Chapter 2

Genetically Modified Potato as a Source of Novel Carbohydrates

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Additional information is available at the end of the chapter

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Abstract

Significant progress has been made in understanding of carbohydrate (starch) biosynthesis through molecular biology and genetic engineering techniques. Genetic modification of plants has a great potential to produce novel carbohydrates with unique properties that cannot be generated by conventional breeding approaches. Starch is the predominant carbohydrate in potatoes and serves as an energy reserve for the plant. Genetic engineering of potato (*Solanum tuberosum* L.) tuber can revolutionise the synthesis of unique starches with altered physical and chemical properties that are engineered to meet the specific industrial requirements. In addition to expression of foreign genes involved in carbohydrate biosynthesis, genes regulating the carbohydrate metabolism, transport and resource partitioning have also been achieved. Here we summarise the recent progress made towards modifications of the biosynthetic pathways by which potato can produce novel carbohydrates. Further, we discuss the prospects of engineering potatoes for production of structural and non-structural carbohydrates.

Keywords: carbohydrate metabolism, starch, genetic engineering, novel carbohydrates, *Solanum tuberosum* L., sucrose transport

1. Introduction

The most abundant bio-compounds on our planet are the carbohydrates which is synthesised by green plants during the process of photosynthesis. More than 100 billion metric tons of CO_2 and H_2O per year are converted into carbohydrates during the process of photosynthesis [1]. The carbohydrates converted into the starch in the plant plastids which is the major storage

carbohydrate found in various types of green plant tissues and organs. Two types of starch are reported to be present in the plants which are distinguishable as the 'transitory starch' and the 'storage starch'. The transitory starch is synthesised and accumulated in chloroplasts of green leaves during photosynthesis and degraded throughout the night to provide substrates for respiration and continued sucrose synthesis to supply to sink tissues [2]. The storage starch is synthesised in amyloplasts and typically associated with sink organs, such as stems, seeds, roots, and tubers. This type of starch is accumulated during different developmental stages and utilised during various periods of dormancy, germination, growth, or other specific processes. The storage starch is deposited in granules rich in amylose content than that observed in the transitory starch [3, 4].

According to the recent reports published by the Food and Agricultural Organisation Statistics (FAO statistics), the potato (*Solanum tuberosum* L.) stands at the world's 4th major crop in terms of yield after rice, wheat and corn, and in terms of area under cultivation, its stands at the 8th position. The potato is grown for its tuber which is considered as is a high-energy staple food around the world and high productivity per unit area due to its intense cultivation. Thus, the potato represents one of the best candidates for alleviating food shortages. Potato belongs to *Solanaceae* family, is the perennial herbaceous plant having white to purple flowers with yellow stamens. Some potato cultivar bears small green fruits, each containing up to 300 seeds. Potato is grown from the botanical seeds or usually propagated vegetatively by planting pieces of tubers containing eyes or dormant buds which develop into new shoots (sprouts) when grown under suitable conditions. Potato tubers are rich in starch, storage proteins and develop by the morphological changes of the underground stem into stolon bearing auxiliary buds and scars of scale leaves. The total carbohydrates in potato tuber range from 1.0 to 7.0 g/kg. The reducing sugar (glucose, fructose) concentrations are higher in young tubers and reduced significantly near the end of the cultivating season. Starch is the prime carbohydrate component of the potato dry matter containing the amylose and amylopectin. Starch has conventionally been used in the food industry to augment the functional properties of various foods. The physicochemical and functional properties of starch system vary with the starch biological origin. The structural characteristics and amylose-to-amylopectin ratio of potato starch also vary among cultivars. Nutritional and processing quality of potato products (frozen and dry) are greatly affected by their starch characteristics and content. Several chemical, physical, and enzymatic modifications have been accomplished to improve the processing operation of potato starch. Potato starch can be used in other industrial applications as a gelling agent, thickener, bulking agent, colloidal stabiliser and water-holding agent. However, due to low shear and thermal resistance and high bent towards degradation hinder its use in some industrial food applications. These limitations are generally overcome by starch modification, which can be achieved through derivatization, such as etherification, esterification, cross-linking, and grafting; decomposition (acid or enzymatic hydrolysis and oxidisation); or physical treatment of starch using heat or moisture or pressure, etc. Most of these modifications are usually recognised as non-toxic by the safety authorities. Several modified potato starches with slow digestibility are being developed that may provide nutritional benefits for humans. These starches have the potential to be used for the treatment of certain medical conditions (e.g., glycogen storage disease and *Diabetes mellitus*). The Food and Drug Administration (FDA) controls and emphasises the type and amount of each chemical used in starch modification, as well as the percentage of the substitution.

