AGRICULTURAL BIOLOGY,
2017, V. 52, № 1, pp. 25-36
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Potato farming: science and technologies

UDC 633.491:664.22:631.523:577.21

doi: 10.15389/agrobiology.2017.1.25rus
doi: 10.15389/agrobiology.2017.1.25eng

TARGET GENES FOR DEVELOPMENT OF POTATO (*Solanum tuberosum* L.) CULTIVARS WITH DESIRED STARCH PROPERTIES (review)

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The authors declare no conflict of interests
Acknowledgements:
Supported by Budget Project of Institute of Cytology and Genetics SB RAS for Potato Program
Received November 7, 2016

Abstract

Starch is an important organic feedstock easily available for human in industrial scale. Optimal physical and chemical properties of amylose and amylopectin molecules comprising starch significantly vary in dependence on the technical scope. Molecular and supramolecular composition as well as structure of the molecules are genetically regulated and may be considered as traits for selection. Combining genes in certain composition one may program potato plant to produce starch of predetermined structure and properties. The main goal of the review is analysis of chain sequence industrial application→starch properties→enzymes→coding genes and discussion of genes and gene compositions programming synthesis of certain starch modifications in potato tubers. Potato genotype may be changed in a controlled manner by classical combination breeding or marker-assisted selection as well as genetic engineering approaches, including the new breakthrough genome editing technologies. Starch biosynthetic pathway in tuber cells requires participation of at least seven main enzymes in cytosol and plastids and of about ten more enzymes in starch granule surface or inner space. Thus, granule-bound starch synthase gene (*GBSS*) knockout drastically increases amylopectin content up to > 98 %. That is the namely reason why cultivars with *GBSS* knockout turned out the first genetically modified forms of potato with corrected starch, field-tested as a technical crop. High amylopectin starch gives gels with high optical clearance, stability during centrifugation, and demonstrates valuable increase of maximum and final gelatinization temperature as well as different rheological behavior. If both *GBSS* and starch synthases genes *SSII* and *SSIII* are inhibited, the starch gives the gel, which is much more stable in prolonged freezing, or multiple freeze—thaw cycles compared to ordinary starch gel. The *SBEI* gene encoding the main starch branching enzyme being inhibited does not increase amylose content in modified potato. But simultaneous inhibition of both *SBEI* and *SBEII* genes results in high (60-89 %) amylose starch with minor amylopectin content. Elevation of *SBEII* expression allows obtaining starch characterized by increased amylopectin branching with shorter end chains. On contrary, amylopectin from potato plants with inhibited SBE synthesis has longer polysaccharide chains with lower branching. *GWD* gene knockout results in amylopectin with reduced phosphate content and, accordingly, reduced viscosity gels from the modified starch. Low phosphate starch demonstrates also a reduced rate of biocatalytic hydrolysis. Overexpression of *SSIV* results in increased tuber starch content in both greenhouse and field grown plants. Starch granule morphology and crystallinity may be corrected on genetic level as well. Typically, morphological traits including physical and chemical properties of starch are regulated by not one or two genes, but a certain gene network. So, discovery of qualitative trait loci and identification of diagnostic markers for them allows application of marker-assisted selection for developing potato cultivars with predetermined starch properties as an optimal feedstock for certain industries.

Keywords: potato, starch, biosynthesis genes, starch synthase, amylose, amylopectin, branching enzyme, physical and chemical properties

Starch is one of the few complex organic substances readily available for human economic activity on an industrial scale. From the point of view of the composition homogeneity, with rich possibilities for modifying its constituent

molecules of amylose and amylopectin [1, 2], starch outperforms other sources of organic raw material, such as oil, which contains mostly simpler hydrocarbon molecules in difficult-to-separate mixtures, or lignocellulose, which requires chemical processes for the separation of its constituent heterogeneous poly- and oligomers. The variety of products that can be obtained from starch as a result of chemical or biotechnological transformations determines the range of fields of its application [3]. It is an accessible, eco-friendly and economical biopolymer that is widely used in a native or modified form (Fig. 1). In Europe, 70-80 % of starch go to non-food needs [4, 5]. In Russia, native starches are widely used in the food industry (confectionery, bakery, in sausage and canned food production), as well as in the production of corrugated board [6].

