Biosynthesis and Modification of Starch By Genetic Engineering With Reference To Rice

Raghavendra A. S.

School of Life Sciences, Department of Plant Sciences, University of Hyderabad, Hyderabad 500046 India. Email: as_raghavendra@yahoo.com,asrsl@uohyd.ernet.in

Abstract

This article is an attempt to present an overview of our understanding of starch biosynthesis and its possible manipulation in rice. The two major parts deal with the reactions or enzymes of biosynthetic pathway leading to starch, and the attempts made to manipulate the starch content in rice. In view of the huge scope for further improvement in the quantity and quality of starch in rice, it is essential to understand starch metabolism in plants, particularly in cereals and apply all relevant knowledge to rice. Some promising areas, in this regard are the flux control, metabolome analysis, and modulation of a set of multiple enzymes/genes, besides the modulation of single enzyme or gene. In this context, knowledge from studies on Arabidopsis starch could be not only relevant but also important for applications to rice starch.

Keywords: Assimilate partitioning, *Oryza sativa*, metabolic engineering, rice, starch, sucrose

Introduction

Rice is an important component of daily food in not only tropical regions but also temperate countries. The rice grains contain large quantities of starch and provide a ready source of carbohydrates for human nutrition. Besides, starch is a key component of plants, involved in regulation of plant growth and nutrition (Geigenberger et al 2011). For e.g. starch-deficient mutants of *Arabidopsis* grow poorly or even die under short-day conditions. Similarly the phosphorus as well as nitrogen nutrition are also tightly linked to starch metabolism. Starch is also of great economic value, being second most abundant biopolymer on earth, after cellulose. It is the most important carbohydrate for feed, and an abundant feedstock for bioethanol production (Zeeman et al 2010).

Starch metabolism in plants has always been of great interest and a popular topic for research. In recent years, it has attained crucial importance with reference to rice. We attempt to present an overview of our understanding of starch biosynthesis and its possible manipulation in rice. In the first part, we describe the reactions or enzymes of biosynthetic pathway leading to starch. The second part deals with attempts made to manipulate the starch content in rice plants and the last part draws attention to the possible future directions.

Biosynthetic pathway of starch in seed endosperm

The starch granules in endosperm of seeds, such as those of rice grains, are composed of two types of starch polymers: amylose and amylopectin. Amylose is an essentially linear glucan that contains only a-1,6-branches and makes up to 20% to 30% of normal starch, while amylopectin is more highly branched. Both these starch forms are polymers of glucose (Glc, or G) entities synthesized exclusively inside plastids (either green chloroplasts of green tissues or colourless leucoplasts of seed endosperms) of higher plants.

The details of starch biosynthesis have been frequently reviewed (Geigenberger 2011; James et al 2003; Jeon et al 2010; Nakamura 2002; Stitt et al, 2010; Zeeman et al 2010). The first and almost an irreversible step involves the conversion of Glc-1-P and ATP to ADP-Glc and inorganic pyrophosphate (PPi), catalyzed by ADP-Glc pyrophosphorylase (AGPase). ADP-Glc acts as the glucosyl donor for different classes of starch synthases (SS), which elongates the 1,4-linked glucan chains of the two insoluble starch polymers amylose and amylopectin (Fig. 1). Five distinct SS classes are known in plants: granule-bound SS, which is responsible for the synthesis of amylose; and soluble SS I to IV, responsible for amylopectin synthesis.

