IMAGING NECTARIES

STEREOMICROSCOPY AND EVALUATION OF NECTAR PRODUCTION

To study the impact of individual genes on nectar production, it is obviously necessary to observe and collect nectar secretions. Unfortunately, precise quantification of total nectar volume in Arabidopsis is difficult at best; however, because the nectar clings to the inner surface of sepals when gently peeled back (Fig. 1a), relative changes in total nectar volume can be determined. We have found this to be a crude, but consistent method for determining relative changes in Arabidopsis nectar volume. This analysis has been used to preliminarily identify altered secretion phenotypes in several nectary-specific gene mutants. For example, as described above, we have identified CELL WALL INVERTASE 4 (AtCWINV4) as an absolutely required factor for nectar production in Arabidopsis, as *cwinv4* T-DNA mutants do not secrete nectar (Fig. 1b) and also display altered starch accumulation patterns (Ruhlmann et al. 2010). Significantly, Arabidopsis can also be useful for studying aspects of nectary biology besides nectar production, including the generation of floral scents (Tholl et al. 2005), and hormonal impacts on floral development (Aloni et al. 2006). Arabidopsis nectar can also be collected and assayed for total sugar and other metabolites (see: http://nectarygenomics.org/sites/default/files/NECTAR%20COLLECTION%20&%20ANALYSIS.pdf

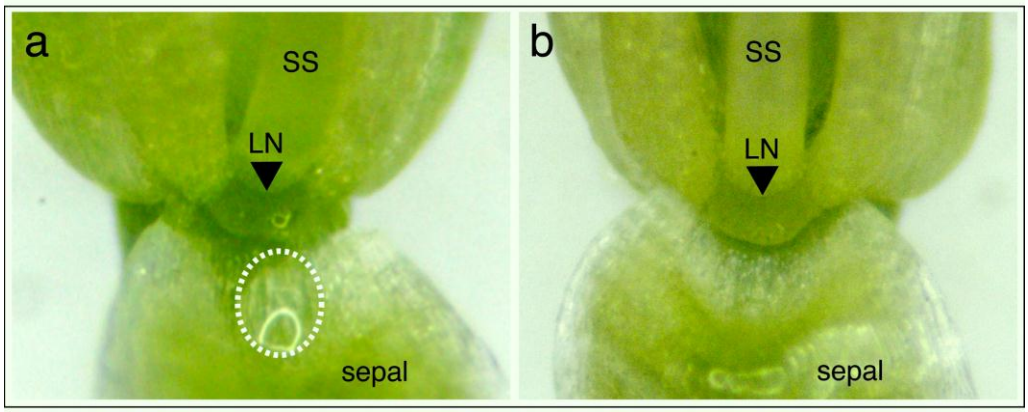

Figure 1. Evaluation of nectar production in Arabidopsis flowers. Gently peeling back the sepal allows for the easy determination of relative increases or decreases in nectar production in Arabidopsis flowers. (a) Nectar droplets accumulating within the sepal cups surrounding lateral nectaries (LN) are consistently present in wild-type plants (circled). (b) *cwinv4* mutants do not secrete nectar (e.g., *cwinv4-1*, SALK_130163). Preliminary analyses indicate that other nectary-specific gene mutants produce little or no nectar, whereas some have increased nectar volumes.

IMAGING NECTARIES

CONFOCAL MICROSCOPY OF NECTARY ULTRASTRUCTURE

Significant changes in intracellular morphology, particularly in ER, Golgi, vacuoles, and plastids, are known to occur in nectaries throughout development. To date, and to our knowledge, all reports of nectary ultrastructure have relied on thin sectioning of nectaries followed by light or electron microscopy. However, a significant advantage of Arabidopsis is that nectaries are very small (~100 microns wide and deep) and occur just inside the sepals at the base of stamen and petals. Thus, simple removal of sepals allows direct access to nectary visualization without further dissection (e.g., Fig. 2). To take advantage of these characteristics, Arabidopsis nectary ultrastructure can be studied *in vivo* by laser scanning confocal microscopy (LSCM, Fig. 2). We have examined a large number of transgenic Arabidopsis lines expressing GFP and YFP fusions (e.g., Fig. 2). Each of these fusion proteins is targeted to a specific subcellular location and all have shown good imaging results in nectaries. Plastids are also readily imaged through chlorophyll autofluorescence. In addition to these fluorescent transgenic lines, imaging can be performed with fluorescent dyes, such as Invitrogen's FM4-64 and FM1-43. With these fluorophores we have been able to image plasma membrane and endomembrane compartments, as well as nectary morphology as a whole. It is expected that observations made via LSCM might be able to help tease apart the general pathways of nectar secretion by providing *in vivo* analysis of nectary ultrastructure and organelle dynamics.

