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THE USE OF CARBOHYDRATE METABOLISM GENES FOR POTATO (*Solanum tuberosum* L.) IMPROVEMENT

(review)

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Abstract

Potato (*Solanum tuberosum* L.) is one of the most important crop species in the world. Its nutritional and industrial qualities depend on starch content in tubers. Starch consists of linear (amylose) and branched (amylopectin) glucose polymers. Three main goals of modern potato breeding programs include increment of tuber starch yield, development of potato cultivars with improved amylose or amylopectin content and prevention of cold-induced sweetening. Nowadays some molecular and biotechnological approaches to vary plant characteristics have been developed. Among them the most popular are marker-assisted selection, transgenic technologies, genome editing. But, regardless of the chosen approach, the fundamental stage of successful work is the proper choice of the target gene, which in turn requires detailed understanding of the metabolic pathways for the synthesis and degradation of carbohydrates in plant tissues. Starch metabolism includes rather big number of reactions and requires synergetic work of a great number of enzymes. Moreover, it should be mentioned that in starch formation and degradation participate not only carbohydrates modifying proteins, but some regulatory proteins that are also involved in such pathways. Taking into account the previously published review (V.K. Khlestkin et al., 2017), in which attention is paid to genes that determine the specific physical, chemical and technological starch properties, in the present review the emphasis is made on the current understanding of the starch biosynthesis and degradation processes and the key genes of carbohydrate metabolism enzymes in potato tubers. In the present review, among proteins involved in plant carbohydrate metabolism we have chosen those that play the key roles in potato tubers starch formation and retention. The key proteins are sucrose synthases, starch-phosphorilases, granule-bound starch synthase, α- and β-amylases, acid vacuolar invertase, as well as invertase and amylase inhibitors. The main candidate genes that may influence potato agronomical traits are described. The future work requires analysis of allelic polymorphism of the candidate genes in a wide range of potato species, cultivars and lines, looking for associations with desired agronomic traits. It will allow us to use these genes for marker-assisted selection and as target genes for gene editing.

Keywords: potato, starch, amylose, amylopectin, cold-induced sweetening, starch metabolism

Potato (*Solanum tuberosum*) is the most important world food, fodder, and technical crop. Potato is under cultivation throughout the entire territory of the Russian Federation, in different climatic zones located on a huge space from the southern borders to the polar circle, being one of the main food products.

Starch is the basis of the nutritive value of potato tubers. According to its structure, food starch can be divided into glycemic and resistant one, which is determined by the quantitative ratio of the two polymers, amylose and amylopectin. Amylose represents a direct chain of glucose molecules that is digested longer. Amylopectin has some branches of small glucose chains and is digested faster. Thus, the energy and dietary characteristics of potato depend on the qual-

itative composition of starch grains. Potato as a technical crop is valuable for starch content, which is used in the production of glue, glucose, bioethanol, bioplastics and other products and materials [1-3]. In this regard, one of the important directions of potato breeding is the increase in the specific weight of tuber starch and the creation of cultivars with an increased content of amylose or amylopectin. It is also important to remember that the economic effectiveness of potato cultivation depends not only on the production volume and tubers starchiness but also on the duration of their storage, where the weak link again is starch. Tubers contain an average of 12-18% of starch and 0.5-1.5% of sugars under normal conditions. Storage temperatures below +3 °C cause a protective response of tubers to overcooling, which is accompanied by intense starch deterioration and the accumulation of reducing sugars (glucose and fructose). This is the so-called cold-induced sweetening process, which worsens the commercial qualities of potato tubers.

Therefore, three main tasks are considered relevant for today: the increase in the starch proportion in potato tubers (starch content), modeling the qualitative composition of tubers starch (the ratio of amylose and amylopectin), and prevention of the cold-induced sweetening process and the decrease in the amount of reducing sugars. To solve them, it is necessary to determine the ways of carbohydrate metabolism in tubers first, to identify the key enzymes that regulate these ways, and to identify alleles of genes coding them associated with economically valuable characteristics of tubers. It will give an opportunity to carry out a targeted selection based on the modeling of tubers carbohydrate metabolism to produce potato with the desired properties.

The carbohydrate composition of potato tubers is a compound-complex feature, which is controlled by a set of genetic and external factors [4]. A few decades ago, the sequence of biosynthesis reactions and starch decay in a plant cell, which seemed to be well studied, was determined at the physiological level [5]. However, the modern analysis of genomic and transcriptomic data showed that the schemes of carbohydrate metabolism of plant cells are much more complicated: there are alternative metabolic pathways, and the same reaction can be catalyzed by different enzymes. A large number of proteins, regulating the activity of the main enzymes of carbohydrate metabolism, and, for example, carrier proteins, which determine the spatial localization of key reactions, were revealed.

Thus, the understanding of carbohydrate metabolism mechanisms will make it possible to carry out targeted selection, choose useful alleles of key genes and obtain new cultivars with the desired properties. Therefore, the search for genes affecting the content of sugars and starch in potato tubers arouses great interest in many researchers nowadays [2-4, 6].

Carbohydrate metabolism in potato tubers. Potato starch consists of two polymers: branched amylopectin and linear amylose, the structural unit of which is α-glucose. Starch synthesis occurs in plastids (mainly in chloroplasts and amyloplasts), where both polymers form insoluble granules. Starch can vary in grain structure, the degree of molecules polymerization, and physicochemical properties [1, 6-7].

The metabolism of starch occurs in leaves (in chloroplasts) as well as in tubers (in amyloplasts). Most reactions proceed predominantly equally, but some organ-specific differences also exist. For example, a consistent change in the processes of starch synthesis and decay takes place in leaves within 24 hours. Potato tubers, in turn, synthesize starch throughout their development, accumulating it as an energy-intensive substrate. In leaves, ATP, necessary for starch synthesis, is formed during photosynthesis, and in the amyloplasts of tubers,

ATP is imported from photosynthetic organs. The substrate for the starch synthesis in leaves chloroplasts is ADP-glucose, formed as a result of the Calvin-Benson cycle, while in developing potato tubers sucrose becomes such a substrate, coming from photosynthetically active leaves [3, 8]. Biochemical differences in the ways of starch biosynthesis in potato leaves and tubers imply the existence of distinctive features in the genetic basis of the discussed metabolic processes in these organs.