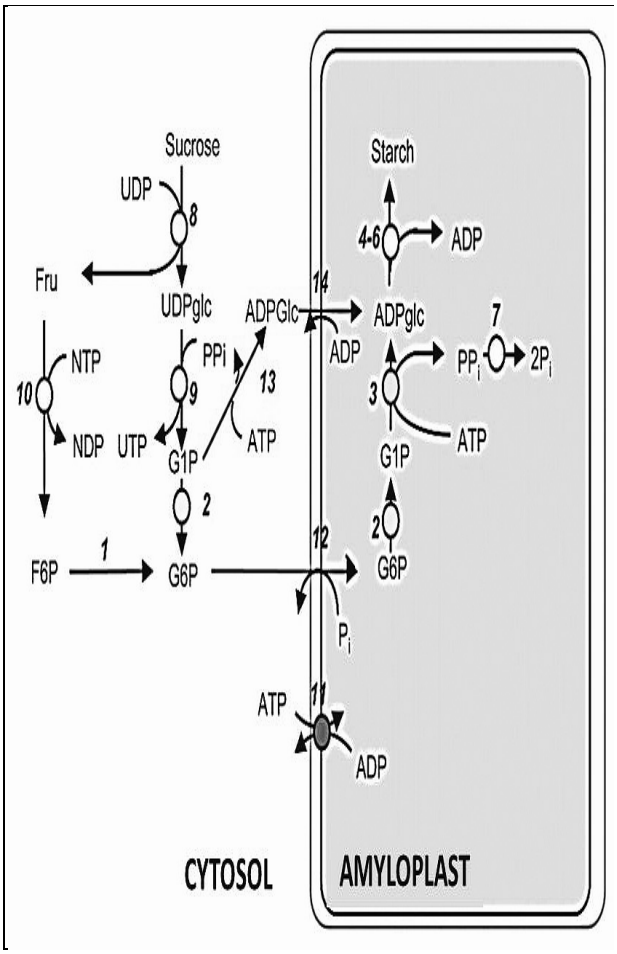

Figure 1. Schematic representation of the pathway of starch biosynthesis, the subcellular compartmentation of reactions in heterotrophic tissues, such as rice endosperm. The reactions of the pathway of starch biosynthesis are catalyzed by the following enzymes: 1 = phosphoglucoisomerase; 2 = PGM; 3 = AGPase; 4 = SS; 5 = SBE; 6 = starch-debranching enzyme; 7 = inorganic pyrophosphatase; 8 = Suc synthase; 9 = UDP-Glc pyrophosphorylase; 10 = fructokinase; 11 = ATP/ADP translocator; 12 = Glc-6-P/Pi translocator; 13 = cytosolic AGPase; and 14 = ADP-Glc/ADP translocator (adapted from Geigenberger 2011)

In most tissues, AGPase is located exclusively in the plastid. In leaves exposed to light, Glc-1-P is synthesized from Calvin-Benson cycle intermediates via plastidic phosphoglucose isomerase and phosphoglucomutase (PGM), while ATP is provided by photophosphorylation at the thylakoid membrane (Fig. 1A). In non-photosynthetic tissues such as potato tubers (Fig. 1), incoming sucrose is mobilized by a series of cytosolic reactions to Glc-6-P, which is imported into the amyloplast in counter exchanged with inorganic phosphate (Pi) by a Glc-6-P/Pi translocator and subsequently converted to Glc-1-P via plastidial PGM. The second substrate of AGPase, ATP, is provided by mitochondrial respiration and imported into the plastid via the envelope ATP/ADP exchanger In contrast, in cereal seed endosperm, AGPase is mainly located in the cytosol, with a total AGPase activity of about 85 to 95% (James et al 2003). ADPGlc synthesized in the cytosol must be imported into the plastid to support starch synthesis.

Starch comprises two D-glucose homopolymers, amylose and amylopectin (Fig. 2). Amylose is essentially a linear molecule, in which glucosyl monomers are joined via a-1,4 linkages. Amylopectin, the more abundant polymer in starch, contains linear chains of various lengths. The key enzymes involved are ADP-glucose pyrophosphorylase (AGP), starch synthase (SS), starch branching enzyme (SBE) and starch debranching enzyme (DBE).

