Diurnal Variations of Starch Granule in Arabidopsis with Two-Photon Fluorescence and Second-Harmonic Generation Imaging

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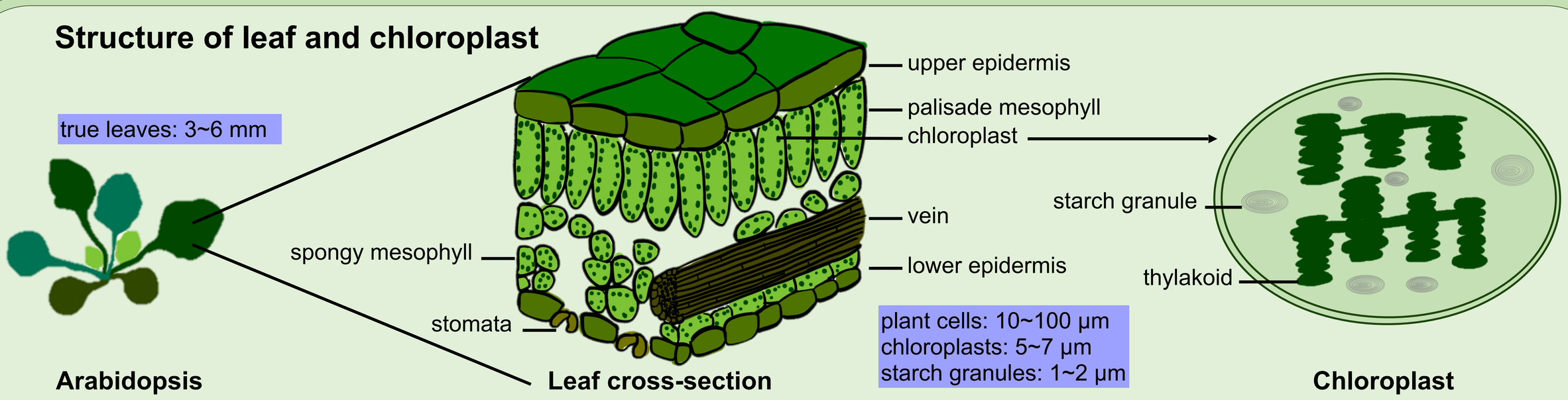
Abstract

Starch is the main storage carbohydrate in higher plants. During the photosynthesis of plants, starch can accumulate temporarily in the chloroplast and form into starch granules. In addition, starch granules are synthesized during the day and degraded at night in the leaves. Currently, one method for examining starch in plants requires dissolving the chlorophyll of the plant and staining the starch with iodine solution. Another method involves cloning a construct that contains target gene of interest, then acquiring transgenic plants that carry the construct by screening. These processes are time consuming or complicated, and in the staining case, provides no spatial information about the distribution of starch granules in leaf. In this study, we used two-photon fluorescence (TPF) and second-harmonic generation (SHG) imaging to quantify starch granules in Arabidopsis leaves during a 24-hour period. The starch granules are visualized by SHG imaging while the chlorophyll and mesophyll are visualized by TPF imaging. Two kinds of Arabidopsis were used. One wild-type (Columbia-0, Col-0) with normal starch production, and one mutant (*starch excess 1, sex1*) with excess starch production. Our data indicated a similar trend of diurnal changes of starch as seen in previous studies that examine starch content by enzymatic assay. With almost no preparation needed, TPF and SHG imaging is potentially a powerful tool for *in situ* diurnal study of leaf.