Despite deceptively simple biochemical reactions, there are still many unresolved issues about carbohydrate metabolism. The presence of many enzymes, the opportunity to carry out reactions by alternative pathways, the sequence of intermediate reactions that is still not determined and their subcellular localization complicate the understanding of the process. For example, it is not clear in which organelles the intermediate stages of starch metabolism happen, which proteins carry out and control the intracellular transport of sugars from the cytosol to amyloplasts. Therefore, several alternative hypotheses exist instead of a single starch metabolism pattern [3, 8-9]. It is also important to understand that different periods of plants life are characterized by different metabolic pathways. In particular, starch biosynthesis in the leaves, when stolons are initiated, during the development of stolons, in the ripened tubers, and in the collected tubers during storage vary significantly.

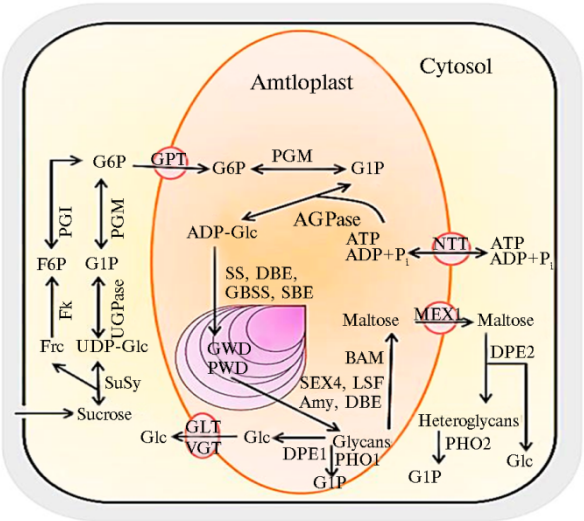
This review will be devoted to the issues of starch biosynthesis in growing potato tubers, in which intense starch accumulation happens.

Sucrose, which is delivered to the cells through the symplast or apoplast, is formed during photosynthesis in the leaves. In the case of the apoplast pathway, sucrose enters the tuber directly through the intercellular space, where it is hydrolyzed to glucose and fructose by apoplast invertases. These generated monosaccharides penetrate into the cells of tubers using hexose transporters. Sucrose enters the cells of the tubers by the symplast way simultaneously, by using sucrose-transporter proteins. By entering the cytosol of tuber cells, sucrose is hydrolyzed by sucrose synthase to UDP-glucose and fructose.

Thus, UDP-glucose accumulates in the cytoplasm of tuber cells. The issue of further UDP-glucose transformations and localization of biochemical reactions remains a controversial one. According to some reports, UDP-glucose is converted into glucose-1-phosphate, which is then converted into ADP-glucose, in its turn, entering the amyloplast and involved in the reactions of polysaccharides biosynthesis there [8]. The alternative model implies that in the cytosol of tuber cells UDP-glucose is first converted into glucose-1-phosphate and then into glucose-6-phosphate; in this form, it is transported using the triose-6-phosphate translocator in the amyloplast. Glucose-6-phosphate is converted into ADP-glucose inside of amyloplasts, from which, under the influence of starch-synthesizing enzymes (starch synthase, starch-branching enzymes, etc.) starch is formed [8, 10]. Both alternative ways imply that ADP-glucose is the direct substrate for the synthesis of amylose and amylopectin in amyloplasts [9]. The residue of ADP-glucose joins the increasing chain with starch synthase (SS, EC 2.4.1.21). While the polysaccharide chain is growing, starch-branching enzymes (BE, EC 2.4.1.18) introduce branching, and amylopectin is synthesized this way [9]. The synthesis of the linear molecule of amylose, in its turn, is carried out by the enzyme of granule-bound starch synthase (GBS, EC 2.4.1.242).

Starch granules with a semi-crystalline structure are formed at the final stage. Although the exact mechanisms of the process are still unclear, it is considered that the final stage of starch granule formation depends on amylopectin only [9]. The very process of starch grains formation is specific for different types of plants and different organs: if there are many small granules in the leaf chloro-

plast, then there are only a few granules in the tuber amyloplast, and they are very large [9].



Potential starch metabolism way in potato tubers [3]. Enzymes: SuSy – sucrose synthase, Fk – fructokinase, UGPase – UDP-glucose-pyrophosphorylase, PGI – phosphoglucoisomerase, PGM – phosphoglucomutase, AGPase – ADF-glucose-pyrophosphorylase, SS – starch synthases, LSF – SEX4-alike enzyme, Amy – amylases, DBE – debranching enzyme; DPE – disproportionating enzyme; PHO – starch phosphorylase, BAM – β-amylases. Translocator proteins: GPT – glucose phosphate transporter, MEX – maltose transporter, NTT – nucleotide translocator; GLT – glucose transporter; VGT – vacuolar glucose transporter. Substances: Frc – fructose, Glc – glucose, UDF-Glc – UDP-glucose, F6P – fructose-6-phosphate, G1P – glucose-1-phosphate, G6P – glucose-6-phosphate, ADP-Glc – ADF-glucose, ATP – ATP, P_i – inorganic phosphorus.

Thus, the starch biosynthesis, starting from the monosaccharide substrates formation and to the starch grains formation, includes many reactions and requires coordinated work of many different enzymes. In addition, it has already been noted that the carbohydrate metabolism involves not only enzymes that modify mono-, di-, and polysaccharides but also regulatory proteins that affect these reactions indirectly, the work of which must also be taken into account.

The primary stage of starch synthesis in tubers depends on the work of saccharolytic enzymes directly, as they contribute to the hexoses accumulation, which is included in starch synthesis further on. However, the final starch accumulation is determined not only by the speed of its synthesis but also by the intensity of its decay, since these processes have been carried out simultaneously and continuously. A sufficient number of enzymes destroying starch, which are specific to the glycoside bond and influence various substrates (amylose, amylopectin, dextran), were described. These enzymes are of different genetic origins and belong to different families [7, 11-14].

Enzymes destroying starch can be divided into two categories, i.e. hydrolytic (α- and β-amylase) and phosphorolytic (α-glycan-phosphorylase). Their comparative activity may vary depending on the stage of development or environmental conditions. Which of enzymes groups has bigger importance is a rather controversial issue. According to some reports, the main contribution to the starch decay is made by the hydrolytic way, although the phosphorolytic one is less energy-consuming [15]. However, starch phosphorylation may not be a sufficient factor by itself [16]. Perhaps, this process makes the starch grains surface more hydrophilic and, thus, more accessible to hydrolytic enzymes, creating selective protein-carbohydrate and protein-protein interactions additionally [14, 16-18].

