and suppression of the gene expression delays tuber germination, which is accompanied by a decrease in the amount of reducing sugars in the peel and core tissue under the tuber buds, as well as a slight change in the phytoglycogen structure and starch granule size [2]. Thus, StAmy23 can stimulate bud germination in dormant tubers by providing sugars through hydrolysis of soluble starch [2].

Interestingly, all (very few) studies of *StAmy23* focus only on the gene activity in potato tubers, including in response to cold stress.

In this work, the expression profile of *StAmy23* in various organs and tissues

of potato plants was determined for the first time. A high level of gene expression was shown in stems and berries, as well as in non-photosynthetic roots and stolons, where the level of *StAmy23* transcription either corresponded to that in tubers or significantly exceeded it.

The aim of this work was to compare the expression of *StAmy23* gene in photosynthetic and non-photosynthetic organs in three varieties of potatoes of Russian and foreign selection, differing in starch content in tubers.

*Materials and methods.* Comparative bioinformatic structural analysis of the mRNA nucleotide sequences encoding StAmy23 homologous proteins in different plant species, deposited in the NCBI Nucleotide collection, and the corresponding amino acid sequences of StAmy23 was performed using the NCBI-BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogeny of StAmy23 was assessed using the Fast Minimum Evolution method (Grishin distance matrix, https://www.ncbi.nlm.nih.gov/blast/treeview/). Based on the performed comparative structural analysis of the coding and complete sequences of  $\beta$ -amylase genes, homologous to *StAmy23*, found in NCBI, cDNA-specific primers were developed for carrying out quantitative real-time PCR (RT-PCR). Forward and reverse primers were selected in such a way that there was at least one intron between them.

To analyze the expression profile of StAmy23 gene in various tissues of potato plants, as well as to determine the content of starch and reducing sugars (glucose and fructose) in tubers, we used the potato variety (*Solanum tuberosum* L.) (early) and Saturna (medium late) of foreign selection, differing, according to originators (https://reestr.gossortrf.ru/), in starch content in tubers: Barin is medium (13.4-14.6%), Saturna high (16.5-21.4%), and Gala low (10.2-13.2%) in the starch level. All three varieties (provided by Lorkh VNIIKH, Moscow Province) belong to canteens and are not used for the industrial production of chips. The plants were grown in 2020 in the field (Lorkh VNIIKH, Moscow Province). In September 2020, two plants of each variety were collected, tubers, tuber skin, pulp of tubers, roots, stolons, stems, leaves, and berries were separated for subsequent analysis of the expression of *StAmy23* amylase gene, tubers (whole with skin and pulp) were also used to determine the content starch and reducing sugars.

To isolate the total RNA, 50-100 mg of tuber tissue, tuber peel, pulp of tubers, roots, stolons, stems, leaves, and berries were used. Total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The resulting preparations were additionally purified from DNA impurities (RNase free DNasy set; Qiagen, Germany) in accordance with the manufacturer's recommendations. The synthesis of cDNA was performed using the GoScript<sup>™</sup> Reverse Transcription System kit (Promega, USA) according to the attached protocol. The concentration of RNA and cDNA was determined on a Qubit 4 fluorimeter (Thermo Fisher Scientific, United States) using appropriate reagents (Qubit RNA HS Assay Kit and Qubit DS DNA HS Assay Kit, Invitrogen, United States). Additionally, the RNA quality was checked by electrophoresis in 1.5% agarose gel using a FastRuler Middle Range DNA Ladder (Thermo Fisher Scientific, USA).

