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LS-8-P-1761 The level of branching activity: a critical parameter to promote the accumulation of starch-like glucans in a branching enzyme mutant of Arabidopsis complemented with the E. coli GlgB ortholog

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Starch and glycogen are the two main storage polysaccharides that accumulate in living cells. Although they are both made of α(1→4)-linked glucose residues branched in α(1→6) position, they differ in structure and properties. The main difference lies in the distribution of the α(1→6) linkages in the macromolecules. In glycogen, these branching points are homogenously distributed whereas in amyllopectin (the major and semicrystalline fraction of starch), they are concentrated in amorphous lamellae. The crystalline regions of amyllopectin are mainly composed of linear glucans that intertwine into parallel double helices. In starch metabolism, the isoamylase-type debranching enzymes (that specifically cleave the α(1→6) linkages) control the distribution of the branching points by removing those in excess or misplaced, allowing the interlacing of the chains, an essential event to confer crystallinity to amyllopectin [Wattebled et al., Plant Physiol. 138 (2005), 184; Wattebled et al., Plant Physiol. 148 (2008), 1309]. This work aimed at establishing the implication of branching enzymes (that introduce the α(1→6) linkages in the glucans) in the branching point distribution in amyllopectin. The Arabidopsis be2- be3- branching enzymes double mutant [Dumez et al., Plant Cell 18 (2006), 2694] has been transformed allowing the expression of the E. coli branching enzyme (GlgB) natively involved in glycogen synthesis in this bacterium. Several transformed plants harboring different levels of GlgB activity were cultivated. Strips of freshly cut leaves harvested at the end of the day were fixed with glutaraldehyde, post-fixed with OsO4 and embedded in Epon resin. Ultrathin sections were cut with a diamond knife, post-stained with periodic acid thiocarbohydrazide silver proteinate (PATAg) and observed by transmission electron microscopy (TEM). Typical images of plastids from the wild-type and three transformants with different levels of GlgB activity are shown in Figure 1. The glucans were specifically stained with PATAg and morphological differences can clearly be seen by comparison with the well-formed starch granules in the wild-type specimen (Fig. 1a). These observations complement chain length distribution profiles (established by HPAED-PAD after complete enzymatic debranching of the molecules) and determination of crystallinity levels (determined by X-ray diffraction) of insoluble polysaccharides. Altogether, our results indicate that replacing the endogenous plant branching enzymes by a protein of bacterial origin can result in the production of a polymer with characteristics close to those of the wild-type amyllopectin. Our results allowed to establish a relation between the level of branching enzyme activity and the structure of the synthesized polysaccharides.

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Fig. 1: TEM images of ultrathin sections of leaf plastids from wild-type (a) and transformed (b-d) Arabidopsis, positively stained with PATAg. The level of ClgB activity in the three transformants varies from high (b) to intermediate (c) and low (d).