Multiple Arabidopsis galacturonosyltransferases play a role in the synthesize polymeric homogalacturonans

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Background

- Pectin is a cell wall glycan with proven roles in biomass yield and recalcitrance. Homogalacturonan (HG), a major pectin, is synthesized by members of the GT8 *Galacturonosyltransferase (GAUT)* gene family. Prior to this work, only 3 of the 15 GAUT family members in Arabidopsis (GAUTs 1, 4 and 11) and the GAUT1:GAUT7 complex had been shown to synthesize HG *in vitro*.

Approach

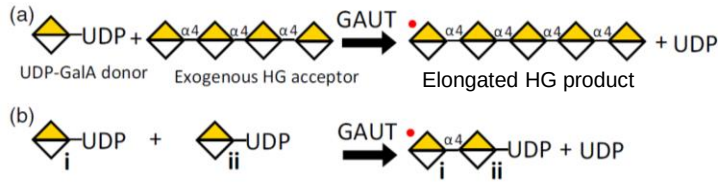
- The GAUT1:GAUT7 complex had been shown to synthesize polymeric HG *in vitro* in the presence and absence of exogenous HG acceptor, the latter known as *de novo* synthesis. Here the full GAUT family was probed *in vitro* to identify and characterize new HG biosynthetic enzymes and the structures of *de novo* synthesized HGs was ascertained.

Outcomes

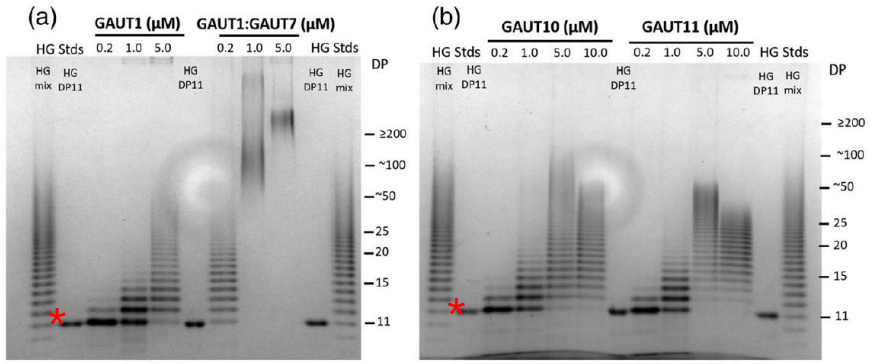
- Three additional GAUTs (10, 13 and 14) were shown to synthesize HG *in vitro*. Analysis of the products revealed that all enzymatically active GAUTs (1, 1:7, 10, 11, 13 and 14) synthesize polymeric HG. GAUT13 and GAUT14 have the highest HG biosynthetic rates of the individual GAUTs while the GAUT1:GAUT7 complex has the highest overall rate of HG synthesis.
- GAUT13 and GAUT14 were shown to catalyze *de novo* synthesize HG. Isolation and characterization of GAUT13 short-chain oligosaccharide *de novo* reaction products identified UDP on the reducing end. These results provide evidence for the initiating mechanism of HG biosynthesis by GAUTs; here, *de novo* synthesis of HG occurs wherein an UDP-GalA molecule is used as the starting acceptor and remains covalently attached to the growing polymer chain.

Significance

- HG is a critical abundant pectic glycan present in all plant cell walls. This study brings the current number of GAUTs with proven HG biosynthetic activity to six (GAUTs 1, 4, 10, 11, 13 and 14).
- Understanding the mechanisms and full suite of enzymes involved in pectin biosynthesis across all stages of plant growth and development is key to developing plants with favorable growth and recalcitrance phenotypes. Our findings provide new gene targets for this purpose in CBI's biofeedstocks, based on their newly proven enzyme activities and mechanisms of HG biosynthesis *in vitro*.



This work identifies new GAUTs that synthesize HG by transfer of GalA from the donor substrate UDP-GalA, an activity called acceptor dependent synthesis (a). We also show that some GAUTs catalyze *de novo* synthesis of HG in the presences of donor substrate alone (b) and that this occurs by retention of UDP on the reducing end of the synthesized HG. Red dot is non-reducing end of product.



Elongation of mid-size HG acceptor (DP of 11, asterisk) by increasing concentrations of GAUT1, GAUT1:GAUT7, GAUT10, GAUT11, GAUT13 and GAUT14 results in the formation of polymeric HG. GAUT13 and GAUT14 produce HG oligomers smaller than a DP of 11. These products were shown to be *de novo* synthesized HG that contains UDP on its reducing end.

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