The analysis of the expression of *StAmy23* gene in roots, stolons, tubers, tuber skin, pulp of tubers, stems, leaves, and berries of potato plants in all three cultivars (Saturna, Gala, and Barin) was performed using the RT-qPCR method. The relative level of *StAmy23* expression was determined in comparison with the transcription of the reference *ef1* genes (primers 5'-ATTGGAAACGGATAT-GCTCCA-3' and 5'-TCCTTACCTGAACGCCTGTCA-3') and *sec3* (5'-GCTTA-TCATACTTCCGATCTCGCA-3') [22, 23]. For RT-PCR, we used 100 ng of cDNA template, a set "Reaction mixture for RT-PCR in the presence of SYBR

GreenI and ROX (LLC Syntol, Russia) according to the manufacturer's recommendations, and a thermal cycler CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, USA). The PCR assay was run in two biological and three technical repetitions under the following conditions: 5 min at 95 °C; 15 s at 95 °C, 50 s at 62 °C (40 cycles).

To quantify total starch, tuber material (including pulp and peel, 500 mg) was homogenized in 4.5 ml of a solution containing dimethyl sulfoxide (DMSO, 33%, v/v) and hydrochloric acid (0.44 M), incubated at 60 °C for 30 min in a water bath cooled to 25 °C, and diluted with water (mQ) in a ratio of 1:5. The pH was adjusted to 4.5 with 5 M sodium hydroxide. The suspension was filtered through Miracloth (Merck, USA); 100  $\mu$ l of the filtrate was used to measure the starch content using the Starch enzyme test (Boeh-ringer Mannheim/R-Biopharm AG, Switzerland) according to the manufacturer's protocol (Eppendorf BioSpectrometer® basic spectrophotometer, Eppendorf, Germany;  $\lambda = 340$  nm).

To assess the concentration of glucose and fructose, 1 g of tuber material (including pulp and peel) was ground in liquid nitrogen, suspended in 10 ml of 80% ethanol, and centrifuged at 16000 g for 15 min. The supernatant was analyzed by high performance liquid chromatography (HPLC) using a Varian ProStar chromatograph (Varian Inc., USA), a 102 M differential refractometric detector for a chromatograph (Styer model, ZAO SKB Khromatek, Russia) and an Agilent Pursuit column 200E PFP (150 mm × 4.6 mm, 5  $\mu$ m, A3050150X046, Agilent, USA). Isocratic elution was performed using acetonitrile:water (75:25 v/v) as mobile phase; flow rate 1.5 ml/min, temperature 30 °C. Biochemical analysis was performed in two biological and three technical repetitions.

For statistical processing of RT-PCR results and analysis of starch and reducing sugars contents, we used the GraphPad Prism v.8 software (GraphPad Software Inc., USA; https://www.graphpad.com/scientific-software/prism/). Data were expressed as mean (*M*) with standard error ( $\pm$ SE) based on two biological and three technical replicates for each cDNA variant and each potato sample. To assess differences in gene expression and carbohydrate content, Welch's t-test (unequal variance) was used (p < 0.05 indicates the statistical significance of the differences).

*Results.* The first stage of the work was bioinformatic analysis of the data on potato amylase StAmy23 available in the NCBI database. Potato *StAmy23* gene, mRNA and protein sequences (2871 nt; 4 exons; LOC102598863 alpha-amylase-like [*Solanum tuberosum* (potato)]; Gene ID: 102598863, chromosome VI) were extracted from GenBank NCBI (https: // www. ncbi.nlm.nih.gov/).

Analysis using NCBI-BLASTP (in the NCBI Non-redundant protein sequences database) showed that the closest homologues of the StAmy23 amylase (Protein ID: XP\_006354888.1) are the  $\alpha$ -amylase proteins of different potato varieties (for example, XP\_004238157.1, 98% identity) and tomato *Solanum lycopersicum* L. (for example, XP\_004235226.1, 91%) (Fig. 1, A). Homologous  $\beta$ -amylase of a more distant solanaceous species *Capsicum annuum* L. (PHT84617.1) has a 91% identity to potato StAmy23 in the amino acid sequence. Phylogenetic analysis based on the amino acid sequence of StAmy23 and amylase homologues confirmed the revealed similarity (see Fig. 1, B)

Based on the performed comparative structural analysis of the sequences of genes and mRNA of  $\beta$ -amylases homologous to *StAmy23* in *Solanum* species found in NCBI, we developed cDNA-specific primers for RT-PCR: StAmy23-F 5'-ATGGCG-CTTGATGAAAGTCAGC-3' and StAmy23-R 5'-CCA-GACTTTGCAATATCAGGAAC-3'.