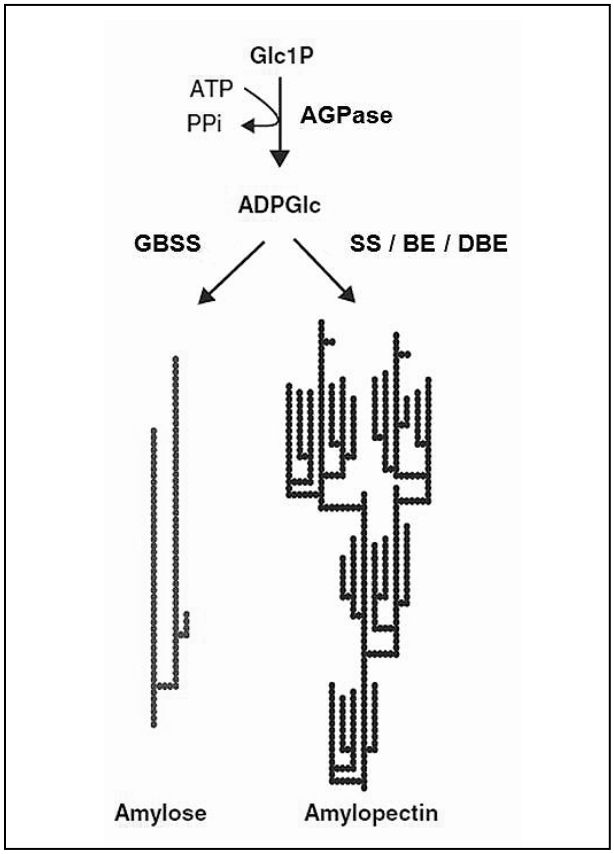

Figure 2. Simplified scheme of starch synthesis. The filled circles in the amylose and amylopectin models represent individual glucosyl residues. Glc1P = glucose 1-phosphate; AGPase = ADPglucose pyrophosphorylase; ADPGlc = ADPglucose; BE = branching enzyme; DBE = debranching enzyme; GBSS = granule-bound starch synthase; SS = starch synthase (adapted from Santelia and Zeeman 2011)

Table 1. Summary of the results of genetic modification of expression of different lebels of different enzymes. These results are mostly from other cereals but are quite applicable to rice.

Target enzyme	Regulation	Effect	End result
AGPase	Upward	increase in photosynthesis and yield	High starch levels
SBE	Downward	increase in photosynthesis and yield; improved granule integrity; increased water absorbance	High starch levels; quality modified
DBE	Downward	increase in photosynthesis and yield	High starch levels
AGPase	Downward	improved granule integrity; increased water absorbance	Modified starch; increased amylopectin
SS	Downward	increase thickness and crispiness	Increased amylose
ATP/ADP translocator	Upward	increased thickness and crispiness, high crop yield	Increased starch; increased amylose
SBE-A	Downward	increased crispiness and thickness	Increased amylose
GBSSI	Downward	increased adhesiveness	Increased amylopectin

The names of abbreviated enzymes can be seen in Fig. 1 and 2. (From information in Casey et al 2000); AGPase = ADPglucose pyrophosphorylase; SBE = starch-branching enzyme; ADPGlc = ADPglucose; BE = branching enzyme; DBE = debranching enzyme; GBSS = granule-bound starch synthase; SS = starch synthase

Manipulation by genetic engineering: direct and indirect

The current knowledge on genetic engineering is derived from studies on different cereal crops, particularly maize. Similar attempts with rice have begun and are getting extensive. These attempts through genetic engineering aim for not only increasing the starch content but also starch quality (Xie and Peng 2011; Zeeman et al 2010). Similarly the direct genetic modification of starch content use the principle of upward or downward regulation of enzymes involved in starch biosynthesis. A major direct target for increasing starch content is the key regulatory enzyme, AGPase and another promising one appears to be the ADP/ATP translocator (Table 1). While major emphasis has been to enhance the starch content, modification of enzymes involved in polymerization glucan residues into either amylose or amylopectin yielded interesting results. Downward regulation of starch-branching enzyme, SBE-A or SS results in enhanced amylose and improved crispiness of starch grains (Tanaka et al 2004; Wei et al 2010). In contrast, downward regulation of SS or GBSSI increases amylopectin and softy nature of rice grains (Slattery et al. 2000; Zhang et al 2011).

The indirect approaches to enhance starch are attempts to increase the overall photosynthetic performance (e.g. by overexpression of Rubisco) and modulation by hormonal status (particularly cytokinins and/or ethylene) and even nutritional status (Peleg et al 2011; Suzuki et al 2012; Zhu et al 2011). Phosphorus nutrition has been found to have profound effect on starch metabolism in rice endosperm (Wasaki et al 2006).

Scope for future work

The scope for further improvements in the quantity and quality of starch in rice is quite vast. It would be essential to understand starch metabolism in plants, particularly cereals and apply all relevant knowledge to rice. Some such promising areas are studies related to flux control, metabolome analysis, and modulation of a set of multiple enzymes or genes, besides already successful modulation of single enzyme or gene. In this context, it would be appropriate to study and exploit enzyme regulation by allosteric metabolites, post-translational protein modifications, protein complex formation and responses to hormonal or light signals (Geigenberger 2011). Knowledge from studies on *Arabidopsis* starch are extremely important for such application to rice starch.