Structure of leaf and chloroplast



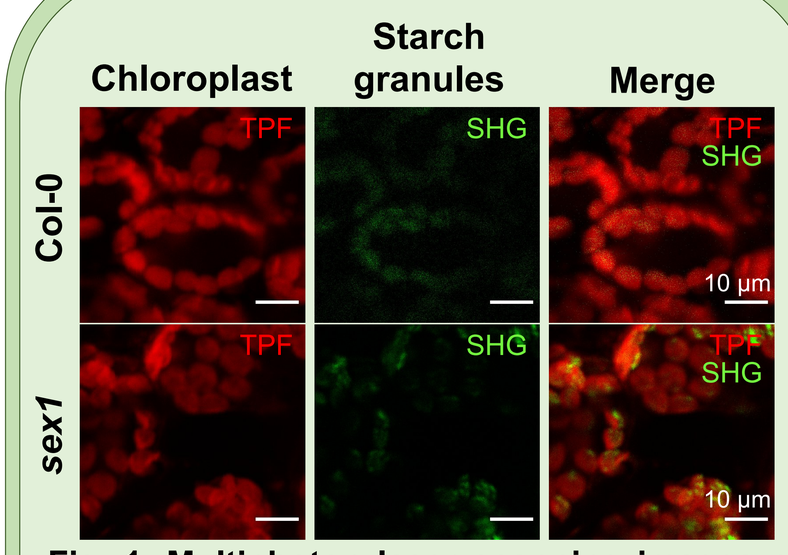


Fig. 1. Multiphoton laser scanning images of chloroplasts inside a fresh leaf.
Cells were viewed under a 20X objective lens in confocal microscope. Autofluorescence of chlorophyll and SHG signals were excited by 1064-nm multiphoton laser light.

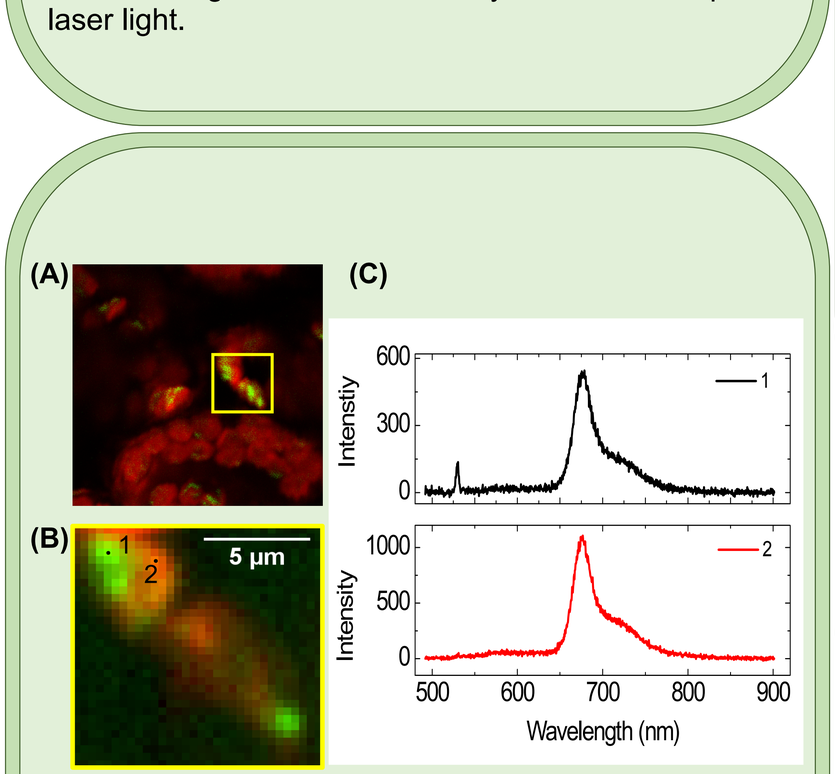


Fig. 2. (A) PMT mapping of chloroplast inside a fresh *sex1* leaf; (B) Spectrum mapping of selected area; (C) Spectrum of selected points.
Autofluorescence of chlorophyll and SHG of starch signals were excited by 1064-nm laser light, and the corresponding emission lights transmitted through 670/30 nm filter (red) and 532/3 nm SHG filter (green).

