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Mutants in Starch Biosynthesis Pathway, Characterization and Application in Corn Breeding

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Starch is the main storage carbohydrate in vascular plants. It serves as the main source of calories in the diet of humans due to its abundance as a naturally occurring component of living terrestrial biomass, which is only surpassed by cellulose. Because starch is the principal constituent of the harvestable organ of many agronomic plants, its synthesis and accumulation also influence crop yields. Starch synthase (SS) uses ADP glucose (ADPG) as the sugar donor molecule to create this insoluble polyglucan. Its two main parts are polymers of α-D-glucose units called amylopectin and amylose. Amylopectin is a larger polymer that is regularly branched with 1,6-branch points, whereas amylose is a linear polymer of up to several thousand glucose residues. These two molecules are assembled together to form a semi-crystalline starch granule.

Corn starch

Corn starch consists of approximately 25% amylose and 75% amylopectin. About 25% amylose and 75% amylopectin make up corn starch. Amylopectin plays a function in the production of crystalline granules and the thickening of paste, while amylose controls the starch's gelling and hardness. Starches derived from various botanical sources vary in composition and physical characteristics, which results in a range of processing capabilities and uses in the non-food and food industries. Non-food sectors such as sizing agents in textile, paper industry, adhesive gums, and biodegradable materials and in food industries, particularly in bakery, thickening, confectionary and emulsification (Burrell, 2003; Mooney, 2009; Slattery et al., 2000). Additionally, starch is employed as a feedstock for the manufacturing of first-generation bioethanol (Goldemberg, 2007).

Starch metabolism and regulation is critically important for the rational design of experimental traits aimed at improving yields in agriculture, and producing more and better

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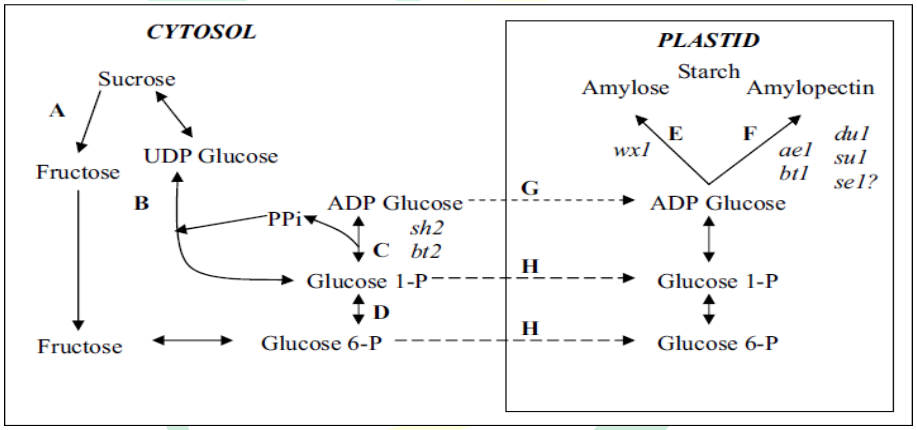
polymers that fit both industrial needs and social demands.Sweet corn is not a subspecies of the species Zea mays or a distinct race of maize. A gene or genes that affect endosperm starch synthesis and its use as a vegetable set it apart from other varieties of maize.

Starch biosynthesis pathway and its endosperm specific mutants

The heterotrophic organs import the sucrose made in the leaves and utilise it as a carbon source for energy production and starch synthesis in the amyloplast.Starch biosynthesis in the cereal endosperm requires the coordinated activities of several enzymeslike Sucrose synthase (SuSy),adenosine 5diphosphate-glucose (ADP-Glc) pyrophosphorylase (AGPase),granule-bound starch synthase (GBSS), soluble starch synthase(SS), starch branching enzyme (BE), starch debranching enzyme(DBE), and plastidial starchphosphorylase(Pho1).



Starch biosynthesis pathway

Role of enzymesin starch biosynthesis pathway

1. Sucrose synthase (SuSy)

This SuSy catalyses the reversible conversion of sucrose and uracil diphosphate into UDPGlc and fructose. Hexokinase, phosphoglucoisomerase and phosphoglucomutase converts fructose to Glc-1-P and UDPGlc to Glc-1-P by UGPase. Thus SuSy is a major determinant of sink strength that highly controls the channelling of incoming sucrose into starch. This SuSy activity is absent in maize sh1 mutant.

2. ADP-glucose pyrophosphorylase (AGPase)

AGPase catalyses the formation of starch monomer, ADPGlc. In higher plants, it is heterotetrameric, with two large (AGP-L) and two tiny (AGP-S) catalytic subunits expressed by distinct genes. The AGP-L subunits are hypothesised to alter the enzymatic regulatory

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features that increase the allosteric response of the small subunit to 3-phosphoglyceric acid (3-PGA) and inorganic phosphate(Pi), whereas the AGP-S subunits are normally responsible for enzymatic complex catalytic activity. The AGP-S has catalytic activity and the AGP-L subunit have enzymatic regulatory abilities enhance the allosteric response of small subunit to 3-phosphoglyceric acid (3-PGA) and inorganic phosphate (Pi). Mutant *bt2* endosperm lacked the 55 kD smaller subunit while mutant *sh2* endosperm lacked the 60 kD larger subunit.