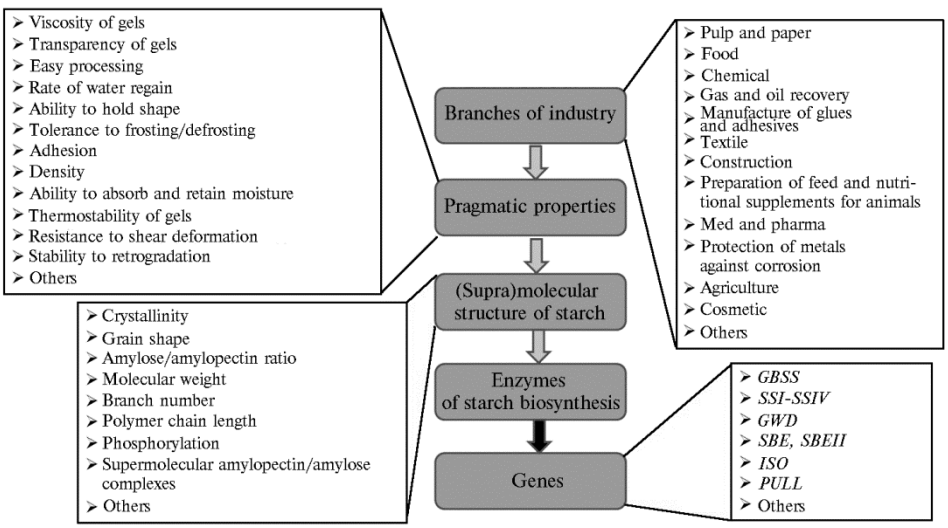

Fig. 1. Molecular and supramolecular characteristics, which are determined by genes controlling the enzymatic biosynthesis of starch in a cell, and responsible for its properties and practical applications. The target genes of starch synthases (*GBSS* and *SSI-SSIV*), H_2O-dikinase (*GWD*), starch branched enzymes (*SBEI* and *SBEII*), isoamylase (*ISO*), pullulanase (*PULL*) are indicated.

Starch is a complex carbohydrate of plant origin, consisting of two types of polymer molecules (amylose and amylopectin), each of which is a homopolymer formed from identical monomer units (glucose residues), with general formula $(C_6H_{10}O_5)_n$. The molecular composition and structure, as well as the supramolecular assembly of these molecules are regulated by the genes of starch biosynthesis through the corresponding proteins and, therefore, can be considered as phenotypic signs for selection. A combination of certain variants of such genes allows the potato plant to be programmed to produce starch with a given structure and properties.

Practical interest is seen in the amylose-amylopectin ratio, their branching and molecular weight, crystallinity, granule size and porosity, the amount of phosphate residues in polymer chains, the rheological and optical properties of starch gels (see Fig. 1). Molecular and supramolecular characteristics of native starch are responsible for qualities, due to which one or another variety of this natural polymer can find its application in a certain branch of industry. An optimal combination of physical and chemical properties of the molecules that make up starch varies considerably depending on the field of its application.

The purpose of this review is to discuss variants of genes or their combinations that program starch biosynthesis in different modifications, depending on the planned practical result with a sequential task definition at the stages as follows: commercial application of starch→starch properties→proteins

(enzymes)→encoding genes.

