Seedless watermelons: from the microscope to the table through the greenhouse

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HIGHLIGHTS

- Pollen development in triploid watermelon plants is highly asynchronous.
- Microspores/pollen grains derived from triploid plants are larger than those from diploid plants and show degradation symptoms and abnormal cell wall development.
- As a consequence, pollen morphology from triploid plants is also variable.

SUMMARY

Seedless triploid varieties of watermelon (*Citrullus lanatus* var. *lanatus*) are very appreciated by consumers, but its production is limited because pollen donor diploid plants and insect-assisted pollination is required. To improve this process it is necessary to better study pollen biology aspects such as pollen viability and germinability during long-term storage, stigma receptivity period, etc. In this work we have compared the morphology and ultrastructure of triploid and diploid plant-derived pollen grains in commercial varieties using diverse microscopy techniques. We have not detected at this stage key macroscopic morphological differences between diploid and triploid flowers. Anther development within the triploid flower is highly asynchronous. Microspores from triploid plants are larger than those from diploid plants and showed symptoms of cytoplasmic degeneration. Pollen grains from triploid plants present different morphologies, contain three isodiametric pores but colpi are sometimes not well developed. Moreover, and also depending on the hydration stage, the pollen surface is sometimes smooth, without the characteristic reticulate pattern present in pollen grains from diploid plants. All these developmental features may lead to infertility of triploid plant-derived pollen.

INTRODUCTION (AND OBJECTIVES)

Some seedless varieties of watermelon (*Citrullus lanatus* var. *lanatus*) have additional commercial value because they are sweet and lack of seeds. Production of these fruits requires triploid (3n) plants to be pollinated effectively with pollen from diploid (2n) donor plants, a duty that is carried out by bees and bumblebees introduced in artificial hives in greenhouses

[1, 2]. As result of double fertilization, triploid plants produce seedless fruits, a process called stenospermocarpy. However, continuous production of seedless watermelon fruits is challenging because: 1) the efficiency of the process is very low, 2) large amounts of viable pollen are required, and 3) donor-derived pollen production and triploid stigma receptivity must be synchronized [3]. Improving this process 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. In this context, the main aim of this work was to characterize the morphological and ultrastructural characteristics of pollen grains from triploid watermelon plants and compare them with those of pollen grains produced by diploid donors.

MATERIALS AND METHODS

Plant material

Triploid and diploid male and female flowers of watermelon (*Citrullus lanatus* var. *lanatus*) were collected at different developmental stages in the greenhouses of the Fundación Finca Experimental UAL-ANECOOP (Almería, Spain). Macroscopic images of whole male and female flowers were taken *in situ* with a Nikon Coolpix 4500 digital camera (Nikon, Japan). Whole flowers were also observed after dissection under a Leica epifluorescence stereomicroscope M165FC (Leica Microsystems, Germany).

Sample preparation for microscopy

Whole flowers were fixed in 4% (w/v) paraformaldehyde, 2% (v/v) glutaraldehyde in 0.1 M cacodylate buffer at pH 7.5 overnight at 4°C. After fixation, samples were washed with several changes of cacodylate buffer, dehydrated in an ethanol series, embedded in Unicryl resin (BBInternational, Cardiff, UK) and polymerized at -20°C under ultraviolet light for two days. A second set of flowers were processed for paraffin embedding at the "Biobanco del Sistema Sanitario Público de Andalucía" (Granada, Spain).

Light microscopy (LM)

Semi-thin (1 µm) resin sections were obtained using a Reichert-Jung Ultracut E microtome (Leica Microsystems). Semi-thin (10 µm) paraffin sections were obtained using a hand operated-rotary microtome. The sections were placed on BioBond-coated slides, dewaxed (paraffin sections) and stained with a mixture of 0.05% (w/v) methylene blue and 0.05% (w/v) toluidine blue according to [4]. Finally, the slides were mounted with Merckoglass (Merck, Germany). Observations were carried out using a Zeiss Axioplan (Carl Zeiss, Germany) microscope. Micrographs were obtained using a ProGres C3 digital camera with the ProGres CapturePro 2.6 software (Jenoptic, LaserOptic Systems, Germany).

