Branched malto-oligosaccharides cause spontaneous starch granule initiation in *A. thaliana* chloroplasts

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Starch granule initiation

Plants store starch in their chloroplasts to use as source of energy during the night. Granules are initiated as chloroplasts grow and divide, creating a balance between chloroplast size and granule number.

Starch granule initiation is a controlled process during which oligosaccharides present in the stroma are extended by the Starch Synthase IV (SS4) protein. The extended oligosaccharides can serve as a substrate for branching enzymes and other starch synthases, and eventually crystallize. This causes them to become more resistant to breakdown, and form a granule initial (Seung, 2019).

The oligosaccharides used for starch granule initiation may result from de-novo synthesis or from starch breakdown. In plants that are deficient in specific starch breakdown enzymes, the initiation system is confronted with altered pools of oligosaccharides.

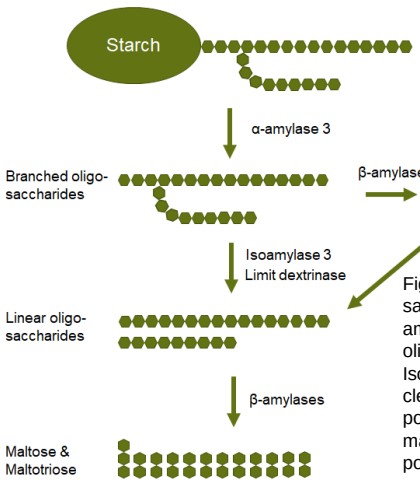


Figure 1: Metabolism of branched oligo-saccharides (simplified model). α-amylase 3 can cleave off branched oligosaccharides from amylopectin. Isoamylase 3 and limit dextrinase cleave off chains at the branching points. β-amylase can cleave off maltose units, but not near branching points.

Branched oligosaccharides

When the two debranching enzymes Isoamylase 3 (ISA3) and Limit Dextrinase (LDA) are missing, starch and branched oligosaccharides accumulate and large numbers of small granules are observed.

Genotype	Starch accumulation	Branched oligosaccharides
Wt Col	-	-
isa3 lda	■	■■
amy3 isa3 lda	■■	-
amy3 isa3	■	-

Data from Streb, 2012

- By comparing *isa3 lda* to *amy3 isa3 lda (*also deficient in α-amylase 3) we can determine whether granule overinitiation is caused by branched oligosaccharides or deficiencies in starch breakdown.
- To determine whether branched oligosaccharides are substrates for the known granule initiation system, or if they bypass it entirely, I am examining plants deficient in ISA3, LDA, and SSIV.

Granule overinitiation in *isa3 lda*

- *isa3 lda* shows a massive overinitiation of granules, increasing with leaf age.
- *amy3 isa3 lda* has fewer granules per chloroplast compared to *isa3 lda.*
- This indicates branched oligosaccharides are indeed responsible for granule over-initiation in *isa3 lda*.

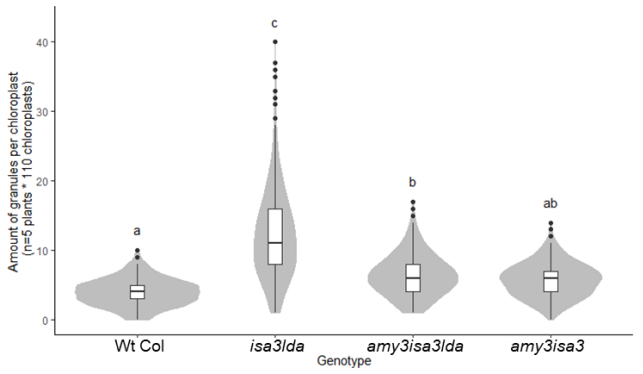
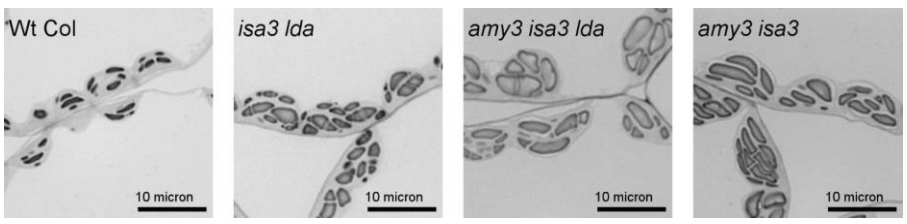


Figure 2: Granule number phenotype in Wildtype and starch breakdown deficient plants. **Left:** The amount of granules per chloroplast. **Bottom:** Light microscopy. Starch granules were stained with Toluidine Blue.



Granule overinitiation independent of SS4

Overinitiation can occur in absence of SS4 (Figure 3). This points towards branched oligosaccharides being used as granule initials without involving the presently described granule initiation system.

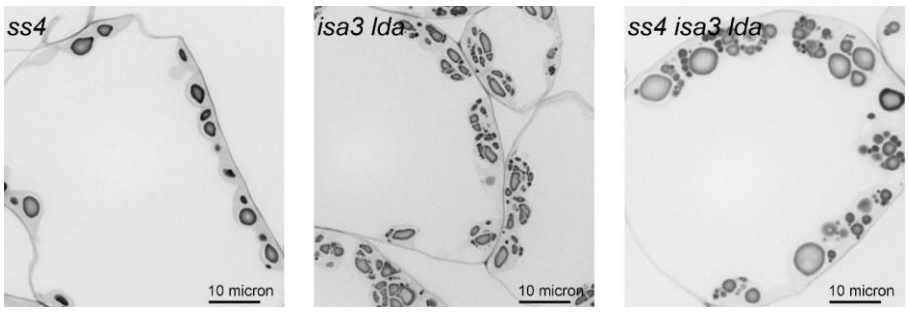


Figure 3: Light microscopy images of *ss4 isa3 lda* mutants. Starch granules were stained with Toluidine Blue. The *ss4* mutant has very few granules, which are large and round. In *ss4 isa3 lda* these granules are even larger, and are joined by numerous small granules.

References

1. Streb, S., Eicke, S., & Zeeman, S. C. (2012). The simultaneous abolition of three starch hydrolases blocks transient starch breakdown in Arabidopsis. *Journal of Biological Chemistry*, 287(50), 41745-41756.
2. Seung, D., & Smith, A. M. (2019). Starch granule initiation and morphogenesis—progress in Arabidopsis and cereals. *Journal of experimental botany*, *70*(3), 771-784.