Starch which is known as the product of the plant photosynthetic carbohydrates, is commercially isolated from a wide range of sources including cereal grains such as corn, wheat, rice, and sorghum; roots and tubers such as potato, sweet potato, cassava, and arrowroot; and stem and pith such as sago. The composition of naturally occurring starch is universal, irrespective of its source, with the main component as amylopectin (75%) and a minor component as amylose (25%). Amylose and amylopectin are synthesised in the plastids, where they assemble into a semi-crystalline granule. The starch found in potato tuber is distinct granules approximately 10–100 μm in diameter [5]. Compared with other commodity starches, the potato starch granules are relatively larger, smooth with a high content of covalently linked phosphate, long amylopectin chains and high-molecular weight amylose. The granules are synthesised by two polysaccharides consisting exclusively of glucose as the monomer component. The glucopyranosyl residues are connected through α-d-(1,4)-linkages forming chains through α-d-(1,6)-branches at the reducing end side linked to similar other chains. The industrial application of starch includes the manufacture of high-quality paper [6] and generation of viscous hydrocolloid systems to be used for food processing [7]. However, the well-ordered and dense structure of the native potato starch granule renders it resistant to enzymatic degradation by hydrolytic enzymes such as amyloglucosidases and α-amylases [8], which is very important in industrial applications. Amylopectin is the major component of starch in general, and in potato it normally constitutes 70–80% by weight [9] regardless of the size of the granules [10]. Approximately 4–6% of linkages are of the α-d-(1,6)-type, making it extensively branched. The weight-average molecular size of amylopectin is on the order of 107 Da [11, 12]; as a result, the macromolecule consists of a huge number of relatively short chains with an average degree of polymerisation (DP) of 21–28 residues [13, 14]. The amylose is considerably smaller than amylopectin and is basically a linear polymer comprising of 2000–5000 residues [5]. This composition may affect the physicochemical properties, such as gelatinization, texture, moisture retention, viscosity, and product homogeneity that are determinants for its industrial applications. Besides the polysaccharide components, potato starch consists of low amounts of material of a non-carbohydrate nature. Less than 0.5% of the granules are proteins [9], apparently involved in starch synthesis. Potato starch also contains phosphorus in the form of phosphate covalently linked to the amylopectin component. It is considered an important factor contributing to potato starch properties.

2. Mechanism underlying starch biosynthesis in potato

The photosynthesis is the primary metabolic mechanism for the carbohydrate biosynthesis which is well-known phenomenon of the plant that takes place with the help of the chlorophyll and light. However, the conversion of carbohydrates to starch granule is a subtle equilibrium between proficient packing of the glucan chains and the prospect of breaking these structures during degradation. Hence, a series of enzyme catalytic activities are required to complete this process in the starch biosynthesis. These include three steps, the first is the activation of the major carbohydrate, the glucose molecule, second step is the elongation of the glucan chain, and the final step is the transfer of linear backbone chains forming branched structures (**Figure 1**). The activation of the glucose residues to form adenosine diphosphoglucose

(ADP-glucose) takes place with the help of enzyme ADP-glucose pyrophosphorylase (AGPase) using the ATP and a molecule of glucose 1-phosphate [15]. This reaction is the rate-limiting step in the starch biosynthesis. In the next step, the elongation of the chain takes place which constitute the amylopectin and finally the starch granule is synthesised with the help of soluble starch synthase (SS) and starch-branching enzymes (SBE). The soluble SS catalyses the elongation of the chain at the non-reducing end in a reaction in which ADP of the ADP-glucose molecule is replaced by the terminal hydroxyl group of the growing glucan chain, creating an elongated linear α-(1,4)-glucan chain. However, only one enzyme is essential recognised as granule bound (GB)-starch synthase (SS) for amylose synthesis. The formation of branched