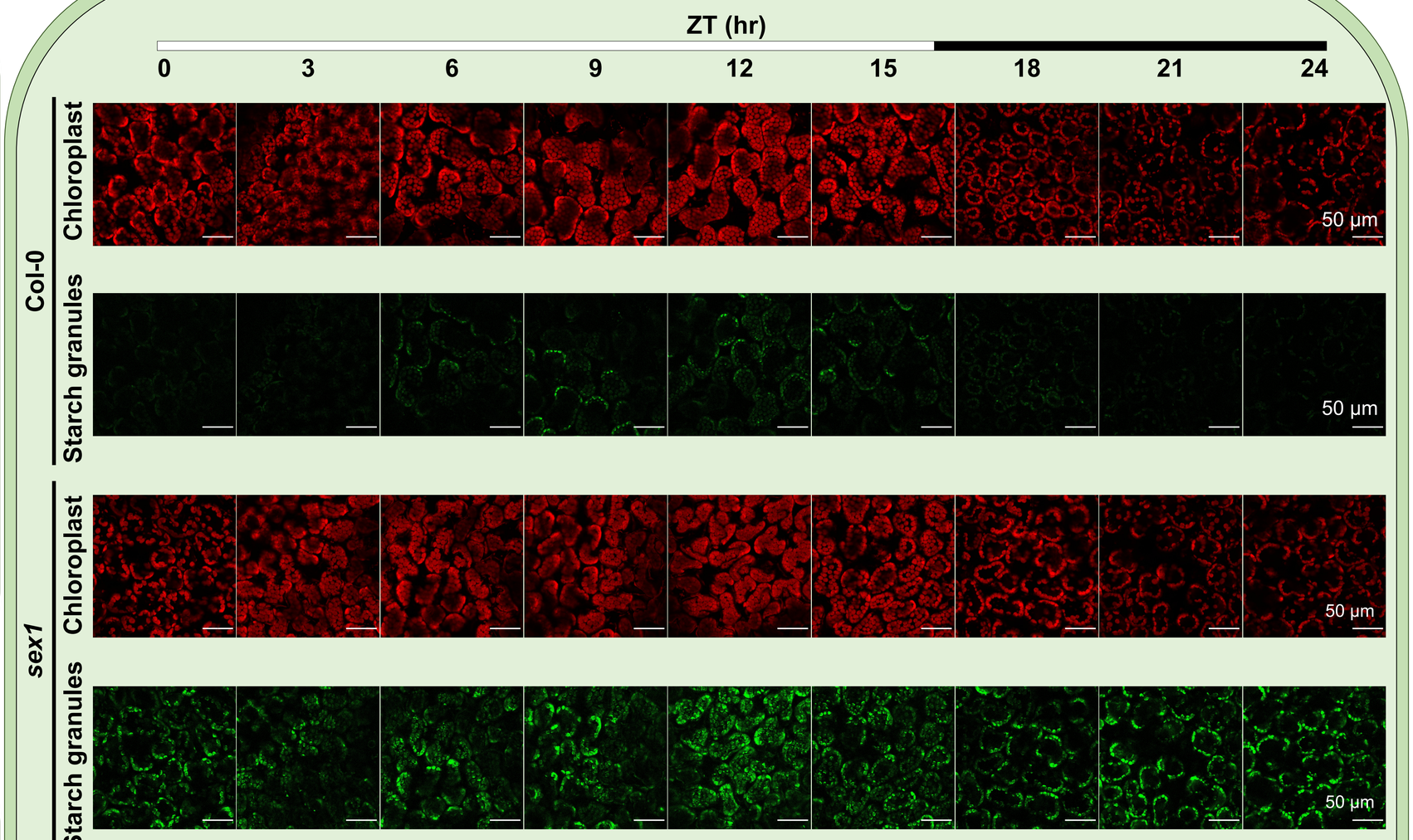


Fig. 3. Diurnal variations of starch granule in Arabidopsis with multiphoton imaging.
The images were used to determine the pixel counts and the intensity of TPF or SHG signal in Col-0 or *sex1* mutant leaves. The seedlings were grown under long day (LD, 16-h light/8-h dark) condition.

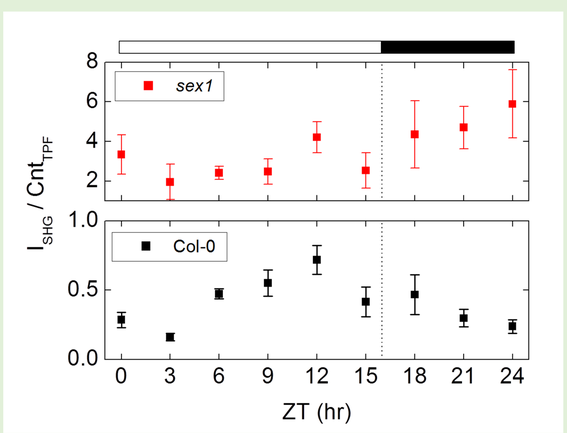


Fig. 4. The ratio of SHG intensity to TPF pixel counts in Col-0 or *sex1* leaves at diurnal condition.
I_{SHG}: summed intensity for pixels above SHG threshold
Cnt_{TPF}: total pixels above TPF threshold

Conclusion

The amount of starch granule determined using TPF and SHG imaging show results that is similar to previously known trend, where the amount of starch synthesis increases during the day and degrades gradually at night. Furthermore, this technique requires minimal sample preparation and can provide information about spatial distribution of starch granules in the chloroplast.

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