FLOWER DEVELOPMENT AND POLLEN MORPHOLOGY IN 
*Cucurbitaceae*

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INTRODUCTION AND OBJECTIVE

Up to date, early flower development and the fundamental changes accompanying pre- and post-fertilization in *Cucurbitaceae* (watermelon, melon, zucchini, cucumber.....) have not been sufficiently described at the morphological level. Some varieties of *Cucurbitaceae* recently emerged as commercial trade, are generating a great success because of their high quality (they are very sweet), and their great agronomic value (large size, resistance to diseases, high yield, harvesting time...). Production of these fruits in the greenhouse requires the plants to be pollinated effectively. Bees and bumblebees are introduced artificially in greenhouses and usually carry out this duty. However, the efficiency of the process is not very high at present and farmers need it to be improved. This requires detailed knowledge of how pollen production takes place, how and when the female flowers reach their receptivity, and whether pollen is viable and can or cannot germinate as well as many other details of fertilization.

The main aim of this work was to characterize the morphological stages of floral development in two plant models: watermelon (*Citrullus lanatus*) and zucchini (*Cucurbita pepo*), by analyzing some structural aspects of their flowers in detail.

MATERIALS AND METHODS

1. Flowers were obtained in the greenhouses of the University of Almería.
2. Photographs of flowers at different developmental stages were taken with a macro lens.
3. Conventional chemical fixation of anthers and gynoecia was performed, using 4% PF and 1% GA in 0.1M phosphate buffer pH 7.2, followed by dehydration in ethanol and infiltration by increasing the concentration of paraffin.
4. Several samples were processed identically, but infiltrated with Unicryl synthetic resin.
5. Semi-thin sections were obtained on a paraffin microtome, and ultra-thin sections were cut on an ultramicrotome.
6. Semi-thin sections were stained with methylene blue/toluidine blue mixture or with...
PAS (staining of polysaccharides).
7. The microscopy techniques used were as follows:
   - Bright-field Light Microscopy (LM: Microscope Zeiss Axioplan),
   - Confocal Laser Scanning Microscopy (CLSM: C-1 Microscope Nikon ), and
   - Transmission Electron Microscopy (TEM: JEOL Electron Microscope JEM1011) at 80 kV.

Figure 1. Photographic documentation of the key methodical steps of the study. (1) Collection of samples in UAL greenhouses, (2) Chemical fixation of the material, (3) Dehydration of the material (4) Infiltration of the material in paraffin and/or resin, (5) Polymerization of paraffin and/or resin, (6) Obtaining semi-thin sections, (7) Confocal microscope observations, (8) TEM observations.

RESULTS

1) FLORAL DEVELOPMENT IN ZUCCHINI
Figure 2. Floral development in zucchini. Top left: female zucchini flower. Upper row A-D: stages of zucchini pistil development. The pistil consists of three carpels fused in one ovary with 3 short styles partially fused at their base, each of which ends with a bilobed stigma. Bottom left: male flower of zucchini. Lower row, figures A'-D' : developmental stages of zucchini stamens. The male flower consists of 3 stamens, each one formed by a filament and a yellowish anther. The 3 stamens are fused at the filament and the anther (Figure 1C'). Pollen is orange-colored.

2) ANther DEVELOPMENT IN ZUCCHINI
Figure 3. Sections of zucchini anther stained with methylene blue/toluidine blue mixture (B) or with PAS (E). Section of an anther showing numerous mature pollen grains in the locule (A). Details of various pollen grains located near the anther wall, surrounded by tapetum cells (B-C). Details of the vegetative cell cytoplasm and the apertural region, showing micro- and macro-spikes decorating the wall of the pollen grain (D). Numerous birefringent granules are observed in the cytoplasm, consisting of starch, as confirmed after specific polysaccharide staining with PAS (E). AL: anther locule, Ap aperture, AW: anther wall, Ex: exine, GN: generative nucleus, PG: pollen grain, St: starch, T: tapetum, VC: vegetative cell cytoplasm, VN: vegetative nucleus.

3) MORPHOLOGY OF THE ZUCHINI AND WATERMELON MATURE POLLENS

Figure 4. Mature pollen of zucchini and watermelon observed with a confocal laser scanning microscope (A and B) and with an epifluorescence microscope (C). (A) Projection of about 50 optical sections of zucchini pollen. The pollen is polypantoporated, nonpolar, with radial symmetry, circular and spherical. It possesses simple pore-type apertures and apertural membrane with operculum. Macro-spikes are present on the pollen surface. (B) Pollen from watermelon is trizoneporate, isopolar with radial symmetry. Simple apertures of the pore-type. (C) Autofluorescence of mature zucchini pollen excited with ultraviolet light. Pollen surface is of reticle-perforated type. Macro-spikes are especially well observed on the pollen surface. Op: operculum, MS: macro-spikes, P: pore. AP: aperture, UB: Ubish bodies.

4) ULTRASTRUCTURE OF ZUCCHINI MATURE POLLEN
Figure 5. Mature pollen of zucchini observed with TEM. Sections were contrasted with uranyl acetate and lead citrate salts. Exine 4 µm of thick, with sexine approximately as thick as the nexine. Complete tectum, columelated infratectum. The pollen surface exhibits regularly distributed macro-spikes with a length of 5-9 µm, and numerous micro-spikes of 1 µm length. The pollen cytoplasm shows abundant starch granules. AP: aperture, Ex: exine, In: intine, mS: micro-spike, MS: macro-spike, Op: operculum, P: pore, St: starch.

CONCLUSIONS

1. The study of floral morphology in Cucurbitaceae is very important given the broad agronomic implications of this family and the frequent fertilization and fruit set problems present among their members.

2. The use of microscopy techniques with different levels of resolution allows us to observe details of the process and to connect them with the reproductive physiology of the plant.

3. Once the basic reproductive structure is known, it will be possible to analyze the behavior of commercial lines, hybrids, plants with different ploidy levels and so on, in order to try to improve some aspects of their reproduction, such as pollen loading, pollination efficiency and finally, their production.

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REFERENCES

MY OWN IDEAS

This chapter compiles all ideas proposed by the high school students in round tables set throughout the experiment.

- The experiments performed are enjoyable and interesting. We have worked with microscopes and other equipment we have never worked with or even seen before. On the whole, the experiments didn’t seem difficult to be performed. The most difficult task of this study was paraffin embedding. What is the most difficult of these studies is how much time has to be used. They are quite aesthetic studies, as they are based in the capture of images from the microscopes, by observing flower elements.
- This kind of research on Cucurbitaceae has an agronomic value: by studying and using them in the products, resistance to virus diseases could be developed.
- By studying Cucurbitaceae, it could be found out which insects attack them, and achieve their resistance, avoiding their destruction by pests.
- The studies developed here could help to reach a higher (and more economically sustainable) yield, allowing better feeding of the population, which is considerably increasing yearly. Such higher production could be obtained by improving pollination efficiency in these plants.
- The analysis carried out could be used to produce new foods, medicines or elements helping society. A company could use this type of studies to create different and healthy foods, and also make money with these products.
- Another application of this study on the reproduction of Cucurbitaceae could be its use as a basis for further studies to be performed on similar species and families.
- Development of seeds could be also analyzed and nice and stunning images about their formation could also be obtained.
- If we know all these reproductive processes in detail, we could determine whether these plants can be mixed, and generate new hybrid fruits or modified plants of high interest.
- Another application could be to study pollen allergy in Cucurbitaceae, and how this may affect population.