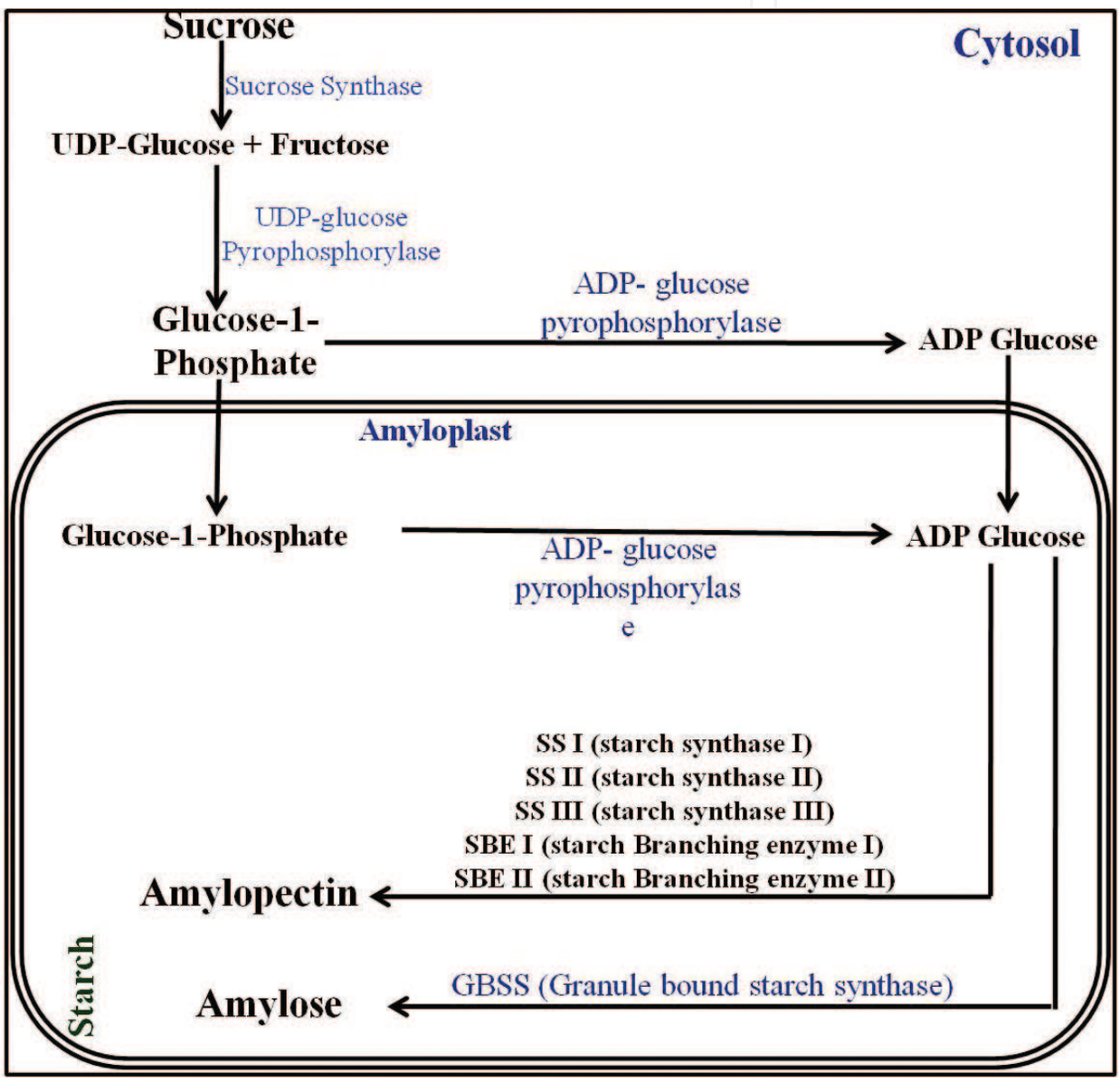

Figure 1. Pathway of starch synthesis. ADP-glucose (ADP-Glc), the donor substrate for both amylose and amylopectin, is synthesised by the ADP-Glc pyrophosphorylase (AGPase). The combined action of different starch synthases (SSI, SSII, and SSIII), branching enzymes (BEI and BEII), and the debranching enzyme (DBE) is necessary for the synthesis of amylopectin. Granule-bound starch synthases (GBSSI and GBSSII) use amylopectin as the acceptor substrate to synthesise amylose, which is formed down-stream of amylopectin.

