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PARTICIPATION OF STOMATA IN WAX SECRETION AND DEPOSITION OF LEAF SURFACE

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Introduction. The epidermis of the aerial organs of higher plats is covered by a cuticle, which is an extracellular thick membrane consisting of a complex cutin matrix and a soluble epicuticular waxes deposited on its surface. The anatomy and chemistry of the waxes of leaves is functioning as a trap to collect water, protect plants against contamination and pests and pathogen attack, and fulfil other important functions (Seymur et al., 1996). During leaf development the wax layer grows by deposition on the leaf surface with special morphological and chemical features that are characteristic of the species and have taxonomic value (Stace, C.A. ,1989). Our observations of the surface of micro-propagated *Agave* plants by SEM show that the first deposition of waxes occurs inside and around the stomata. Micro-propagated plants lack epicuticular waxes and are therefore a good system to study the early stages of wax deposition. The SEM images of waxes around stomata from different species (own unpublished results) also suggest that waxes, or wax precursors are expelled as emulsion in water phase or in gas phases (O_2 and CO_2) to the surface of leaf through the stomata, during the process of water evaporation and oxygen/carbon dioxide gas exchange and are deposited on the more cold leaf surface by condensation (by facilitated diffusion).

The **objective** of our study was to prove this idea experimentally, applying a cold trap to condense waxesfrom the surface of the leaves and compare their distribution with the one of the stomata on the leaves.

Methodology. Leaves collected from the field and during stages of micropropagation were fixed in 2.5% glutaraldehyde dissolved in 0.2 M sodium cacodylic buffer (pH 7.0-7.4) or in 2.5% formaldehyde dissolved in 0.2 M K/Na phosphate buffer (pH 7.0-7.4). After 24 hours fixation, samples were dehydrated in ethanol series (30, 50, 70, 96, and 100%) and dried in a critical point dryer (SAMDRI PVT-795 or

SAMDRI PVT-2, Tousimis Research Corp., Rockville, MD, USA), then affixed to double-sided adhesive tape and SEM stubs, coated with platinum or gold in a dental vacuum DESK II Sputter Coater at 20-25 nm (Tousimis Research Corporation). Samples were analyzed in a one of three scanning electron microscopes (JSM 5800 LV and JSM 6360 LV, JEOL Company, Toyko, Japan; S-3000 N, Hitachi High Technologies America, Schaumburg, IL, USA) with 5-15 kV accelerating voltage.

Construction of a cold trap (our *know how*): thermocouple, thermo conductor, thermometer. The temperature of the cold trap was ~ -7-8°C and the temperature inside the leaf was 24°C.

Results. Using a "cold trap" we could demonstrate that wax deposits into- and close to stomata on the plant leaf surface. The distribution of the condensed waxes on the cold trap does appear that they originated from the opened (and may be young) stomata. These data suggest that stomata take part in the transfer of waxes through the movement of water vapours, oxygen and carbon dioxide. It means that we have typical facilitated transport coupled with active energy consuming transport of mono-valence (K/Na) and bi-valence (Ca/Mg) cations. Wax compounds are transporting in state of emulsifiable concentrate. The electron microscopy has consistently failed to reveal transcuticular pores in the sense of open channels, which via wax and other materials could flow freely (Jeffree, 1996).

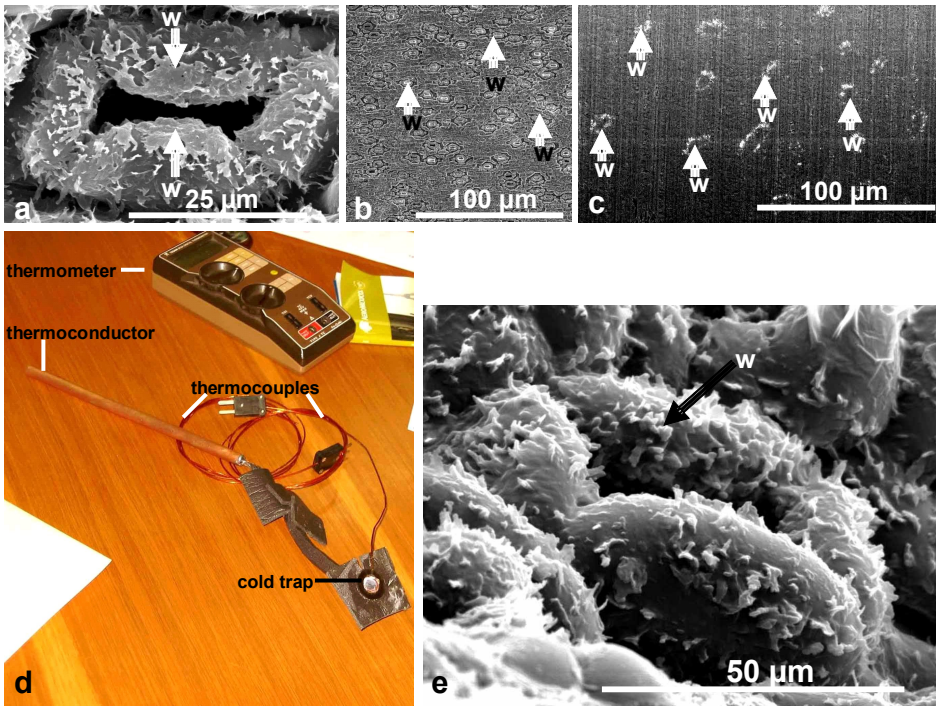

Fig. 1. Results of collecting wax (w) from the leaf surface of *Agave sisalana.*

(**a, e**) Wax deposit inside stoma of the leaf epidermis under high magnification; (**b, c**) Wax deposit under low magnification; **d** -cold trap.

Conclusions. New consumed function of plant stomata is the remarkable illustration of biological alternating sequences – functional metamorphosis of plant organs: the same morphological building block – plant stomata - play the broad spectrum of roles, carrying out numerous metabolic and interstratified (reversal, alternating) functions. We have the correct example of spatial and temporal differences in plant epidermal evolution.

References

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