Starch grains turn into branched or linear forms of polyglycans during the process of decay. Further on, the branched forms are converted into linear glycans as a result of the work of enzymes that remove branching, for example, isoamylase (EC 3.2.1.68) or dextrinase (dextrin 6-α-glucanohydrolase; EC 3.2.1.142) that are specific to the α-1.6-glycosidic bond. At the final stage, linear glycans can be destroyed by β-amylase (EX 3.2.1.2) or starch synthase (EC 2.4.1.21) to neutral sugars [12].

As a result of consistent starch decay, metabolites (triosephosphate, maltose, glucose) are formed in the amyloplasts; then, they are transported to the cytosol with the help of specific transporters [19]. They are involved in the glycans metabolic way there, exposed to the cytosolic phosphorylase – transglucosidase (DPE2, disproportionating enzyme, EC 2.4.1.25), turning into the hexoses phosphates eventually, which, in their turn, are required for the sucrose biosynthesis.

Key genes of carbohydrate metabolism in potato tubers. Many starch metabolism genes are united into genetic families [9]. Different members of one family may play different roles in photosynthesizing and storage organs [3]. The activity of starch metabolism enzymes is regulated both at the transcriptional level (e.g., by circadian rhythms or the presence of sugars) [3] and at the posttranslational level, which includes protein-protein interactions and protein phosphorylation [8].

A major study to identify all genes associated with starch metabolism in the potato genome was carried out in 2017 [3]. As a result, 77 genomic loci coding enzymes of starch metabolism were identified. For comparison, the genome of mustard weed (*Arabidopsis thaliana*) has 46 known genes of starch metabolism, 44 of which have homologs in the potato genome [3]. In addition, new isoforms of many enzymes have been found in the potato genome [3].

The potato genes encoding starch metabolism enzymes that are currently known are given in the table [3, 20-22].

It was shown that among 77 described genomic loci associated with starch metabolism in the potato plant, some genes are expressed in the leaves only, others in all starch synthesizing organs, and the third, the most interesting ones (in the materials of this review) in tubers. In all appearance, the latter group may include genes associated with economically valuable features [3]. The bioinformatic analysis of transcription data [3] revealed several genes, the expression of which is specific for potato tubers. The highest level of tuber-specific expression was observed in genes of the glucose-6-phosphate translocator *GPT2.1*, sucrose synthase *SuSy4*, phosphoglycan phosphatase *SEX4*, starch synthase *SS5* and starch-branching enzyme *SBE3*.

It is known that the most intensive starch synthesis occurs in the process of tubers formation [23]. Therefore, not only genes with high expression in tubers deserve special attention but also those genes, the expression of which grows while the tubers are initiated and developed [3] because they can be regulators of the tubers formation process. Such genes include sucrose synthase *SuSy4,* starch-branching enzyme *SBE3,* glucose-6-phosphate translocator *GPT2.1* and dextrinase *LDE* genes [3]. It is interesting that the level of transcription for the phosphoglycan phosphatase *SEX4* gene, characterized by high expression in the very tubers, is inversely related to the intensity of the tuber growth and the starch synthesis [3].

On the basis of modern concepts of carbohydrate metabolism in tubers, one may offer several candidate genes to solving the above-formulated main objectives of modern potato breeding (the increase in the starch content, the increase in the amylose or amylopectin content, inhibition of cold-induced sweetening). Let us analyze them in details.

Genes determining starch content. Among the genes, the expression of which correlates with the tubers growth, the most interesting one is the sucrose synthase *SuSy4* gene. The largest amount of information was collected for *SuSy4*, indicating its key influence on the starch content in potato tubers [3, 24-27].

Proteins of the sucrose synthase family (EC 2.4.1.13) catalyze the reaction of reversible hydrolysis of sucrose in the presence of UDP to UDP-glucose

and fructose and are found in all higher plants [28]. In the plant cell, SuSy4 is present in the soluble form in the cytosol [24]. Sucrose synthase is the main enzyme resolving sucrose in the endosperm of cereals and potato tubers; it provides a substrate for starch synthesis in the storage organs. Super expression of the sucrose synthase gene *SuSy4* in potato plants leads to the increase in the starch content in tubers and the increase in yield [30].

Genes encoding carbohydrate metabolism enzymes of potato (*Solanum tuberosum* L.) (cit. ex. 3 with supplements)

Protein/protein family	Genes	Expression specificity
ADP-glucose pyrophosphorylase large subunit	*AGPL1, AGPL2, AGPL3*	*AGPL1* in leaves
ADP-glucose pyrophosphorylase small subunit	*AGPS1.1, AGPS1.2, AGPS2*	
Alpha-amylase	*AMY1.1, AMY1.2, AMY23, AMY3, AMY3-like*	*AMY1.1* in leaves
Alpha-glucan phosphorylase	*PHO1a, PHO1b, PHO2a, PHO2b*	*PHO1b* in leaves, *PHO1a* in tubers
ATP-ADP antiporter	*NTT1, NTT2*	
Beta-amylase	*BAM1, BAM2, BAM3.1, BAM3.2, BAM4, BAM6.1, BAM6.2, BAM6.3, BAM7, BAM9*	*BAM3.1* in leaves
Branching enzyme	*SBE1.1, SBE1.2, SBE2, SBE3*	*SBE3* in tubers, expression accelerates with the growth of tubers
Disproportionating enzyme	*DPE1, DPE2*	
Glucan water dikinase	*GWD*	
Glucose transporter	*GLT1*	
Glucose-6-phosphate translocator	*GPT1.1, GPT1.2, GPT2.1, GPT2.2*	*GPT2.1* in tubers, expression accelerates with the growth of tubers
Granule bound starch synthase	*GBSS1*	Expression in tubers is higher than in leaves
Inorganic pyrophosphatase	*PPase, PPase-like*	
Isoamylase	*ISA1.1, ISA 1.2, ISA2, ISA3*	
Limit dextrinase	*LDE*	Organ specificity is not shown, but *LDE* expression accelerates with the growth of tubers
Maltose excess	*MEX1*	
Phosphoglucan phosphatase	*LSF1, LSF2, SEX4, SEX4-like*	*SEX4* in tubers, expression decreases with the growth of tubers
Phosphoglucan water dikinase	*PWD*	
Phosphoglucoisomerase	*PGI, PGI-like1, PGI-like2*	
Phosphoglucomutase	*PGM1, PGM2.1, PGM2.2, pPGM*	
Starch Synthase	*SS1, SS2, SS3, SS4, SS5, SS6*	*SS5* in tubers
Sucrose Synthase	*SuSy1, SuSy2, SuSy3, SuSy4, SuSy6, SuSy7*	*SuSy4* in tubers, expression accelerates with the growth of tubers
Triose-phosphate/phosphate translocator	*TPT, TPT-like*	
UDP-glucose pyrophosphorylase	*UGPase1, UGPase2*	
Vacuolar Glucose Transporter	*VGT3-like*	
Vacuolar invertase	*Pain-1*	
Invertase inhibitor	*INH1, INH2*	
Amylase inhibitor	*SbAI*	

N o t e. Gaps mean the absence of data..