α-(1,6)-linkages in starch is catalysed by the SBE. In this reaction, an α-(1,4)-linkage within the chain is cleaved and an α-(1,6)-linkage is formed between the reducing end of the cleaved glucan chain and a C-6 linked oxygen of an adjacent chain. The starch-branching enzyme has been reported to exist as multiple enzyme isoforms. The action of debranching activities during biosynthesis seems to be important for correct assembly of the starch granule [16]. This process is generally more complicated as compared to glycogen biosynthesis involving a multitude of different homologous enzymes probably responsible for synthesising specific structures of the starch granule in different tissues and at different developmental stages. Apparently, many of these enzymes interact to form enzyme complexes or metabolomes to channel and direct substrates and products.

New cultivars of potato with better yield, disease resistance, have been developed since long time with the help of breeding techniques. Following the advancement of genetic engineering tools, several other potato cultivars with desired yield, dry matter, protein and antioxidant quality, cooking texture (such as waxy, floury), flesh colour, and abiotic stress tolerant plant have also been developed. The demand for starches with special properties useful for industrial food processing has led to the introduction of modified starches using the genetic engineering techniques. Though, there are a lot of information available in the literature on chemical modification of starch, however, genetically modified potato with altered carbohydrates, starch or amylose/amylopectin content, have also been developed. Some of the genetically modified potato starches are being used in the industry under strict control, however, these transgenic varieties of potatoes are not permitted for food use in several countries because of the concerns related to consumer health and the environment. Until these genetically modified potatoes have been given proper clearance by the food authorities and acceptance by the consumers, they may have a good scope for their use in non-food or other industrial applications. We will first describe attempts to alter starch structure to improve starch functionality, then explain how increasing knowledge of the regulation of starch biosynthesis is being used to increase starch production, and finish by summarising new methods for increasing the genetic diversity in crops as well as methods for fine-tuning gene expression in plants in order to bring improved starch-based products with value-added consumer benefits to the marketplace.

3. Genetic engineering of potato for starch modification

Starch modification involves efforts to both achieving enhanced starch production and modifies the composition or component structure to impart specific properties to suit final product. The most widely referred target for starch modification is the alteration in the amylose-amylopectin ratio. Several plants have been genetically modified in their starch biosynthetic pathway to yield high-amylose and high-amylopectin starches.

3.1. Genetic engineering of potato for starch synthase enzyme

It is evident that the starch synthase (SS; ADP-glucose:α-1,4 glucosyl transferase) catalyses transfer of the glucosyl moieties from ADP-glucose to the non-reducing end of an α-1,4-glucan [17]. The potato SSs enzymes catalyse the same reaction represented as

ADP-glucose + (1,4-alpha-D-glucosyl) (n) = ADP + (1,4-alpha-D-glucosyl) (n + 1). Potato is reported to contain four different SSs isoforms known as SSI, SSII, SSIII and GBSSI. The SSI does not have multiple isoforms in plants while other SSs are present in multiple isoforms, suggesting a presumably unique and important role of SSI in starch biosynthesis [18]. However, the precise role of SSI is still not well-defined. Though the activity of SSI enzyme was repressed to non-detectable quantity in transgenic potato plants silenced for SSI gene, neither amylopectin structure nor starch granule morphology was changed. The reason for no detectible changes might be because SSI is mainly expressed in the potato leaf tissue and mainly involved in the synthesis of transitory starch in the leaves [19]. The SSI prefers the shortest amylopectin chains as substrate, and it is particularly responsible for synthesising amylopectin short chains [20, 21]. It was found that a short glucose chain of 6–7 residues are apparently the substrates for SSI enzyme, which then extend to a length to 8–12 glucose residues inside the amylopectin cluster [22]. Despite these findings, antisense down regulation of SSI in potato tubers did not alter the starch structure, signifying other SSs may fairly balance for the lack of SSI in tubers. The SSIII is the key SS in potato tubers and accounts for just about 80% of soluble starch synthase activity; although a minute fraction of the SSIII activity is found there to the starch granules [23, 24]. Down regulation of SSIII via antisense techniques in transgenic potato tubers led to about 80% loss of SSIII activity, and therefore, alterations in the morphology of starch granule were observed [25]. However, the significance of the individual SS isoforms and their distribution between stroma and starch granules within the plastids is very species-dependent and these differences probably provide to deviations seen in the structure of starches created from diverse plant species [26]. Interestingly, the apparent redundant function of SSII and SSIII in amylopectin biosynthesis is also reported by researchers [27]. The exact role of other SS, the SSIV is still not yet known, however, the development of *Arabidopsis* mutants lacking SSIV and abnormalities in their granule initiation is well studied [28]. The investigations have revealed two additional discrete isoforms of SSIV, that is, SSIVa and SSIVb, which are present in cereal endosperms and leaves, respectively [29]. To the best of our knowledge, an enzyme like any known SSIV has not been reported/or characterised in potato. Blast information using potato genome sequence database (www.potatogenome.net) and sequence based comparison showed that this putative enzyme belongs to the same family as other SSs, the GT5 glycosyltransferase family [30].