REFERENCES

Geigenberger P. 2011. Regulation of starch biosynthesis in response to a fluctuating environment. *Plant Physiol* 155: 1566–1577.

James MG, Denyerz K and Myers AM. 2003. Starch synthesis in the cereal endosperm. *Curr Opin Plant Biol* 6: 215–222.

Jeon J-S, Ryoo N, Hahn T-R, Walia H and Nakamura Y. 2010. Starch biosynthesis in cereal endosperm. *Plant Physiol Biochem* 48: 383-392.

Nakamura Y. 2002. Towards a better understanding of the metabolic system for amylopectin biosynthesis

in plants: rice endosperm as a model tissue. *Plant Cell Physiol* 43: 718-725.

Peleg Z, Reguera M, Tumimbang E, Walia H and Blumwald E. 2011. Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnol J* 9: 747-758.

Santelia D and Zeeman SC. 2011. Progress in *Arabidopsis* starch research and potential biotechnological applications. *Curr Opin Biotechnol* 22: 271–280.

Slattery C J, Kavakli IH and Okita TW. 2000. Engineering starch for increased quantity and quality. *Trends Plant Sci* 5: 291–298.

Stitt M, Lunn J and Usadel B. 2010. *Arabidopsis* and primary photosynthetic metabolism: more than the icing on the cake. *Plant J* 61: 1067–1091.

Suzuki Y, Fujimori T, Kanno K, Sasaki A, Ohashi Y and Makino A. 2012. Metabolome analysis of photosynthesis and the related primary metabolites in the leaves of transgenic rice plants with increased or decreased Rubisco content. *Plant Cell Environ* In Press. doi: 10.1111/j.1365-3040.2012.02494.

Tanaka N, Fujita n, Nishi A, Satoh H, Hosaka Y, Ugaki M, Kawasaki S and Nakamura Y. 2004. The structure of starch can be manipulated by changing the expression levels of starch branching enzyme IIb in rice endosperm. *Plant Biotechnol J* 2: 507-516.

Wasaki J, Shinano T, Onishi K, Yonetani R, Yazaki J, Fujii F, Shimbo K, Ishikawa M, Shimatani Z, Nagata Y, Hashimoto A, Ohta T, Sato Y, Miyamoto C, Honda S, Kojima K, Sasaki T, Kishimoto N, Kikuchi S and Osaki M. 2006. Transcriptomic analysis indicates putative metabolic changes caused by manipulation of phosphorus availability in rice leaves. *J Exp Bot* 57: 2049-2059.

Wei C , Qin F, Zhou W, Yu H, Xu B, Chen C, Zhu L, Wang L, Gu M and Liu Q. 2010. Granule structure and distribution of allomorphs in C-type high-amylose rice starch granule modified by antisense RNA inhibition of starch branching enzyme. *J Agric Food Chem* 58: 11946-11954.

Xie G and Peng L. 2011. Genetic engineering of energy crops: a strategy for biofuel production in China. *J Integr Plant Biol* 53: 143-150.

Zeeman SC, Kossmann J and Smith AM. 2010. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu Rev Plant Biol* 61: 209–234

Zhang G et al. 2011. Double repression of soluble starch synthase genes *SSIIa* and *SSIIIa* in rice (*Oryza sativa* L.) uncovers interactive effects on the physicochemical properties of starch. *Genome* 54: 448-459.

Zhijun C, Xin Z, Xiuping G, Ning S, Ling J, Long M and Jianmin M. 2011. Double repression of soluble starch synthase genes *SSIIa* and *SSIIIa* in rice (*Oryza sativa* L.) uncovers interactive effects on the physicochemical properties of starch. *Genome* 54: 448-459.

Zhu G, Ye M, Yang J, Peng X and Zhang J. 2011. Regulation of expression of starch synthesis genes by ethylene and ABA in relation to the development of rice inferior and superior spikelets. *J Exp Bot* 62: 3907–3916.

Citation: Raghavendra AS. 2013. Biosynthesis and modification of starch by genetic engineering with reference to rice. In: Muralidharan K and Siddiq EA, eds. 2013. *International Dialogue on Perception and Prospects of Designer Rice*. Society for Advancement of Rice Research, Directorate of Rice Research, Hyderabad 500030, India, pp 143-146.