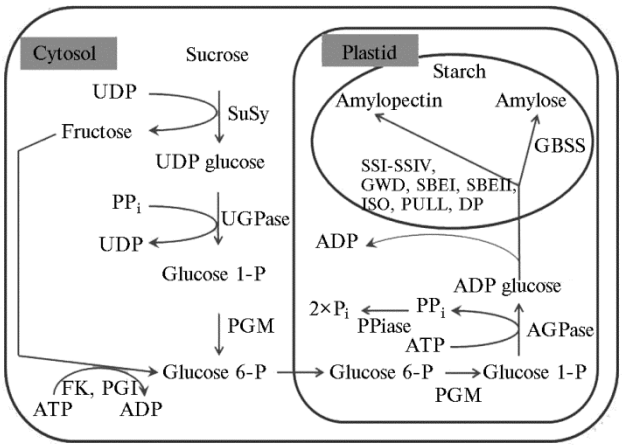

Fig. 2. A diagram of starch biosynthesis in a cell of the potato tuber pulp (7, 8): SuSy — sucrose synthase; UGPase — UDP-glucose pyrophosphorylase; FK — fructokinase; PGM — phosphoglucomutase; PGI — phosphoglucose isomerase; PPiase — pyrophosphatase; AGPase — ADP glucose pyrophosphorylase; GBSS — granule-bound starch synthase; SSI-SSIV — starch synthases; SBEI-SBEII — starch branching enzymes; GWD — H_2O-dikinase; ISO — isoamylase; PULL — pullulanase; DP — disproportionating enzyme; PP_i — pyrophosphate; P_i — phosphate.

The biosynthesis of starch in the tuber pulp cells occurs with the participation of 7 major enzymes in the cytosol and plastids and about 10 more enzymes on the surface or inside the starch grains located in plastids (Fig. 2).

Understanding mechanisms of biosynthesis allows to influence the process in order to adjust the physical and chemical properties of starch so that they better match the needs of certain industries. This effect can be implemented either using the combining selection in its classical concept and diagnostic DNA markers (MAS, marker-assisted selection), or with the involvement of genetic engineering, including for the production of non-transgenic plants with predetermined properties based on safe technologies for genomic editing [9, 10]. Information on target genes is needed for marker-assisted selection and genomic editing. One of the challenges with biological production of starch with predetermined properties is that the measured physical and chemical properties (signs) of starch are usually formed by the gene network as a result of different loci performance. Sometimes it is feasible to identify several of the most important controlling genes, the inhibition or activation of which leads to a significant change in a certain property of starch, and only in exceptional cases a simple “one gene-one chemical trait” correlation can be observed.

Despite the fact that the legislative regulation of the Russian Federation currently does not support the commercial production of genetically modified plants, their production in a laboratory setting is advisable for a number of reasons [11] One of them is the confirmation of the relation between the expression of one or more genes and the properties manifested in the organism obtained. Let us consider the known examples of the influence of the genetic modification of *Solanum tuberosum* L. potato on some of the physical and chemical properties of starch.

High content of amylopectin. Potato starch contains about 80 % of branched amylopectin polysaccharide and 20 % of linear amylose polysaccharide. The main gene responsible for the synthesis of amylose is *GBSS* which encodes granule-bound starch synthase. A modification of this gene dramatically changes the ratio of amylose and amylopectin (up to the amylopectin content > 98 %). It was the genotypes with knockout of the indicated *GBSS* gene that were obtained earlier than other genetically modified forms of potato with altered starch and tested in the field as a technical crop for industrial use, e.g. Amflora (EH92-527-1) produced by BASF Plant Science GmbH (Germany) [12, 13] or the variety by Avebe U.A. (Netherlands) [14]. More recently, a

knockout of the *GBSS* gene was successfully performed using CRISPR/Cas9, the state-of-the-art system for genomic editing. The approach used by the authors allowed the site-directed mutagenesis to be carried out without inserting a foreign DNA into the potato genome. This means that modified, but not transgenic potato plants were obtained. At the same time, in 2 % of the regenerants, genetic editing took place in all four alleles of the *GBSS* gene. The presence of at least one functional allele of the *GBSS* gene is sufficient for a significant amount of amylose to be synthesized in the tuber, and only inhibition of all four alleles results in the formation of starch containing mainly amylopectin [15], which significantly affects the technical characteristics of starch. Starch with a high content of amylopectin produces gels with enhanced optical transparency and stability during centrifugation, and also shows an increase (by 5-6 °C) in the maximum and final gelatinization temperature [16] and a change in rheological properties [17]. If, in addition to *GBSS*, the genes of the *SSII* and *SSIII* starch synthases are inhibited, the tuber starch would be low in the amylose content, with the shortened terminal chains of amylopectin. Gels obtained from such starch are much more stable than those obtained from conventional starch, both during prolonged freezing and in several freeze-thaw cycles [18]. Low amylose starch is better suited for obtaining thickening agents for the textile and pulp and paper industries [19].

