Type of presentation: Poster

LS-2-P-2030 Morphology of leaf starch granules of Arabidopsis thaliana wild type and mutants (sex1 & nex1)

Annunziata M. G.[1], Maximova E.[1], Stitt M.[1], Hartmann J.[2], Lunn J. E.[1]

[1]Max Planck Institute of Molecular Plant Physiology, 14476 Potsdam–Golm, Germany, [2]Max Planck Institute of Colloids and Interfaces, 14476 Potsdam–Golm, Germany

Email of the presenting author: maximova@mpimp-golm.mpg.de

Starch, together with sucrose, is the main end product of photosynthesis in many plants. The main pathways of starch synthesis and degradation are well known but our understanding of their regulation is still fragmentary. The aim of this work was to identify novel starch excess mutants to provide new insights into the pathway and regulation of starch metabolism. We identified a T-DNA mutant with insertion in a gene encoding a protein not previously linked to starch metabolism which we called NEX1. To characterize the *nex1* mutant in detail we are comparing its phenotype with already known starch excess mutants via: 1) metabolite profiling through a 24-h diurnal cycle; 2) analysis of starch granule morphology; 3) transcript profiling (qRT-PCR) of ~80 starch related genes; 4) growth analysis. To investigate the morphology of the leaf starch granules from *Arabidopsis thaliana* (L.) Heynh. wild type (WT) and mutants (*sex1* & *nex1*) we performed transmission electron microscopy (TEM). Pieces (2 mm^2) of mature leaves were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer and post-fixed in 1% OsO_4. The tissues were embedded in Spurr's epoxy resin and examined with an EFTEM (Zeiss, Germany). Some ultrastructural alterations were observed in mesophyll cells in WT plants and mutants (*sex1* and *nex1*) during day and night. Differences were found in plastids, especially, in the size and shape of starch granules, as well as in the organization of thylakoid membranes, predominantly in granal stack, size and number. Electron micrographs showed that starch grains occupy most of the plastid volume in mutants at the end of the day. Starch granules of WT plants are smaller, flat and a little elongated [Fig. 1]. In contrast, in the *sex1* mutant, which accumulates up to three times more starch than WT, the granules are flattened-elongated, running parallel to the long axis of the plastid with transverse crystalline regions (dark part in starch granules) and amorphous (lighter part - less crystalline material) [Fig. 2]. The starch granules in *nex1* are highly variable in size, but are generally larger than WT granules, and more rounded than both WT and *sex1* granules [Fig. 3]. The *nex1* mutant contains only slightly more starch than WT plants at the end of the day, but degrades little or none of its starch at night. The biochemical reason for this defect is unknown. The genetic lesion in the *nex1* mutant might lead to a deficiency in one or more of the enzymes that are required for starch degradation. Another possibility is that the *nex1* lesion causes synthesis of abnormal starch granules that are resistant to hydrolysis by β-amylase or the action of other starch degrading enzymes.

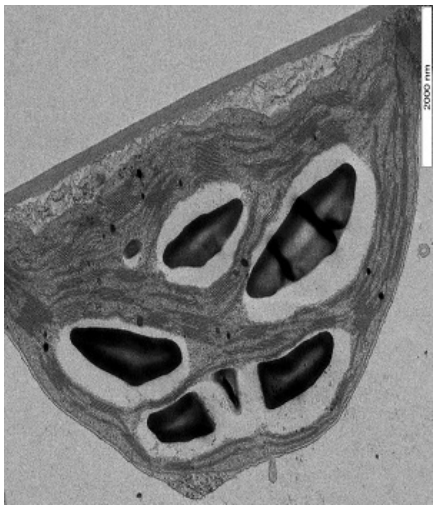

Fig. 1: Transmission electron micrographs of mesophyll cells in Arabidopsis thaliana WT Col0 leaves at the end of the day - fragment of the cell. Notice a few starch grains in stroma of the plastids. Starch granules of WT plants are smaller, flat and a little elongated compared with mutants (sex1 & nex1). Scale bar: 2000 nm

Fig. 2: Transmission electron micrographs of mesophyll cells in Arabidopsis thaliana sex1 mutant leaves at the end of the day - fragment of the cell. Notice a strong starch accumulation. The starch granules are flattened-elongated with transverse crystalline regions appearing as dark bands, and occupy most of the plastid volume. Scale bar: 2000 nm

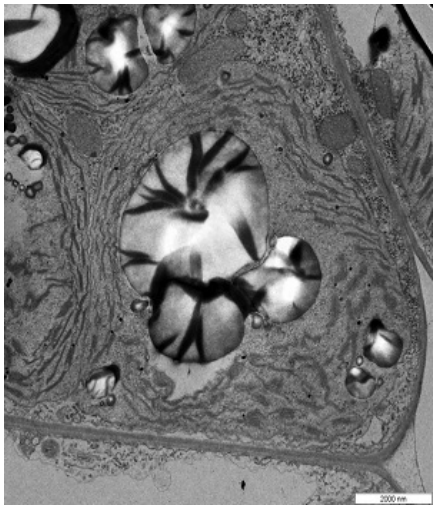

Fig. 3: Transmission electron micrographs of mesophyll cells in Arabidopsis thaliana nex1 mutant leaves at the end of the day - fragment of the cell. Starch granules in plastids are rounded and very variable in size ranging from very small to big compared with wild type and sex1 mutant granules. Scale bar: 2000 nm