The second stage of work was the analysis of the expression profile of

*StAmy23* gene in various tissues of potato plants in comparison with the content of starch and reducing sugars (glucose and fructose) in the tubers of the same plants in order to search for a possible relationship between these characteristics.



Fig. 1. Structural and phylogenetic analysis of potato  $\alpha$ -amylase (*Solanum tuberosum* L.) StAmy23: A — the degree of conservatism of the amino acid sequence between species (highly conserved sequences are highlighted in red, less conservative in blue, variable regions in gray, deletions of variable regions — in white with a narrow red stripe); B — dendrogram of evolutionary relationships between potato StAmy23 and the nearest known homologues proteins (NCBI accession numbers are indicated on the left in the amino acid sequence alignment diagram). Structural comparison was performed based on the data of the search for the closest homologues of StAmy23 (Gene ID: 102598863) in the NCBI database using the BLASTP tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic dendrogram was constructed as a result of analysis and visualization of BLASTP data for structural comparison of StAmy23 homologues using the Fast Minimum Evolution method (Grishin distance matrix, https://www.ncbi.nlm.nih.gov/blast/treeview/).

The three chosen potato varieties (Saturna, Gala, and Barin) differ in the content of starch in the tubers (https://reestr.gossortrf.ru/). The samples were grown in the field conditions, and freshly harvested tubers (including pulp and peel) were used to determine the starch and reducing sugar content (Table). Biochemical analysis showed the highest and closest starch content in the tubers of the Gala and Saturn varieties, while in the Barin variety it was almost 2 times lower (see Table). The results obtained differed from the data of the variety originators who reported that the tubers of the Gala variety were the lowest in the starch content compared to the Saturna and Barin varieties, and the Barin tubers occupy an intermediate position between the Gala and Saturna varieties (see Table). The mismatch may be due to the influence of weather conditions during the growth of the samples analyzed in our work. The greatest amount of reducing sugars was found in the tubers of the Saturna variety, the smallest in the Gala variety, which coincided, respectively, with the highest and lowest starch content among the samples. The tubers of the Gala variety contained 4.5 and 24.5 times less glucose and fructose, respectively, than the tubers of the Barin and Saturna varieties (see Table).

Content of starch and reducing sugars in freshly harvested tubers of the studied potato (*Solanum tuberosum* L.) varieties  $(M\pm SE)$ 

Variety	Starch (as per the official characteristics of the variety), %	Starch, mg/g tissue	Reducing sugars (glucose/fructose), mg/g tissue
Saturna	16.5-21.4	$1111.34 \pm 0.23$	0.217±0.021/0.175±0.070
Barin	13.4-14.6	$6.3 \pm 0.05$	$0.056 \pm 0.007 / 0.016 \pm 0.009$
Gala	10.2-13.2	1111.34±0.34	$0.016 \pm 0.001 / 0.000$

In the same samples, we investigated the transcriptional activity of *StAmy23* gene using our developed primers StAmy23-F/StAmy23-R. Previously Hou et al. [4] and Zhang et al. [20] studied the expression of *StAmy23* in leaves, stems, and tubers of potato varieties resistant and sensitive to cold saccharification and showed its presence in all three types of tissues. The level of gene expression in freshly harvested tubers did not differ between cultivars, while in leaves, *StAmy23* mRNA was more actively transcribed in a resistant cultivar [20]. The expression of this gene in other parts of the plant was not evaluated. Therefore, it was of interest to evaluate the *StAmy23* transcription profile in six different organs of potato plants, i.e., in leaves, stems, berries, roots, stolons, and tubers (pulp and rind separately). In addition, a comparison of gene expression in three cultivars with different starch and reducing sugars content in tubers (see Table) could reveal a possible relationship between the *StAmy23* transcription level and carbohydrate accumulation at the time of po-tato harvest.