Genetic modification in SSs expression reported to an impact on the yield, fine structure and physical properties of the starch. Typically, downregulation of synthase activity does not have much impact on the starch yield possibly because reduced activity in one SS isoform may result in compensatory increases in the activity of another isoform. In case of the transgenic potato with antisense expression of the SSII + SSIII, surprisingly the potato tuber's capability to polymerise glucans from ADP-Glc was mainly compromised. The downregulation of only one SSI activity in potato resulted in reduction of the amylose content. As a result, the physical properties of the starch were also changed, the melting temperature of the granule was increased, and the constancy of the starch solution after gelatinization enhanced due to lack of retrogradation. When SSII was down-regulated in potato, small changes were observed in the chain length distribution. This lends some support to the hypothesis that each synthase may

play a distinct role in elongating side chains of a specific length. The physical properties of the potato starch down-regulated in the SSII activity were also altered, both the melting temperature of the granule and the peak viscosity were decreased. Furthermore, the phosphate content of the antisense SSII starch was also decreased [19]. This is most probably associated with the strictly lowered peak viscosity. This is in consistent with the result that inhibition of an enzyme involved in starch phosphorylation (R1) may also lead to a crumple of the peak viscosity [31]. When SSII plays a more predominant role in assembling the starch granule in potato (for example, when SSIII expression was inhibited, then an increase in the phosphate content, as well as in peak viscosity is also observed) [3]. Thus, there seems to be a relationship between SSII and starch phosphorylation. It is tempting to speculate that the N-terminal extension of SSII may interact with a starch-phosphorylating enzyme. Another possibility is that SSII can introduce phosphorylated glucose residues into nascent glucan chains or preferentially synthesises branch lengths more suitable for phosphorylation. The heterologous gene expression of SSs was also reported in transgenic potato tubers. The cassava granule bound (GB)-SSI was expressed in the amylose-free (amf) potato mutant. Although the GBSSI activity in these starch granules was comparable to that of wild-type granules, the amylase content was only restored to 60% of that of wild-type ones. The fine structure and the physical properties of potato starch can be altered by introduction of a glycogen synthase A (glgA, EC 2.4.1.21) gene. It is worth noting that both the degree of phosphorylation and the peak viscosity were lowered, which agreed with the data for antisense SSII starch.

3.2. Genetic engineering of potato for starch-branching enzyme

Differences in amylopectin branching affect granule crystallinity, which together with differences between species in granule size and shape result in altered thermal, pasting and biophysical properties [32, 33] The two starch-branching enzyme (SBE) isoforms, SBEI and SBEII have been reported in the potato. Amylopectin branching by SBEI and SBEII enzymes form branch points by cleaving α-(1 $\rightarrow$ 4)-linkages and reattaching the glucan chain via an α-(1 $\rightarrow$ 6)-linkage. The changes in SBE activity change the number and size distribution of amylopectin branches. SBEI is the major form of SBE in the potato tuber; however, antisense downregulation of SBEI did not change the amylose content, although small changes in the physical properties of the starch such as the gelatinization onset could be measured in differential scanning calorimetry [34]. The second form, SBEII was subsequently discovered, and when expression of this gene was reduced in the potato tuber, the amylose content was elevated to about 35% even though this SBE isoform made up less than 2% of the SBE activity of the tuber [3]. A combined downregulation of both these SBE isoforms directed to a noteworthy further increase up to 70% or higher [35]. It is now well known that the starch biosynthesis takes place by a group of enzymes including different starch synthases and starch-branching and -debranching enzymes. The role of all these enzymes has been investigated using the gene silencing or genetic knockouts techniques have been used to analyse the role of all these enzymes; however, there are only some example of over-expression existed which is probably due to the problems in cloning large genomic fragments for over-expression or severe toxicity of functional cDNAs to bacteria during cloning. A promising study on the function of potato starch-branching enzyme (SBEII) using over-expression in potato tubers was done recently