High content of amylose. To switch off the synthesis of amylopectin, which is a branched polymer, it is necessary to inhibit the *SBE* gene which encodes a starch branching enzyme (SBE). However, repression of *SBEI*, the major branching enzyme gene, in practice did not result in an increase in the amylose content in the modified potato [20]. A knockout of the *SBEII* gene responsible for the synthesis of the minor form of the SBEII branching enzyme, resulted in a moderate increase in the relative content of amylose (by 38 %) [21]. And only concurrent inhibition of both *SBEI* and *SBEII* genes made it possible to obtain starch with a high portion of amylose (60-89 %) and an addition of a small amount of branched amylopectin. Thus, the SBEII enzyme is required for the synthesis of normal branched amylopectin, and possibly enhances the SBEI activity, though the role of SBEI has not yet been elucidated in detail, given some differences in the mechanisms of action of these enzymes [5, 22]. Field trials conducted for a number of years have confirmed that in potato varieties obtained by inhibition of both branching enzymes the high amylose content of starch still persists.

Inhibition of the *SBEI* and *SBEII* genes also results in a 5-6-fold increase in the number of phosphate groups in the molecules. In addition, with an increase in the amylose content in starch from 20 to 60-80 %, its accumulation in tubers becomes noticeably decreased (from 22-23 to 10-15 %), and the granule size also reduces (from 50-63 to 32-39 μm) [23].

The branching of amylopectin. In potato, only two isoforms of SBE are responsible for the branching of the amylopectin molecule: the major SBEI and the minor SBEII branching enzymes. The construction of a cis-gene, consisting of complementary and genomic DNA fragments (cDNA and gDNA) of the *SBEII* gene under the control of a strong promoter of another potato gene, *GBSS*, made it possible to enhance the expression of the SBEII enzyme. In the modified forms of potato, the branching of the amylopectin molecules was higher (a degree of polymerization 6-12, mostly closer to 6), and the chains are shorter than in the selection varieties [24]. And on the contrary, with suppressed synthesis of SBE, the degree of amylopectin branching decreases [25], and the number of elongated phosphorylated chains with a degree of polymeri-

zation above 14 increases.

When the expression of the *SBE* gene is inhibited, starch acquires the ability to gelatinize in lowered temperatures (by 3 °C) and lower urea concentration (gelation agent), its viscosity is reduced and the volume in water regain is increased [24].

The content of phosphate groups. Some polysaccharide chains in starch granules contain covalently attached phosphate groups. In industry, starch is purposely phosphorylated by treating with orthophosphoric acid, which results in obtaining a food additive E1410, i.e. starch with an increased content of phosphate groups. Phosphorylated starch with negatively charged phosphate groups, which are repelled from each other in solution, much more rapidly increases its viscosity, gives more transparent and viscous gels less prone to retrogradation. In addition, phosphate residues can effectively bind to metal ions, making phosphorylated starch an effective ligand.

In potato starch, 0.2-0.5 % glucose monomers are phosphorylated which is several times higher than in starches obtained from other sources [26]. The most important enzyme for phosphorylation of starch is α-glucan-H_2O-dikinase [27, 28], encoded by the *GWD* gene. It has been reported that knocking out the *GWD* gene results in the synthesis of amylopectin with reduced amounts of phosphate groups and, correspondingly, decreased viscosity of gels obtained from such a starch [27, 29]. It has also been established that the rate of biocatalytic hydrolysis of starch, which is characterized by the reduced content of phosphate groups, decreases [30]. For arabidopsis, another phosphorylating enzyme, phosphoglucan-H_2O-dikinase (PWD), has been shown, which converts the C6-phosphorylated fragment of the molecule to C3-phosphorylated [31]. However, so far there has been no data on whether an enzyme with a similar effect is available in potatoes.

Importantly, with a decrease in the number of the glucan polymer chain branches, i.e. when the activity of the SBE branching enzymes is inhibited, the degree of chain phosphorylation increases (20), sometimes up to 5-6-fold [5].