The results of RT-PCR assay showed an increased *StAmy23* gene transcription in tuber pulp compared to leaves in the cultivars Saturna and Gala, while it decreased in the cultivar Barin (Fig. 2). Together with the data of Zhang et al. [20] who reported that, during the harvesting period, the level of gene expression in tubers is either lower or the same as in leaves, it can be assumed that each potato cultivar has its own *StAmy23* transcription profile. The presence of *StAmy23* mRNA in all analyzed tissues and an unexpectedly high, in comparison with tubers, gene expression in vegetative organs and berries suggest the absence of a pronounced specificity of the work of the *StAmy23* enzyme in relation to certain plant tissues. The level of *StAmy23* transcription in non-photosynthetic roots and stolons either corresponded (cv. Saturna), or significantly (1.8-8.0 times) exceeded (cv. Barin, Gala) that in tubers. High expression also occurred in photosynthetic stems and berries, although its level in leaves was relatively low (see Fig. 2).



Fig. 2. Expression profile of the StAmy23 gene in leaves (1), stems (2), fruits (3), roots (4), stolons (5), and tubers (underlined) in pulp (6) and peel (7) in potato (Solanum tuberosum L.) cultivars Saturna (a), Barin (b) and Gala (c). ef1 and sec3 were the reference genes. The analysis was carried out in two biological and three technical replicates, the values of  $M\pm$ SE are given. Letters a, b and c denote statistically significant differences in gene expression between cultivars (p < 0.05). For example, bc means that the level of gene expression in the marked tissue in cv. Saturna (a) is significantly different

When comparing the expression levels of StAmy23 between cultivars with

from that in cv. Barin (b) and cv. Gala (c).

different starch and sugar contents, it turned out that cultivar Gala had the highest transcriptional activity of *StAmy23* in stems, while cultivar Saturna had the lowest transcriptional activity (see Fig. 2). In the studied cultivars, the leaves were characterized by a similar level of expression with a slight advantage of cultivar Barin. The *StAmy23* expression in berries was the highest in the Barin variety, in roots — in the Gala variety, and in the stolons — in the Barin and Gala varieties. In tuber, a fleshy storage organ the *StAmy23* expression level was the highest in the Gala variety and the lowest in the Barin variety. The studied cultivars did not differ in the transcriptional activity of the *StAmy23* gene in the tuber skin (see Fig. 2).

Comparing the data on transcriptional activity (see Fig. 2) and the results of biochemical analysis (see Table), it can be assumed that there is a positive relationship between the *StAmy23* transcription level and starch content, but not the content of reducing sugars in tubers. Thus, the level of *StAmy23* expression is the highest in cultivar Gala and medium in cultivar Saturna, while the starch content in these cultivars is equally higher than in cultivar Barin with the lowest gene expression level (see Table, Fig. 2). The highest amount of glucose and fructose was found in the Saturna cultivar with medium *StAmy23* expression level, and the lowest in the Gala cultivar with the highest *StAmy23* expression (see Table, Fig. 2).

However, based on the analysis of only three cultivars, it is too early to draw assumptions about a relationship between the transcriptional activity of *StAmy23* and the carbohydrate content in tubers. For strict conclusions, it will be necessary to study a sample of potato samples that are contrasting in the content of starch and sugars in tubers.

It should also be taken into account that the activity of  $\beta$ -amylase StAmy23 is supplemented by the action of  $\beta$ -amylases and starch phosphorylases and can be suppressed by inhibitors of amylases [24, 25]. Therefore, it would be most correct to look for correlations between the cumulative expression profile of genes encoding these enzymes and the starch content.

For example, the SbAI amylase inhibitor at the post-translational level regulates the activity of amylases [26, 27]. An inverse relationship was shown between the expression of the *SbAI* gene and the content of reducing sugars in tubers [28]. It was previously revealed that the level of *SbAI* expression in potato tubers of cv. Barin is significantly (approximately 3 times) lower than in cv. Severnoe siyanie, suitable for the production of crisp potatoes [25]. Based on these data, the unsuitability of tubers for frying in all three varieties studied by us in this work may be associated with a low level of *SbAI* transcription and, as a consequence, with sensitivity to cold saccharification.