[36]. The transgenic potato lines with SBEII over-expression were generated. Compared with wild-type, starch from these tubers possessed an increased degree of amylopectin branching, with more short chains of degree of polymerisation (DP) 6–12 and particularly of DP6. Further, the increased ratio of short to long amylopectin branches facilitated gelatinisation, which occurred at a reduced temperature (by up to 3°C) or lower urea concentration.

3.3. Genetic engineering of potato for modification of carbohydrates (starch) in storage organs

The initiation of starch biosynthesis in storage organs inevitably requires the mobilisation of sucrose into glucose-6-phosphate (G6P), import of G6P into the amyloplast through inorganic phosphate (Pi) exchange, and subsequent conversion of G6P into glucose-1-phosphate (G1P) by plastidial phosphoglucomutase. The first committed step to starch synthesis is the formation of ADP-glucose (ADP-Glc) through ATP activation of G1P, catalysed by ADPG pyrophosphorylase (AGPase). AGPase has a heterotetrameric structure with two small subunits and two large subunits. The AGPase enzymes are reported to be allosterically regulated with 3-phosphoglyceric acid (3PGA) being the main activator and Pi the main inhibitor.

The AGPase enzymes were reported to present in several plant tissues and bacterial extract [37]; however, the localisation and regulation of ADP-Glc synthesis and import of Glc-1-P are highly variable among species and in different organs within a species [38, 39]. As a key factor of this rate-controlling step, AGPase was extensively used to develop different transgenic plants including potato where the rate of ADP-Glc formation and starch accumulation is under tight control of the gene expression. The amylose content was severely reduced in transgenic potato that contained a lower expression of AGPase [40, 41]. The transgenic potato lines were developed using the *Agrobacterium* mediated transformation method where the expression of ADP-glucose pyrophosphorylase (AGPase) was inhibited by introducing a chimeric gene with the coding region of one of the subunits of the AGPase linked in an antisense orientation to the *CaMV 35S* promoter. Limited inhibition of the AGPase enzyme was achieved in leaves and almost complete inhibition in tubers. This resulted in the lowering of starch formation in tubers, which also proved that AGPase has a distinctive role in starch biosynthesis in plants. Biochemical analysis of these tubers revealed a reduction (up to 30%) in the dry weight of tubers and the accumulation of soluble sugars in tubers resulting in a significant increase of the total fresh weight tuber. However, the tuber induction enhanced with increase in the number of tuber per stolon. The molecular analysis of these antisense lines showed that there was no significant change in the RNA level of other starch biosynthetic enzymes, except an increase in the RNA level of the major sucrose synthesising enzyme known as the sucrose phosphate synthase. In addition, the inhibition of starch biosynthesis was complemented by a massive reduction in the expression of the major storage protein species of potato tubers, supporting the idea that the expression of storage protein genes is in some way connected to carbohydrate formation in sink storage tissues. There are some reports also exist where a mutant *Escherichia coli* AGPase gene (glgc16) were over-expressed in some plants for enhancing the starch accumulation, such as potato [17] and maize [42].