Another important gene, for which the association with high starch content in tubers is shown, is α-glycan phosphorylase. α-glycan phosphorylases (starch phosphorylase, EC 2.4.1.1), the members of the glycosyltransferase family 35 (GT35), play a significant role in the carbohydrate metabolism of plants, animals, and prokaryotes [31-32]. Analogs of α-glycan phosphorylases in plants are also known as starch phosphorylases. This enzyme carries out phosphorolytic starch degradation and catalyzes the reaction of reversible transfer of the glycosyl residue at the end of α-1.4-D-glycan chain in the presence of phosphate to form glucose-1-phosphate. All plants have two different forms of starch phosphorylases – plastidic and cytosolic ones. In its turn, the potato plastidic starch phosphorylase PHO1 is encoded by two homologous genes, which are characterized by the tissue-specific expression: *PHO1b* is expressed in leaves mainly, *PHO1a* – in tubers [3, 33-34]. Even though starch phosphorylases can carry out the reactions of both starch destruction and

starch synthesis, it is shown in vitro that the plastidic form plays a more significant role in the process of starch destruction [35-36]. However, in vivo the evidence of this fact is absent. Also, some information about the ability of starch phosphorylase to synthesize oligosaccharide primer, which is then completed by starch-synthase, was obtained in vitro [15, 37-38].

Genes determining the qualitative composition of starch. As it was said before, the field of the starch use is extensive, and in relation to the specific tasks, it is necessary to obtain starch with different physical and chemical properties, which are determined by the quantitative ratio of amylose and amylopectin directly. Starch with a high content of amylopectin (glycemic starch) has an increased value of nutritional energy and is used for the production of infant and dietetic nutrition. In the industry, such starch is also preferable (economically beneficial) as a raw material for the production of glucose-fructose syrups and bioethanol. High-amylose (resistant) starch is more resistant to the influence of α-amylases, whereby it is used in the production of bioplastics. By having a low glycemic index, such starch is also valuable in dietetics [39]. Starch qualitative composition depends on the work of two enzyme groups – starch synthases (including granule-bonded starch synthase) and starch-branching enzymes.

The genes of six starch synthases isoforms (*SS1*, *SS2*, *SS3*, *SS4*, *SS5*, *SS6*) and the homologous gene of granule-bonded starch synthase *GBSS1* [3] were found in the potato genome. Starch synthases SS synthesize amylopectin polysaccharides and can be found either in the dissolved form or joined to a starch granule. Genetic and biochemical information proves that every isoform of starch synthase SS (EC 2.4.1.21) plays its unique role in the process of amylopectin synthesis. It is considered that SS1, SS2, and SS3 isoforms work one by one directly, synthesizing short, middle, and long chain correspondingly. It is also known that 80% of starch synthase activity in potato tubers is from SS3 [9]. *SS5* isoform starch synthase gene is characterized by the tuber-specific expression, although there is confirmation in vivo of the SS5 direct impact on the starch accumulation and yield of potato. At the same time, the homologous gene *SS5* of corn presumably controls starch accumulation at the stage of grain ripening [3, 40]. However, it is believed that the activity of starch synthase (with the exception of SS5) in tubers does not exceed the same in leaves greatly and the agronomic significance of genes encoding these enzymes is not as important as that of the homologous gene *GBSS1* [3].

Granule-bound starch synthase GBSS1 (EC 2.4.1.242) controls amylose biosynthesis in the forming of starch granules. Many investigations indicate the important economic value of this enzyme [41-44]. GBSS1 join the starch granule directly. *GBSS1*expression in tubers is a little bit higher than in leaves. *GBSSI* was revealed and characterized in many potato cultivars [36, 45-46]. Inactivation of this gene allows obtaining potato, the tubers of which contain amylopectin mainly [47-50].

Starch branching enzyme SBE (EC 2.4.1.18) influences the accumulation of the particular form of starch polysaccharides. SBE catalyzes the formation of points for α-1.6-branches in the polysaccharide chain with different frequency and length of the branched chain. Starch branching enzyme activity was revealed in potato first. The polysaccharide structures formed by the starch branching enzyme are then modified by enzymes that remove branching (DBEs, debranching enzymes, EC. 3.2.1.68), and thus insoluble granules are formed. The activity of the starch-branching enzyme affects the degree of branching of amylopectin directly [52-53].

Many plant species have differences in the expression of particular classes of the starch-branching enzyme [39]. Mutant plants with SBE activity defi-

ciency have the indicative phenotype due to inhibition of starch synthesis and accumulation of large amounts of sucrose and other soluble sugars [39]. For example, pea (*Pisum sativum* L.) has wrinkled fruits, and starch content is reduced by 50% [54]; for corn, the mutation of *amylose extender* (*ae-*) is known, which is accompanied by the decrease in starch synthesis by 20% [55]. At the same time, the starch of such plants consists of amylose mainly, and amylopectin found in them is small branched. High amylose starch in potato was obtained only by inhibiting the activity of several isoforms of the starch-branching enzyme at the same time [56].

Genes determining resistance to cold-induced sweetening. In case of storage at temperatures below +10 °C, reducing sugars accumulate in potato tubers, which, when interacting with α-amino acids, lead to the accumulation of acrylamide and deterioration of taste [57-59]. Therefore, the prevention of potato cold-induced sweetening is extremely important for the food industry [60-62]. Cold-induced sweetening occurs due to the hydrolysis process of polyglycan chains by amylases and the destruction of sucrose by invertases.