The method of association mapping revealed genetic markers associated with the degree of phosphorylation of starch at C3 and C6 positions in the residues of D-glucose. A significant association of the trait with certain SNP (single nucleotide polymorphism) in the genes encoding α-glucan-H_2O-dikinase (GWD), starch branching enzyme (SBEI) and starch synthase (SSIII) has been shown. In addition, a link between a polymorphism of a simple sequence repeat (SSR) within the gene encoding the SBEII branching enzyme has also been found. At the same time, the SNP in the *GWD* gene is associated exclusively with C6 phosphorylation, in the *SSIII* gene with C3 phosphorylation, and the polymorphic sites in *SBEI* and *SBEII* are associated with both C3 and C6 phosphorylation [32].

Consequently, potato modifications aimed at enhancing the expression of the *GWD* gene and/or inhibiting the *SBE* and *SSIII* genes activity would allow to produce much-needed in industry starch with an increased content of phosphate groups, bypassing the stages of chemical processing of natural raw materials, i.e. to obtain an environmentally friendly product with a reduced production cost.

Starch content. The starch content of tuber depends on the enzymatic reactions aimed at both the synthesis and the cleavage of starch. Three main stages of starch formation are controlled by three enzymes: ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS) and starch branching enzyme (SBE). And it is AGPase (encoded by the *glgc-16* gene) that catalyzes the reaction which limits the rate of starch biosynthesis. Indeed, the expression of the *glgc-16* transgene in potato plants increased the starch content by 30 % [33, 34].

Enhanced expression of SSIV increased the amount of starch in the tubers of transgenic forms both in the greenhouse and in the field from 94 g to 98-137 g per plant, providing an increase in the product yield from 4.25 to 4.40-6.10 t/ha (depending on the cultivar) [35].

The cleavage of the polymer chains of starch is regulated by amylases. Due to their activity, the starch content of potato tuber decreases and storage time increases. Recently, it has been shown that the *SbAl* gene inhibits amylase, slowing the hydrolysis of starch and controlling its content in tuber. At the same time, the amount of reducing sugars decreases. As a result, chips made from such tubers have a less intense brown colour [34]. Phosphate groups in amylopectin molecules promote the hydrolysis of starch, therefore their reduced amount leads to the accumulation of this polysaccharide. In potatoes, the overexpression of a gene that regulates the phosphorus content in starch in arabidopsis (*AtPAP2*, encodes purple acid phosphatase) results in the increased yield (2-3 times) of tubers and their size due to solids and starch content [36].

Breeding for regulation of the activity of genes responsible for the phosphorylation and synthesis of polymer chains of starch, as well as the inhibition of amylases, can be applied to obtain technical varieties of potatoes with a high content of starch.

Crystallinity of granules. Tuber starches, including potato starches, are characterized by the presence of B-polymorphs in crystalline domains [37], usually well-structured, compact and responsible for the formation of starch granules with a visually smooth surface. It is believed that it is the supramolecular organization of the amylopectin branches in starch that is responsible for its crystallinity [38]. Due to the well-ordered structure, the starch granules are very resistant to amylolytic degradation [39]. In fact, there were no differences in the dimensions of amylopectin crystallites, the thickness of the crystalline lamellae of the granules, and the polymorphic structure of starch from potato tubers deficient for the expression of the *GBSS* (encodes starch synthase controlling the amylose synthesis) or *GWD* genes and unmodified plants. The reduced content of the *GBSS* or *GWD* encoded enzymes affects only the pattern of the defects in amylopectin crystallites [40]. The impact of starch synthase (SSII) in rice [41] associated with starch granules (GBSS) in the representatives of *Chlamydomonas* [42] and in the SGP (starch granule protein) in wheat [43] on the crystallinity of starch granules has been described in the literature, although experimental findings on potatoes are limited. Therefore, it is still rather difficult to propose a strategy for increasing the bioavailability and reactivity of starch grain through selection methods or gene modification. However, it should be borne in mind that facilitating these processes on an industrial scale could lead to a reduction in energy costs, saving of water, reagents and operational time, and also provide the deeper processing of starch.