Transgenic potato lines were developed with collective expression of invertase and glucokinase which indicated an intense decrease in starch accumulation and a stimulation of glycolysis [43]. The aim was to surge starch increase in potato tubers by enriching their capacity to metabolise the sucrose. As a first step, the precise expression of a yeast invertase in the cytosol of transgenic tubers led to a 95% decrease in sucrose content that was complemented by a larger accumulation of glucose and a reduction in starch. In the next step, a double transgenic potato lines were developed where the bacterial glucokinase from *Zymomonas mobilis* was introduced by the transformation method into an invertase-expressing transgenic potato aiming to transport the glucose into the metabolism. The double transgenic lines obtained showed up to three folds glucokinase activity than in the parent invertase transgenic line and which did not accumulate glucose. Surprisingly, there was an additional intense reduction (up to 35%) in starch content was observed in the transgenic lines than the wild-type control plants. The biochemical analysis of growing tuber tissue revealed great expansions in the metabolic intermediates of glycolysis, organic acids and amino acids, 2–3 folds increases in the maximum catalytic activities of key enzymes in the respiratory pathways, and 3–4 folds increases in carbon dioxide production. These variations occur in the lines expressing invertase, and are highlighted following introduction of the second transgene, glucokinase. It was determined that the expression of invertase in potato tubers leads to an improved flux via the glycolytic pathway at the expense of starch synthesis and that heterologous over-expression of glucokinase augmented this change in partitioning.

The investigations were done to analyse the extent to which starch synthesis in potato tubers is controlled by the activity of AGPase [44]. The transgenic potato was developed with the down regulation of AGPase gene. In the biochemical assay, the fluxes of carbohydrate metabolism were measured in tubers. It was found that the reduction in AGPase activity led to a reduction in starch accumulation, and an increase in sucrose accumulation. The control coefficient of AGPase on starch accumulation in intact plants was estimated to be around 0.3. The fluxes of carbohydrate metabolism were measured in tuber discs from wild-type and transgenic plants by investigating the metabolism of [U-(14) C] glucose. In tuber discs, the control coefficient of AGPase over starch synthesis was estimated to be approximately 0.55, while the control coefficient of the enzyme over sucrose synthesis was −0.47. The values obtained suggested that AGPase activity shows substantial control over tuber metabolism in potato.

3.4. Genetic engineering of potato to modify the starch granule size

Starch granule size is an important feature that determines the suitability for many of the food and non-food uses. Starch granule size is highly species-specific and starch granules can appear in many different forms. The large sizes of starch granules in potato tubers are advantageous in application demanding the high viscosity but limit its suitability for noodle making. In order to alter the granule size using the genetic engineering approaches, the transgenic potato lines were developed by over-expression of the *cyclodextrin glycosyltransferase* gene isolated from *Bacillus circulans* bacterial strain which is a multiple tandem starch-binding domain (SBD). Biochemical analysis of transgenic potato tuber starch revealed large number of small starch granules without affecting the starch yield [45, 46].

Two starch phosphorylases in plants, plastidic phosphorylase A (Pho 1a) and cytosolic phosphorylase (Pho 2), catalyse reversible transfer of glucose from glucose-1-phosphate to a-glucan chain-releasing phosphorus (pi). A gene was cloned which translated protein counterpart was involved in starch metabolism identified by its ability to bind the potato starch granules [31]. The gene was introduced as RNAi construct in the potato using the *Agrobacterium* mediated transformation method. Biochemical analysis revealed that the reduction in the protein level of transgenic potato augmented with reduction in the phosphate content of the starch. The complementary result is obtained when the same gene was expressed in *E. coli*, as this leads to an increased phosphate content of the glycogen. It was assumed that this protein might be responsible for the incorporation of phosphate into starch-like glucans, a process that is not understood at the biochemical level. The reduced phosphate content in potato starch have some secondary effects on its degradability, as the respective plants show a starch excess phenotype in leaves and a reduction in cold-sweetening in tubers. In a mapping study in potato, Pho 1a emerged as a candidate gene linked to starch gelling and starch granule size [47]. In another study, it was found that the antisense inhibition of isoamylase in potato induces massive numbers of small granules in tubers, suggesting that the debranching activity is necessary to prevent excessive granule initiation [48]. On the other hand, it has been observed that mutation of starch synthase IV in *Arabidopsis* increases the starch granule size in leaf [28]. However, despite these observations, we really have a poor understanding of what controls granule size and shape.