As it was said before, starch degradation can be carried out either hydrolytically or phosphorolytically. The hydrolytic way is catalyzed by α-amylases (AMY, alpha-amylase, EC 3.2.1.1) and β-amylases (BAM, beta-amylase, EC 3.2.1.2). Both families include proteins with many isoforms. Nowadays, at least five genes of α-amylases and at least ten genes of β-amylases were identified in the potato genome [3]. α-amylases hydrolyze α-1.4-glycan bonds to form various linear and branched maltooligosaccharides. Two genes of α-amylases – *StAmy1* and *StAmy23* work in potato tubers. In case of low-temperature storage, only amylase StAmy23 is active [63]. β-amylases realize hydrolysis of the non-reducing end of glycan chains associated with α-1.4-glycoside bonds, with the formation of β-maltose [64]. It is shown that the activity of β-amylases of potato increases in the first week of storage at +4 °C significantly [65]. The expression of β-amylases is also closely correlated with the accumulation of reducing sugars in potato tubers stored at positive temperatures of 3-5 °C [66], thus, confirming the importance of β-amylases in the process of cold-induced sweetening. It is considered that among the known genes of β-amylases, *StBAM1* and *StBAM9* have the highest level of transcription in tubers [63].

The sucrose hydrolysis by invertases with the formation of glucose and fructose [4] also leads to the formation of reducing sugars during the storage of potato tubers. Nowadays, it is clearly shown that the main role in cold-induced sweetening of potato is played by acidic vacuolar invertase (Pain-1) (beta-fructofuranosidase, EC 3.2.1.26), catalyzing the irreversible hydrolysis of sucrose. Inactivation of the *Pain-1* gene reduces the accumulation of reducing sugars in tubers at low temperatures [22, 67-70]. This gene was identified in potato, its structure and expression were studied, and single-nucleotide substitutions (SNPs) were found to determine the activity of the enzyme [71-74].

Potato cultivars resistant to cold-induced sweetening have a low transcription of the vacuolar invertase gene, but some lines show high expression of this gene at low enzyme activity [69]. It was found that, in addition to the regulation of the vacuolar invertase work at the transcriptional level, post-translational modification of protein occurs with the participation of inhibitors [75]. Therefore, it is necessary to consider a group of enzymes that indirectly affect the cold-induced sweetening process separately, although they have no affinity for a glycosidic bond and do not interact with sugars and polyglycans. This group should include inhibitors of invertase and amylase.

The sequences of invertase inhibitors' genes were determined for different plant species [76]. Two inhibitors, the St-Inh (*INH1*) and StInvInh2

(*INH2*), were found in the cultivated potato species, affecting the invertase activity and, consequently, the cold-induced sweetening of tubers, which was confirmed by the effect of their overexpression in potato tubers [77]. The potato haploid genome contains one copy of the *INH1* and *INH2* genes localized on the 12th chromosome in tandem orientation and subjected to alternative splicing, and the gene products inhibit apoplast (INH1) and vacuolar (INH2) invertase [76]. It was shown in vitro that *INH2* [78] has the greater inhibitory effect, which is confirmed by a significantly higher level of *INH2* expression in potato genotypes resistant to cold-induced sweetening than in the sensitive ones. In addition, the association of some splice variants of the *NAT2* gene and the variability of its promoter region with the degree of exposure of potato tubers to cold-induced sweetening was reported [76, 79].

Another example of post-translational regulation of genes involved in the process of cold-induced sweetening is associated with the work of the amylase inhibitor. The activity of amylase for potato inhibits by the SbAI gene, which was first cloned from the *Solanum berthaultii* species [21]. The growth of SbAI activity leads to the suppression of amylases and, consequently, to the decrease in the accumulation of reducing sugars in tubers [21]. The presence of protein-protein interactions between SbAI and StAmy23, StBAM1, and StBAM9 [21] proteins was shown with the help of a dihybrid system. Therefore, amylase inhibitor is considered a key regulator of cold-induced sweetening processes of potato tubers caused by the amylase activity.

So, the molecular and biotechnological approaches (marker-mediated selection, derivation of transgenic plants, genomic editing, etc.) already allow changing the desired characteristics of plants. However, regardless of the used approach, the fundamental step that determines the successful result of the work is the right choice of the target gene. In this review, the key enzymes that directly and indirectly can carry out the most important stages of the starch synthesis and decomposition in tubers are identified in a large number of proteins involved in the carbohydrate metabolism in potato tubers. The range of encoding these enzymes candidate genes, allelic variants of which can be associated with economically valuable traits of potato, was determined. Further work requires analysis of allelic variants of these candidate genes in a wide range of cultivars, lines, and samples of wild potato species and identification of associations with the required agronomic traits. It will allow using them as target genes for the development of molecular markers and editing sites for the selection of cultivars with specified characteristics.

REFERENCES

1. Khestkin V.K., Pel'tek S.E., Kolchanov N.A. Target genes for development of potato (*Solanum tuberosum* L.) cultivars with desired starch properties (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2017, 52(1): 25-36 (doi: 10.15389/agrobiology.2017.1.25eng).
2. Li L., Tacke E., Hofferbert H., Lübeck J., Strahwald J., Draffehn A. Validation of candidate gene markers for marker-assisted selection of potato cultivars with improved tuber quality. *Theor. Appl. Genet.*, 2013, 126(4): 1039-1052 (doi: 10.1007/s00122-012-2035-z).
3. Van Harsselaar J., Lorenz J., Senning M., Sonnewald U., Sonnewald S. Genome-wide analysis of starch metabolism genes in potato (*Solanum tuberosum* L.) *BMC Genomics*, 2017, 18: 37 (doi: 10.1186/s12864-016-3381-z).
4. Duarte-Delgado D., Juyy D., Gebhardt C., Sarmiento F., Mosquera-Vásquez T. Novel SNP markers in *InvGE* and *SssI* genes are associated with natural variation of sugar contents and frying color in *Solanum tuberosum* Group Phureja. *BMC Genet.*, 2017, 18: 23 (doi: 10.1186/s12863-017-0489-3).
5. Frommer W., Sonnewald U. Molecular analysis of carbon partitioning in solanaceous species. *J. Exp Bot.*, 1995, 46: 587-607.
6. Pfister B., Zeeman S. Formation of starch in plant cells. *Cell. Mol. Life Sc*i., 2016, 73(14): 2781-2807 (doi: 10.1007/s00018-016-2250-x).
7. Martin C., Smith A. Starch biosynthesis. *Plant Cell*, 1995, 7(7): 971-985 (doi: 10.1105/tpc.7.7.971).