Morphology of granules. Of interest, the expression of a foreign starch branching enzyme (SBE from *Escherichia coli*) does not enhance the branching of amylopectin in potatoes, but significantly changes the morphology of the starch granules: hummocky granules with deep pores are formed in an amylose-containing mutant, and tuberous agglutinated granules in the amyloseless line [43, 44].

Not every species of bacteria or yeast can destroy the supramolecular organization of polymer molecules of starch to involve them into biochemical transformations. Due to the limited availability of this polysaccharide, the degree of its modification during chemical processing also remains low, and to increase it, a temperature higher than the gelation temperature is required. In starch granules with the altered morphology, increased surface area and a larger pore

size, the reaction centers are more accessible for chemical reagents and enzymes, and therefore may exhibit increased reactivity, including at low temperatures.

Genetic markers of quantitative traits. Despite the fact that the properties of starch in potato tubers can be modified by affecting a certain number of genes, the general nature of displaying the expression activity of DNA regions during formation of quantitative traits is much more complicated. Using the method of interval mapping of genomes in a population of diploid potatoes, it was shown that starch phosphorylation is regulated and/or controlled by five quantitative trait loci (QTL) on chromosomes 2, 5 and 9, and the content of amylose by six loci on chromosomes 2, 3, 5, 7 and 10. Similarly, loci controlling the starch grain size, the starch content of potato tuber and the temperature of starch gelatinization were discovered [45, 46]. Many of the identified QTLs coincided by their localization with the known genes that encode the enzymes of starch biosynthesis, but, in addition, loci have also been discovered in which no one of the starch biosynthetic genes was previously mapped. The nucleotide sequences corresponding to these QTLs have not yet been deciphered and the molecular mechanisms through which they influence the alterations in the properties of starch are still not clear. However, the stable association of such loci with traits and the presence of DNA markers closely linked to these loci makes it possible to use the results obtained for marker-assisted selection. Indeed, for tetraploid potato, three markers had been identified, associated with the traits of productivity, i.e. the yield of tubers per hectare and the starch content of tuber [47], which were later used for marker-assisted selection [48]. Another study showed that single nucleotide substitutions in the genes encoding the Pain1 and InvCD141 invertase enzymes, the SSIV starch synthase, the StCDF1 transcription factor and the LapN aminopeptidase are associated with the yield of tubers and starch and starch content of tuber [49]. Identified SNPs can also be used for controlled selection of potato forms possessing the specified technological properties. In addition, markers associated with the desired quantitative traits in potato would be possibly found in its plastid and mitochondrial genomes. For example, it has been shown that the W/y-type cytoplasm in European potato varieties correlates with a high content of starch [50]. Findings on the effects of the starch biosynthesis genes on its properties are summarized in Table 1.

1. The genes of starch biosynthesis, the corresponding enzymes, their effects on the properties of starch in potato (*Solanum tuberosum* L.) (7, 50-52)

Gene	NCBI No.	Linkage group	NCBI Reference	Product	EC	Starch properties
SuSy	102577594	VII, XII	Baroja-Fernández E. et al. Plant Cell Physiol, 2003, 44(5): 500-509	Sucrose synthase	2.4.1.13	Content in tubers
UGPase	102577726	XI	Katsube T. et al. Biochemistry, 1991, 30(35): 8546-8551	UDP-glucose pyrophosphorylase	2.7.7.9	Content in tubers
PGM	102585015	N/A	None	Phosphoglucomutase	5.4.2.2	Content in tubers
FK	102577816	VI	Smith S.B. et al. Plant Physiol., 1993, 102(3): 1043	Fructokinase	2.7.1.4	Content in tubers
PGI	102577825	N/A	None	Phosphoglucose isomerase	5.3.1.9	Content in tubers
PPiase	102584131	VIII, IX, XII	du Jardin P. et al. Plant Physiol., 1995, 109(3): 853-860	Pyrophosphatase	3.6.1.1	Content in tubers
AGPase	102577790 (small subunit)	I, IV, VII, VIII, XII	du Jardin P. et al. Plant Mol. Biol., 1991, 16(2): 349-351	ADP-glucose-pyrophosphorylase	2.7.7.27	Content in tubers
GBSS	102577459	VIII, II	van der Leij F.R. et al. Mol. Gen. Genet., 1991, 228(1-2): 240-248	Granule-bound starch synthase	2.4.1.21	Amylose Content
SSI	102600045	III	Kossmann J. et al. Planta, 1999, 208(4): 503-511	Starch synthase	2.4.1.21	Content in tubers
SSII	102583115	N/A	Kossmann J. et al. Planta, 1999, 208(4): 503-511	Starch synthase	2.4.1.21	Content in tubers
SSIII	102577674	II	Marshall J. et al. Plant Cell, 1996, 8(7): 1121-1135	Starch synthase	2.4.1.21	Content in tubers