4. Potato starch engineering for industrial application

The principal industrial productions of starches are based only on four main resources such as maize, cassava, wheat, and potatoes, which represent 76, 12, 7, and 4%, respectively. The other botanical resources represent less than 1%. The main production areas are North America, China, Europe, Southeast Asia, and South America with 33, 33, 18, 11, and 5%, respectively. Along with the better understanding of starch structure and enzymes involved in starch biosynthesis, many of the genes that encode these enzymes have been cloned and transformed into plants using *Agrobacterium tumefaciens* to modify the starch metabolism. Transgenic plants have been generated by down regulation (antisense or co-suppression approaches) or over-expression of endogenous gene or expression of heterologous genes, where starch properties and morphology have been altered. The possibility to produce tailor-made starches in planta has broadened the functionality of starches in industrial applications. The in planta modified starches, such as the amf starch, are often of better quality relatively to the chemically derivative which excludes the use of hazardous chemicals and leads to energy savings in the production process (of up to 60% for, e.g., synthetic polymer replacers). Potato starch shows a naturally high degree of phosphorylation compared to starches from other crops. It is universally acknowledged that starch with longer polymer chains tends to contain higher levels of phosphate because the longer chains provide a better substrate for the phosphorylating enzyme. The presence of phosphate in potato starch results in the stable-paste properties and transparent gels. Hence, potato starch is preferred for use in paste products and as an

ingredient in noodles. The transgenic potato developed by simultaneous inhibition of starch-branching enzymes (SBE A & B) [35] revealed that the phosphate content of high-amylose starches increased up to five fold as compared to the wild-type plant starch. A crucial enzyme glucan water dekinases (GWD) responsible for phosphorylating starch has been identified in potatoes, and regulating the expression of this gene could change the phosphate content and viscosity of potato starch [49].

5. Stress tolerant transgenic potato over-expressing modified carbohydrates as signalling factors

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stresses are serious threats to agriculture besides their deteriorative impact to the environment. Drought and salinity are the most important environmental stress factors that limit food production worldwide, and may cause a serious salinisation on more than 50% of all arable lands by the year 2050 [50]. In China, almost half of the land is arid or semi-arid and the crop production is strongly affected by drought and salinity seasonally even if in the irrigated farm land. Trehalose is a non-reducing disaccharide of glucose. A plant that produces trehalose is often highly tolerant to desiccation stress. Genetic engineering of potato has done for trehalose biosynthesis in potato by introducing the *otsA* and *otsB* genes from *E. coli*, which encode trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, respectively [51]. The plants also report to contain sucrose-non-fermenting-1- (SNF1) linked protein, analogous to that of the protein kinase (SNF-) yeast-signalling pathway. The role of SNF1related protein in sugar signalling has been analysed experimentally by developing the transgenic potato expressing an antisense SNF1-related protein kinase. Analysis of these plants revealed that the SNF1-linked protein plays a role in transduction of the sugar signal, triggering the initiation of sucrose synthase in potato leaves [52]. It has been well established that the plants share with few yeast elements involved in sugar sensing, however, several aspects of sugar receptors are likely to be unusual to higher plants. It has been reported that the abiotic stress factors may bring forth the synthesis of stress-related hormones particularly ABA and ethylene, which appear to be implicated in sugar-sensing mechanisms [53, 54]. The level of complexity in sugar signalling and protein involved depicted that despite the successful explanation of some sugar-sensing signalling mechanisms in recent years, added efforts are required to achieve a complete picture of sugar sensing in plants and thus, increase our knowledge of the mechanisms for plant abiotic stress tolerance and adaptation.

6. Perspectives of GMO starches

In an era towards a bio-based economy, the knowledge on how to improve complex carbohydrates such as starch is essential. A deeper understanding of the starch biosynthetic pathway, how storage starch granules are formed and how the composition, size, and shape can be changed and optimised for different bio-products, is of great importance for food and

non-food applications. Despite its great importance, the development and commercialisation of crops with altered starch properties using biotechnological approaches is being hampered by regulatory hurdles. The very high costs and the great deal of time needed, associated with the regulation of genetically modified crops (GMOs), are major problems. Although there is currently one GMO potato variety in the market, the *Amflora*, the commercialisation of this variety has been challenged by farmers and environmental organisations. The development of new methods in plant breeding that would circumvent these regulatory problems would be of greatly stimulated the development of novel starches [55]. The identification of genetic marker associated with starch properties and the exploitation of new mutations in tilling populations are other tools with great potential for uncovering key genes determining starch properties [47]. Another bottleneck to produce improved starches is associated with the difficulties in predicting beforehand the effect of a (trans) gene. The understanding of mechanism by which starch granules are made in the form of dense granules would be a great step forward in the synthesis of tailored starches for different bio-based applications.

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