8. Bahaji A., Li J., Sánchez-López Á., Baroja-Fernández E., Mucoz F., Ovecka M., Almagro G., Montero M., Ezquer I., Etxeberria E., Pozueta-Romero J. Starch biosynthesis, its regulation and biotechnological approaches to improve crop yields. *Biotechnol Adv.*, 2014, 32(1): 87-106 (doi: 10.1016/j.biotechadv.2013.06.006).
9. Zeeman S., Kossmann J., Smith A. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu. Rev. Plant Biol.*, 2010, 61: 209-234 (doi: 10.1146/annurev-arplant-042809-112301).
10. Naeem M., Tetlow I., Emes M. Starch synthesis in amyloplasts purified from developing potato tubers. *The Plant Journal*, 1997, 11(5): 1095-1103 (doi: 10.1046/j.1365-313X.1997.11051095.x).
11. Smith A., Zeeman S., Thorneycroft D., Smith S. Starch mobilization in leaves. *J. Exp. Bot.*, 2003, 54(382): 577-583 (doi: 10.1093/jxb/erg036).
12. Smith A., Zeeman S., Smith S. Starch degradation. *Annu. Rev. Plant Biol.*, 2005, 56: 73−98 (doi: 10.1146/annurev.arplant.56.032604.144257).
13. Zeeman S., Smith S., Smith A. The diurnal metabolism of leaf starch. *Biochem. J.*, 2007, 401(1): 13-28 (doi: 10.1042/BJ20061393.
14. Orzechowski S. Starch metabolism in leaves. *Acta Biochim. Pol.*, 2008, 55(3): 435-445.
15. Dauvillée D., Chochois V., Steup M., Haebel S., Eckermann N., Ritte G., Ral J., Colleoni C., Hicks G., Wattebled F. Plastidial phosphorylase is required for normal starch synthesis in *Chlamydomonas reinhardtii. Plant J.*, 2006, 48(2): 274-285 (doi: 10.1111/j.1365-313X.2006.02870.x).
16. Fettke J., Albrecht T., Hejazi M., Mahlow S., Nakamura Y., Steup M. Glucose 1-phosphate is efficiently taken up by potato (*Solanum tuberosum*) tuber parenchyma cells and converted to reserve starch granules. *New Phytol.*, 2010, 185(3): 663-675 (doi: 10.1111/j.1469-8137.2009.03126.x).
17. Yu T., Kofler H., Häusler R., Hille D., Flügge U., Zeeman S., Smith A., Kossmann J., Lloyd J., Ritte G. The Arabidopsis *sex1* mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not in the chloroplast hexose transporter. *Plant Cell*, 2001, 13(8): 1907-1918 (doi: 10.1105/TPC.010091).
18. Hejazi M., Fettke J., Haebel S., Edner C., Paris O., Frohberg C., Steup M., Ritte G. Glucan, water dikinase phosphorylates crystalline maltodextrins and thereby initiates solubilization. *The Plant Journal*, 2008, 55(2): 323-334 (doi: 10.1111/j.1365-313X.2008.03513.x).
19. Zeeman, S., Smith, S., Smith A. The breakdown of starch in leaves. *New Phytol.*, 2004, 163(2): 247-261 (doi: 10.1111/j.1469-8137.2004.01101.x).
20. Liu X., Song B., Zhang H., Li X.Q., Xie C., Liu J. Cloning and molecular characterization of putative invertase inhibitor genes and their possible contributions to cold-induced sweetening of potato tubers. *Mol. Genet. Genomics*, 2010, 284(3): 147-159 (doi: 10.1007/s00438-010-0554-3).
21. Zhang H., Liu J., Hou J., Yao Y., Lin Y, Ou Y., Song B., Xie C. The potato amylase inhibitor gene SbAI regulates cold-induced sweetening in potato tubers by modulating amylase activity. *Plant Biotechnol. J.*, 2014, 12(7): 984-993 (doi: 10.1111/pbi.12221).
22. Clasen B., Stoddard T., Luo S, Demorest Z., Li J., Cedrone F., Tibebu R., Davison S., Ray E., Daulhac A., Coffman A., Yabandith A., Retterath A., Haun W., Baltes N., Mathis L., Voytas D., Zhang F. Improving cold storage and processing traits in potato through targeted gene knockout. *Plant Biotechnol. J.*, 2016, 14(1): 169-176 (doi: 10.1111/pbi.12370).
23. Kloosterman B., Vorst O., Hall R., Visser R., Bachem C. Tuber on a chip: differential gene expression during potato tuber development. *Plant Biotechnol. J.*, 2005, 3(5): 505-519 (doi: 10.1111/j.1467-7652.2005.00141.x).
24. Appeldoorn N., de Bruijn S., Koot-Gronsveld E., Visser R., Vreugdenhil D., van der Plas L. Developmental changes of enzymes involved in conversion of sucrose to hexose phosphate during early tuberisation of potato. *Planta*, 1997, 202(2): 220-226 (doi: 10.1007/s004250050122).
25. Fu H., Kim S., Park W. High-leve1 tuber expression and sucrose inducibility of a potato *Sus4* sucrose synthase gene require 5′ and 3′ flanking sequences and the leader intron. *The Plant Cell*, 1995, 7(9): 1387-1394.
26. Viola R., Roberts G., Haupt S., Gazzani S., Hancock R., Marmiroli N., Machray G., Oparka K. Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. *Plant Cell*, 2001, 13(2): 385-398 (doi: 10.1105/tpc.13.2.385).
27. Zrenner R., Salanoubat M., Willmitzer L., Sonnewald U. Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). *The Plant Journal*, 1995, 7(1): 97-107 (doi: 10.1046/j.1365-313X.1995.07010097.x).
28. Avigad G. Sucrose and other disaccharides. In: *Encyclopedia of plant physiology*. T.A. Loewus, W. Tanner (eds.). Springer-Verlag, Heidelberg, 1982.
29. Koch K. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr. Opin. Plant Biol.*, 2004, 7(3): 235-246 (doi: 10.1016/j.pbi.2004.03.014).
30. Baroja-Fernández E., Mucoz F., Montero M., Etxeberria E., Sesma M., Ovecka M., Bahaji A., Ezquer I., Li J., Prat S., Pozueta-Romero J. Enhancing sucrose synthase activity in transgenic potato (*Solanum tuberosum* L.) tubers results in increased levels of starch, ADPglucose and UDPglucose and total yield. *Plant Cell Physiol.*, 2009, 50(9): 1651-166 (doi: 10.1093/pcp/pcp108).
31. Rathore R., Garg N., Garg S., Kumar A. Starch phosphorylase: role in starch metabolism and biotechnological applications. *Crit. Rev. Biotechnol.*, 2009, 29(3): 214-224 (doi: 10.1080/07388550902926063).