Confocal laser scanning microscopy (CLSM)

Whole mature pollen grains were dispersed in an anti-fading Citifluor (Sigma, USA)/water (1:1) solution and observed in a Nikon C-1 confocal laser scanning microscope (Nikon, Japan).

Transmission electron microscopy (TEM)

Ultrathin sections (70-90 nm) were cut on a Reichert-Jung Ultracut E microtome (Leica Microsystems), mounted on 200-meshed nickel grids and stained with 2% (w/v) uranyl acetate followed by 1% (w/v) lead citrate. Observations were carried out with a JEOL TEM-1011 (JEOL, Japan) transmission electron microscope at 80 kV.

RESULTS

Flower morphology of triploid watermelon plants

Watermelon is a monoic species with unisexual flowers on the same individual. Macroscopic observations revealed significant differences in morphology between male and female flowers (Figure 1). Male triploid flowers contain five atypical stamens and possess a rudimentary unfertile ovary (Figure 1A).

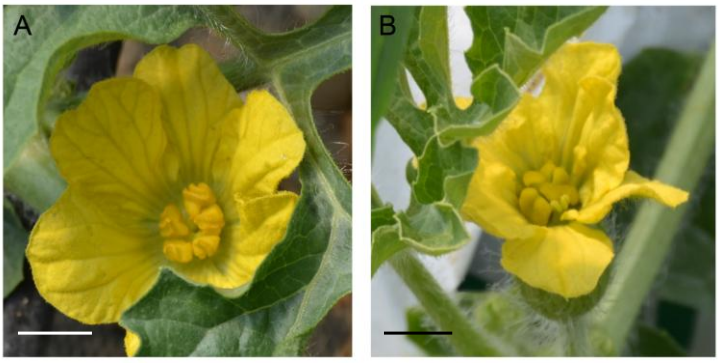

Figure 1. Unisexual flowers of triploid watermelon (*Citrullus lanatus*) plants at anthesis. A) Male flower. B) Female flower. Bars= 1 cm.

On the other hand, female triploid flowers have a tricarpelar and inferior ovary (Figure 1B). The ovary contains numerous orthotropous ovules and the placentation is of parietal type (Figure 2). We did not find noticeable morphological differences between diploid and triploid flowers.

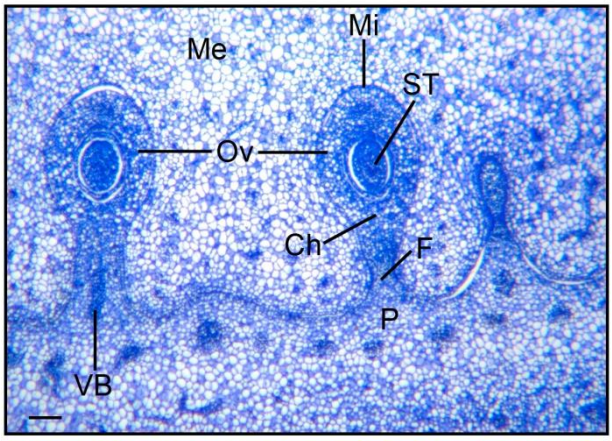

Figure 2. Morphological characteristics of a triploid watermelon ovary. Ch, chalaza; F, funiculus; Me, mesodermis; Mi, micropyle; Ov, ovule; P, placenta; ST, sporogenous tissue; VB, vascular bundle. Bar= 100 µm.

Watermelon pollen morphology

Anther development within the flower is asynchronous since different anthers contain microspore/pollen at different stages of development (Figure 3). Moreover, microspores from triploid watermelon plants are bigger than those from diploid plants (Figure 3E, H).

Pollen grains from diploid and triploid watermelon plants are tricolporate, and the pollen exine is of reticle-perforated type (Figure 4A). Pollen grains from triploid plants may vary in their morphology. They contain three isodiametric pores but colpi might be not well developed

(Figure 4B). Moreover, the pollen surface is sometimes smooth, and may lack the characteristic reticulate pattern present in pollen grains from diploid plants (Figure 4B).