3. Starch synthases (SSs)

This enzyme catalyse the transfer of a glucose unit precursorADPGlc, to the non-reducing end of a pre-existing (1→4)-α-glucan primer, which will eventually form amylose or amylopectin.It is classified as

a)Granule-bound starch synthase

b) Soluble starch synthase

a. Granule-bound starch synthase (GBSS)

The two GBSS isoforms are GBSSI and GBSSII. In plant tissues other than those used for storage, where transient starch builds up, GBSSII works. The majority of GBSSI is restricted to storage tissues like the seed endosperm. The Waxy (wx) locus in cereal species is responsible for encoding GBSSI. The endosperm starch of wx mutants contains less amylose or none at all. Additionally, it is believed that GBSS isoforms contribute to the production of amylopectin, specifically the extralong unit chain (ELC) component.

b. Soluble starch synthase

This enzyme has been grouped into four classes(SSI, SSII, SSIII and SSIV). SSI appears to be primarily responsible for thesynthesis of short glucan chain. Monocots contain the SSIIa and SSIIb classes of SSII genes. SSIIa is absent in the maize sugary2 (su2) mutants. SSIII produces the relatively long chains of amylopectin. maize dull1 mutants lack SSIII. A plastid's capacity to hold starch granules may be regulated by SSIV.

4. Starch-branching enzymes (SBEs)

SBEs generate new branches on starch molecules, by cleaving internal (1→4)-α bonds of a branch chain and create a new α-(1→6)-α-glucosidic linkage. SBEI and SBEII are the two classes of SBE found in cereals**.** SBEI produces longer chains with DP 16 by lesser branched polyglucans.SBEII generates shorter chains with DP 12.Dicots only have one

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SBEII enzyme, but SBEII can be further divided into SBEIIa and SBEIIb isoforms in monocots.

5. Starch debranching enzymes (DBEs)

DBEs disbranch the (1→6)-α-glycosidic linkages of starch. Two groups of DBEs are: a. Isoamylase (ISA) with three forms (ISA, ISA2 and ISA3), b. Pullulanase. Maize sugary1 (su1)mutants deficient in ISA1

6. Plastidialphosphorylase (*Pho*1)

This *Pho*1 enzyme has role in the formation of primers for starch biosynthesis in the endosperm.

Classes of mutants

1. Class one mutations

Affect cytosolic reactions early in the process of starch synthesis, before starch is synthesized. Example ***brittle1 (bt1), brittle2 (bt2) and shrunken2 (sh2)***and these are called as "**super sweet" or "ultra-sweet"** varieties

2. Class two mutations

Affect reactions within the amyloplast directly involving starch granule assembly. Example ***amylose extender1 (ae1), dull1 (du1), sugary (su1), and waxy1 (wx1)***

Characteristics of mutants

1. Sugary1 (su1)

This Gene is located on short arm of chromosome 4.Sucrose three times and water-soluble polysaccharide(WSP) ten times of ordinary maize. WSP gives smooth texture and creaminess. The sugars quickly convert to starch so they are best eaten soon after harvest.

***2.* Shrunken2 (sh2)**

It is Located on long arm of chromosome 3. Sucrose six times and water-soluble polysaccharide(WSP) similar of ordinary maize. Longer shelf life than conventional sweet corn. Mature kernel dry and shrivel which affects seedling germinationand plant growth.

3. sugary enhancer1 (se1)

It is recessive modifier of the *sugary1* (*su1*). It resides on the long arm of chromosome 4. *su1se1* double mutants have total sugar level comparable to *shrunken2*based super sweet hybrids. While retaining the characteristic creamy texture conferred by phytoglycogen that is unique to traditional *su1su1* hybrids.

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4.Brittle 1 and Brittle 2

Brittle 1 and *brittle* 2 express similar phenotype as *sh2* because of obstruction in pathway at next or same step with gene *sh2*. *sugary1 (su1), Shrunken2 (sh2), sugary enhancer1 (se1), brittle1 (bt1) and brittle2 (bt2)* used in breeding sweet corn

5. Waxy1 (*wx1*)

*wx1 is*located on short arm of chromosome 9 and it reduces amylose synthesis, resulting in more amylopectin. Sticky maize and Used in the textile, corrugating, and adhesive industries

6. Amylose extender1 (ae1)

ae1 leads to accumulation of up to 50% amylose due to the less amylopectin production. Resistant starch with Low Glycaemic index. Manufacture of photo dissociative plastics that can potentially help control serious "white pollution"

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