32. Nighojkar S., Kumar A. Starch phosphorylase: biochemical, molecular and biotechnological aspects. *Genet. Eng. Biotechnol.*, 1997, 17(4): 189-202.
33. Sonnewald U., Basner A., Greve B., Steup M. A second L-type isozyme of potato glucan phosphorylase: cloning, antisense inhibition and expression analysis. *Plant Mol.* Biol., 1995, 27(3): 567-576.
34. Albrecht T., Koch A., Lode A., Greve B., Schneider-Mergener J., Steup M. Plastidic (Pho1-type) phosphorylase isoforms in potato (*Solanum tuberosum* L.) plants: expression analysis and immunochemical characterization. *Planta*, 2001, 213(4): 602-613.
35. Preiss J., Levi C. Starch biosynthesis and degradation. In: *The biochemistry of plants. V. 3.* J.B. Pridham (ed.). Academic Press, NY, 1980.
36. Newgard C., Hwang P., Fletterick R. The family of glycogen phosphorylases: structure and function. *Crit. Rev. Biochem. Mol. Biol.*, 1989, 24(1): 69-99 (doi: 10.3109/10409238909082552).
37. Dai W., Deng W., Cui W., Zhao Y., Wang X. Molecular cloning and sequence of potato granule-bound starch synthase. *Acta Botanica Sinica*, 1996, 38(10): 777-784.
38. Satoh H., Shibahara K., Tokunaga T., Nishi A., Tasaki M., Hwang S.K. Mutation of the plastidial α-glucan phosphorylase gene in rice affects the synthesis and structure of starch in the endosperm. *Plant Cell*, 2008, 20: 1833-1849 (doi: 10.1105/tpc.107.054007).
39. Tetlow I., Emes M. A review of starch-branching enzymes and their role in amylopectin biosynthesis. *IUBMB Life*, 2014, 66(8): 546-558 (doi: 10.1002/iub.1297).
40. Liu H., Yu G., Wei B., Wang Y., Zhang J., Hu Y., Liu Y., Yu G., Zhang H., Huang Y. Identification and phylogenetic analysis of a novel starch synthase in maize. *Front. Plant Sci.*, 2015, 6: 1013 (doi: 10.3389/fpls.2015.01013).
41. Hovenkamp-Hermelink J., Jacobsen E., Ponstein A., Visser R., Vos-Scheperkeuter G., Bijmolt E., de Vries J., Witholt B., Feenstra W. Isolation of an amylose-free starch mutant of the potato (*Solanum tuberosum* L.). *Theor. Appl. Genet.*, 1987, 75(1): 217-221 (doi: 10.1007/BF00249167).
42. Jacobsen E., Hovenkamp-Hermelink J., Krijgsheld H., Nijdam H., Pijnacker L., Witholt B., Feenstra W. Phenotypic and genotypic characterization of an amylose-free starch mutant of the potato. *Euphytica*, 1989, 44(1-2): 43-48 (doi: 10.1007/BF00022597).
43. Visser R., Somhorst I., Kuipers G., Ruys N., Feenstra W., Jacobsen E. Inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs. *Mol. Gen. Genet.*, 1991, 225(2): 285-296.
44. Van der Steege G., Nieboer M., Swaving J., Tempelaar M. Potato granule-bound starch synthase promoter-controlled GUS expression: regulation of expression after transient and stable transformation. *Plant Mol. Biol.*, 1992, 20(1): 19-30.
45. Rohde W., Becker D., Kull B., Salamini F. Structural and functional analysis of two waxy gene promoters from potato. *Journal of Genetics & Breeding*, 1990, 44: 311-315.
46. Van de Wal M., Jacobsen E., Visser R. Multiple allelism as a control mechanism in metabolic pathways: GBSSI allelic composition affects the activity of granule-bound starch synthase I and starch composition in potato. *Mol. Genet. Genomics*, 2001, 265(6): 1011-1021 (doi: 10.1007/s004380100496).
47. Visser R., Stolte A., Jacobsen E. Expression of a chimaeric granule-bound starch synthase-GUS gene in transgenic potato plants. *Plant Mol. Biol.*, 1991, 17(4): 691-699 (doi: 10.1007/BF00037054).
48. Kuipers G., Vreem J., Meyer H., Jacobsen E., Feenstra W., Visser R. Field evaluation of antisense RNA mediated inhibition of GBSS gene expression in potato. *Euphytica*, 1992, 59(1): 83-91 (doi: 10.1007/BF00025364).
49. Flipse E., Keetels C., Jacobson E., Visser R. The dosage effect of the wild type GBSS allele is linear for GBSS activity, but not for amylose content: absence of amylose has a distinct influence on the physico-chemical properties of starch. *Theor. Appl. Genet.*, 1996, 92(1): 21-127 (doi: 10.1007/BF00222961).
50. Heilersig B., Loonen A., Janssen E., Wolters A., Visser R. Efficiency of transcriptional gene silencing of GBSSI in potato depends on the promoter region that is used in an inverted repeat. *Mol. Genet. Genomics*, 2006, 275(5): 437-449 (doi: 10.1007/s00438-006-0101-4).
51. Haworth W., Peat S., Bourne E. Synthesis of amylopectin. *Nature*, 1944, 154: 236-238 (doi: 10.1038/154236a0).
52. Hizukuri S. Polymodal distribution of the chain lengths of amylopectin, and its significance. *Carbohyd. Res.*, 1986, 147(2): 342-347 (doi: 10.1016/S0008-6215(00)90643-8).
53. Bertoft E., Seetharaman K. Starch structure. In: *Starch: origins, structure and metabolism.* I.J. Tetlow (ed.). Society for Experimental Biology, London, 2002.
54. Bhattacharyya M., Smith A., Ellis T., Hedley C., Martin C. The wrinkle-seeded character of peas described by Mendel is caused by a transposon-like insertion in a gene encoding starch branching enzyme. *Cell*, 1990, 60(1): 115-122 (doi: 10.1016/0092-8674(90)90721-P).
55. Boyer C., Daniels R., Shannon J. Abnormal starch granule formation in *Zea mays* L. endosperms possessing the amylose-extender mutant. *Crop Sci.*, 1976, 16: 298-301.
56. Schwall G., Safford R., Westcott R., Jeffcoat R., Tayal A. Production of very-high-amylose potato starch by inhibition of SBE A and B. *Nature Biotechnology*, 2000, 18: 551-554 (doi: 10.1038/75427).