In addition, if we talk about correlations with the content of glucose and fructose, then the activity of genes for the enzymes of sucrose hydrolysis (invertase, sucrose synthase) and inhibitors of invertase should also be taken into account [29-31]. The processes of glucose utilization in plant cells, including the synthesis of starch and sucrose, should also be considered. It should also be kept in mind that the source of glucose in tubers can be not only maltose resulted from the cleavage of starch by amylases [32], but also sucrose which is produced in photosynthetically active leaves and moves from them to tubers [33].

The way of decomposition of starch in storage organs differs from the way of decomposition of starch in leaves [7]; however, in both cases, amylases are involved in the process. It is known that the leaves of dicotyledons (including potatoes) are rich in starch, while the leaves of cereals (for example, barley) are rich in sugars [24, 34]. This suggests that the main role of transient starch in potato leaves is mainly to maintain growth processes at night, as has been shown for *Arabidopsis thaliana* [35-37]. The presence of *StAmy23* gene transcription in photosynthetic tissues of potato plants indicates that the encoded enzyme is involved in the regulation of starch metabolism not only in storage organs (berries and tubers), but also in vegetative tissues to maintain plant growth and response to abiotic stresses.

So, in this work, we performed a bioinformatic phylogenetic analysis of  $\alpha$ -amylase StAmy23, which determined the closest homologues of the enzyme. They are  $\alpha$ -amylases of representatives of the *Solanaceae* family, primarily of the species and varieties of potatoes, tomatoes and peppers. Biochemical analysis of freshly harvested tubers of three potato cultivars (Barin, Saturna, and Gala) revealed differences between cultivars in the content of both starch and reducing sugars. Thus, in the Barin variety, the starch content (6.3 mg/g tissue) was 2 times lower than in the Gala and Saturna varieties, which have similar high starch contents (approximately 11.34 mg/g tissue). The amount of reducing sugars (glucose/fructose) was found to be the smallest in Gala tubers; tubers of varieties Barin and Saturna contained 4.5 and 24.5 times more glucose/fructose, respectively, (0.056/0.016 and 0.217/0.175 vs. 0.016/0.000 mg/g tissue in variety Gala). We have determined the expression profile of the *StAmy23* gene in the stems and leaves of the analyzed varieties, as well as for the first time in other organs and tissues of a potato plant (roots, stolons, berries; in tubers, separately in the peel and pulp). It was shown that the level of StAmy23 transcription in roots and stolons either corresponds (in the Saturna variety) or exceeds (in the Barin and Gala varieties) that in the tubers. The highest expression level of the StAmy23 gene in berries was found in the Barin variety (0.29), in the roots and tubers in the Gala variety (0.55)and 0.17), and in the stolons in the Barin and Gala varieties (0.31 and 0.33). It is assumed that there is a positive relationship between the level of StAmy23 transcription and the starch content (but not reducing sugars) in tubers. The transcriptional activity of the StAmy23 gene in all analyzed tissues of the potato plant indicates the possible participation of  $\alpha$ -amylase StAmy23 in the hydrolysis of starch not only in storage, but also in vegetative (including photosynthetic) organs to maintain the physiological processes of growth and plant response to stress. The data obtained are promising for expanding the understanding of the fundamental processes underlying carbohydrate metabolism of plant cells, including its changes in response to stress. From a practical point of view, the research results can be used in breeding potato varieties for the selection of donors of economically valuable traits (for example, resistance to cold saccharification of tubers, resistance to low cultivation temperatures, high or low starchiness of tubers). Continuation of research will be associated with the study of the cumulative effect of  $\alpha$ - and  $\beta$ -amylases on various characteristics of plants of different varieties and types of potatoes.

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