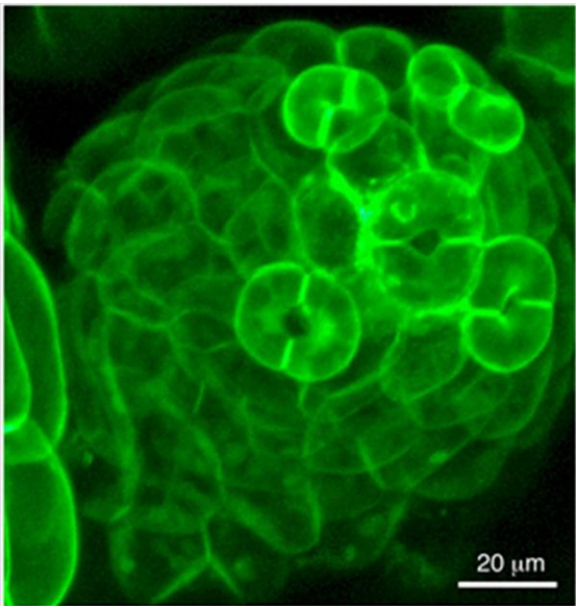

Fig. 2. Analysis of Arabidopsis nectary structure and ultrastructure via laser scanning confocal microscopy. *In vivo* imaging of Arabidopsis nectaries is possible with GFP fusion proteins and fluorescent dyes. In this example, laser scanning confocal microscopy was used to examine an Arabidopsis lateral nectary expressing plasma membrane localized GFP (GFP:LTI6b; described in Cutler et al. 2000). The image shown was compiled from a z-stack of 72 individual photos. Sample preparation consisted simply of removing sepals from the flower prior to imaging. Available software also allows the creation of time-lapse movies to follow membrane and organelle dynamics. Modified stomata are clearly visible and serve as the presumed sites of nectar secretion.

IMAGING NECTARIES

SCANNING ELECTRON MICROSCOPY

As with stereo and confocal microscopy of nectaries, imaging is simply performed by removing sepals to reveal nectaries. In our analyses, we flash freeze flowers in liquid nitrogen and imaging under low vacuum conditions (~65 kPa) and an accelerating voltage of 15 kV, with a JEOL Ltd. JSM-6490 scanning electron microscope at the University of Minnesota Duluth Imaging Facility. Under these conditions no prior fixation or coating is required. One example of lateral and median nectaries imaged by SEM can be found in Figure 3.

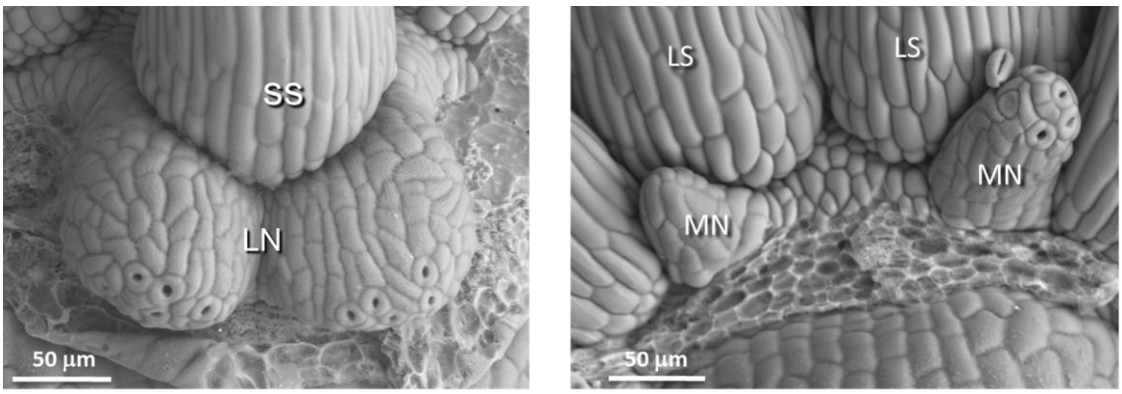

Figure 3. SEM imaging of a lateral (LN, left) and median nectary (MN, right). SS=short stamen, LS = long stamen.

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