Table 1 (continued)

GWD	102577510	V	Lorberth R. et al. Nat. Biotechnol.. 1998, 16(5): 473-477	H_2O-dikinase	2.7.9.4 2.7.9.5	Phosphorylation
SBEI	102596498	IV	Kossmann J. et al. Mol. Gen. Genet., 1991, 230(1-2): 39-44	Starch branching enzyme	2.4.1.18	Branching of amylopectin chains
SBEIIA *SBEIIB*	102590711	IX	Larsson C.T. et al. Plant Mol. Biol., 1998, 37(3): 505-511	Starch branching enzyme	2.4.1.18	Branching of amylopectin chains
ISO	102577466	XI	Sun C. et al. Plant Mol. Biol., 1999, 40(3): 431-443	Isoamylase (hydrolysis at branching points)	3.2.1.68	Isomerization of starch
PULL	102581262	N/A	None	Pullulanase	3.2.1.41	Isomerization of starch
DP	Different options	IV	None	4-α-Glucotransferase	2.4.1.25	Isomerization of starch

N o t e. NCBI — National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/).

As a reminder, despite the multiple-factor nature of the impact of many genes involved in the biosynthesis of starch, in some cases, through their inhibition or the other way around overexpression, it was possible to change certain properties of starch (Table 2).

2. The effect of inhibition and expression of the genes encoding the biosynthesis of potato starch on its physicochemical properties

Gene or gene combination	Inhibition or expression outcome	Change in physicochemical properties
GBSS	Inhibition: Decrease in amylose content	Increase in the gel optical transparency and stability during centrifugation, increase (by 5-6 °C) in the maximum and final gelatinization temperature
GBSS, *SSII*, *SSIII*	Inhibition: Decrease in amylose content, shortening of the terminal chains of amylopectin	Gel stability during freezing or freeze-thaw cycles, water regain and ability to absorb water
SBEI	Inhibition: Moderate increase in amylose content, increase in phosphate content, increase in amylopectin chain length	Not established
SBEI and *SBEII*	Inhibition: Significant increase in amylose content, increase in phosphate content	Decreases in gelatinization temperature, gel viscosity, increased volume in water regain, increase in reducing sugars content and amount of phosphate groups
SBEII	Expression: Increase in amylopectin branching, decrease in degree of polymerization of external chains of amylopectin	Decrease in gelatinization temperature, viscosity, increase in water regain volume
GWD	Inhibition: Decrease in amount of phosphate groups	Decrease in gel viscosity, reduced rates of biocatalytic hydrolysis
glgc-16	Expression	Increase in starch content of tuber
SSIV	Expression	Increase in starch content of tuber
SbAI	Expression. Inhibition of amylase activity, slowing down the starch hydrolysis, decreased amount of reducing sugars	Increased starch content of tuber, decreased effect of brown colouration during heat treatment

Therefore, the impact of most genes involved in starch biosynthesis in potato is multi-faceted: on the one hand, each gene is involved in the regulation of the manifestation of several starch physicochemical properties, and on the other, almost every trait is affected by several genes. Genetic engineering methods allow verifying assumptions about the role of a particular gene, as well as directly evaluating the outcome using instrumental procedures. If the outcome coincides with the expectations, the approach can be further used in traditional or marker-assisted selection for obtaining technical varieties in which starch will serve as the optimal raw material for the relevant industries.

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