Morphology and structure of starch-like glucans in a branching enzyme mutant of Arabidopsis complemented with the glycogen branching enzyme from *E. coli*

Laura Boyer¹, Ofilia Mvundza Ndjindji², Christine Lancelon-Pin², Adeline Courseaux¹, Fabrice Wattebled¹, Xavier Roussel¹, Christophe D’Hulst¹, Bruno Pontoire³, Jean-Luc Putaux²

¹UGSF, UMR CNRS 8576, Université Lille 1, Villeneuve d’Ascq, France
²CERMAV, UPR CNRS 5301, Grenoble Université Alpes, Grenoble, France
³UR1268 Biopolymères Interactions Assemblages, INRA, Nantes, France

Starch and glycogen are the main storage α(1,4)α(1,6)-glucans that accumulate in living cells. However, amylopectin (the branched fraction of starch) and glycogen have different branching degrees and α(1,6) branch point distribution. The present study aimed at establishing the implication of branching enzymes (BEs) in the α(1,6) linkage pattern in amylopectin. The Arabidopsis *be2-be3* BE double mutant that accumulates maltose instead of starch has been transformed, allowing the expression of the *E. coli* BE (GlgB) involved in glycogen synthesis. Transformed plants harboring increasing levels of GlgB activity were cultivated. Ultrathin sections of resin-embedded strips of freshly cut leaves harvested at the end of the day were treated with periodic acid thiosemicarbazide silver proteinate (PATAg), that specifically stain polysaccharides, and observed by TEM. Differences in morphology and size of the synthesized polyglucans were seen by comparison with the well-formed starch granules in the wild-type specimen. These observations complement chain length distribution profiles (established by HPAE-PAD after enzymatic debranching of the molecules) and measurement of crystallinity index (from X-ray diffraction data) of the insoluble polyglucans. Our results show that replacing the endogenous plant BEs by a bacterial ortholog restored the production of a polymer with characteristics close to those of wild-type amylopectin. Moreover, we have established a relation between the level of BE activity and the structure of the synthesized polysaccharides.