57. Tareke E., Rydberg P., Karlsson P., Eriksson S., Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.*, 2002, 50(17): 4998-5006 (doi: 10.1021/jf020302f).
58. Shepherd L., Bradshaw J., Dale M., McNicol J., Pont S., Mottram D., Davies H. Variation in acrylamide producing potential in potato: segregation of the trait in a breeding population. *Food Chem.*, 2010, 123(3): 568-573 (doi: 10.1016/j.foodchem.2010.04.070).
59. Hou J., Zhang H., Liu J., Reid S., Liu T., Xu S., Tian Z., Sonnewald U., Song B., Xie C. Amylases StAmy23, StBAM1 and StBAM9 regulate cold-induced sweetening of potato tubers in distinct ways. *J. Exp. Bot.*, 2017, 68(9): 2317-2331 (doi: 10.1093/jxb/erx076).
60. Xin Z., Browse J. Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant, Cell & Environment*, 2000, 23(9): 893-902 (doi: 10.1046/j.1365-3040.2000.00611.x).
61. Mottram D., Wedzicha B., Dodson A. Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature*, 2002, 419: 448-449 (doi: 10.1038/419448a).
62. Halford N., Curtis T., Muttucumaru N., Postles J., Elmore J., Mottram D. The acrylamide problem: a plant and agronomic science issue. *J. Exp. Bot.*, 2012, 63(8): 2841-2751 (doi: 10.1093/jxb/ers011).
63. Zhang H., Hou J., Liu J., Xie C., Song B. Amylase analysis in potato starch degradation during cold storage and sprouting. *Potato Res.*, 2014, 57(1): 47-58, (doi: 10.1007/s11540-014-9252-6).
64. Weise S., Kim K., Stewart R., Sharkey T. β-Maltose is the metabolically active anomer of maltose during transitory starch degradation. *Plant Physiol.*, 2005, 137(2): 756-761 (doi: 10.1104/pp.104.055996).
65. Cottrell J., Duffus C., Paterson L., Mackay G., Allison M., Bain H. The effect of storage temperature on reducing sugar concentration and the activities of three amylolytic enzymes in tubers of the cultivated potato, *Solanum tuberosum* L. *Potato Res.*, 1993, 36(2): 107-117 (doi: 10.1007/BF02358725).
66. Wiberley-Bradford A., Busse J., Bethke P. Temperature-dependent regulation of sugar metabolism in wild-type and low-invertase transgenic chipping potatoes during and after cooling for low-temperature storage. *Postharvest Biol. Tec.*, 2016, 115: 60-71 (doi: 10.1016/j.postharvbio.2015.12.020).
67. Bhaskar P., Wu L., Busse J., Whitty B., Hamernik A., Jansky S., Buell C., Bethke P., Jiang J. Suppression of the vacuolar invertase gene prevents cold-induced sweetening in potato. *Plant Physiol.*, 2010, 154(2): 939-948 (doi: 10.1104/pp.110.162545).
68. Ye J., Shakya R., Shrestha P., Rommens C. Tuber-specific silencing of the acid invertase gene substantially lowers the acrylamide-forming potential of potato. *J. Agric. Food* Chem., 2010, 58(23): 12162-12167 (doi: 10.1021/jf1032262).
69. Liu X., Zhang C., Ou Y., Lin Y., Song B., Xie C., Liu J., Li X.Q. Systematic analysis of potato acid invertase genes reveals that a cold-responsive member, StvacINV1, regulates cold-induced sweetening of tubers. *Mol. Genet. Genomics*, 2011, 286(2): 109-118 (doi: 10.1007/s00438-011-0632-1).
70. Wu L., Bhaskar P., Busse J., Zhang R., Bethke P., Jiang J. Developing cold-chipping potato varieties by silencing the vacuolar invertase gene. *Crop Sci.*, 2011, 51(3): 981-990 (doi: 10.2135/cropsci2010.08.0473).
71. Draffehn A., Meller S., Li L., Gebhardt C. Natural diversity of potato (*Solanum tuberosum*) invertases. *BMC Plant Biol.*, 2010, 10(1): 271 (doi: 10.1186/1471-2229-10-271).
72. Slugina M., Snigir E., Ryzhova N., Kochieva E. Structure and polymorphism of a fragment of the Pain-1 vacuolar invertase locus in *Solanum* species. *Mol. Biol. (Russia)*, 2013, 7(2): 215-221 (doi: 10.7868/S0026898413020146).
73. Slugina M.A., Khrapalova I.A., Ryzhova N.N., Kochieva E.Z., Skryabin K.G. *Doklady Akademii nauk*, 2014, 454(1): 100 (doi: 10.7868/S0869565214010253) (in Russ.).
74. Slugina M., Shchennikova A., Kochieva E. TAI vacuolar invertase orthologs: the interspecific variability in tomato plants (*Solanum* section *Lycopersicon*). *Mol. Genet. Genomics*, 2017, 292(5): 1123-1138 (doi: 10.1007/s00438-017-1336-y).
75. Rausch T., Greiner S. Plant protein inhibitors of invertases. *Biochim. Biophys. Acta*, 2004, 1696(2): 253-261 (doi: 10.1016/j.bbapap.2003.09.017).
76. Brummell D., Chen R.K.Y., Harris J.C., Zhang H., Hamiaux C., Kralicek A.V., McKenzie M.J. Induction of vacuolar invertase inhibitor mRNA in potato tubers contributes to cold-induced sweetening resistance and includes spliced hybrid mRNA variants. *J. Exp. Bot.*, 2011, 62(10): 3519-3534 (doi: 10.1093/jxb/err043).
77. Glaczinski H., Heibges A., Salamini R., Gebhardt C. Members of the Kunitz-type protease inhibitor gene family of potato inhibit soluble tuber invertase in vitro. *Potato Res.*, 2002, 45(2-4): 163-176 (doi: 10.1007/BF02736112).
78. Liu X., Cheng S., Liu J., Ou Y., Song B., Zhang C., Lin Y., Li X., Xie C. The potato protease inhibitor gene, St-Inh, plays roles in the cold-induced sweetening of potato tubers by modulating invertase activity. *Postharvest Biol. Tec.*, 2013, 86: 265-271 (doi: 10.1016/j.postharvbio.2013.07.001).
79. Liu Q., Guo Q., Akbar S., Zhi Y., El Tahchy A., Mitchell M., Li Z., Shrestha P., Vanhercke T., Ral J.P., Liang G., Wang M.B., White R., Larkin P., Singh S., Petrie J. Genetic enhancement of oil content in potato tuber (*Solanum tuberosum* L.) through an integrated metabolic engineering strategy. *Plant Biotechnol. J.*, 2017, 15(1): 56-67 (doi: 10.1111/pbi.12590).