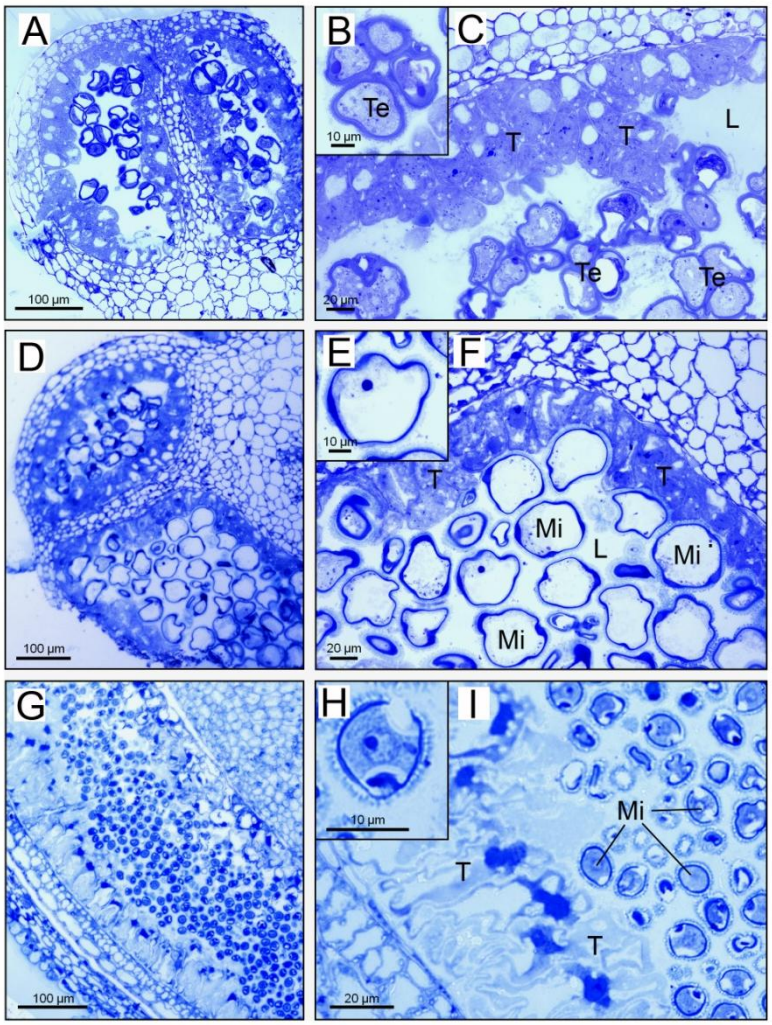

Figure 3. Comparison of anther development between diploid and triploid watermelon plants. A-F) Light microscopy toluidine blue-stained sections of two triploid anthers at the stages of tetrad (A-C) and microspore (D-F), respectively. G-I) Sections of a diploid anther at the stage of microspore. L, anther locule; Mi, microspore; T, tapetum; Te, tetrad.

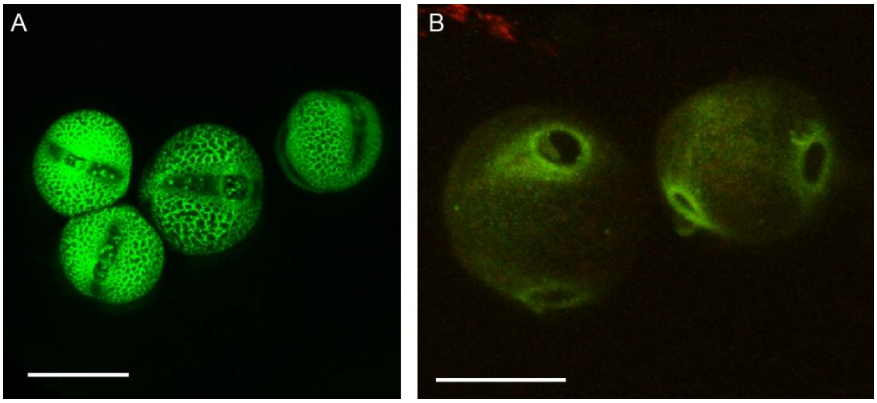

Figure 4. Different morphologies detected in pollen from triploid watermelon plants. Bars= 50 µm.

Watermelon pollen ultrastructure

The exine comprises two layers, the outer sexine and the inner nexine (Figures 5A-B). The sexine is sculptured with numerous tecta and bacula. The cytoplasm shows numerous amyloplasts filled with starch granules (Figure 5B). Microspores from triploid plants showed symptoms of cytoplasmic degeneration (Figures 5C-D).

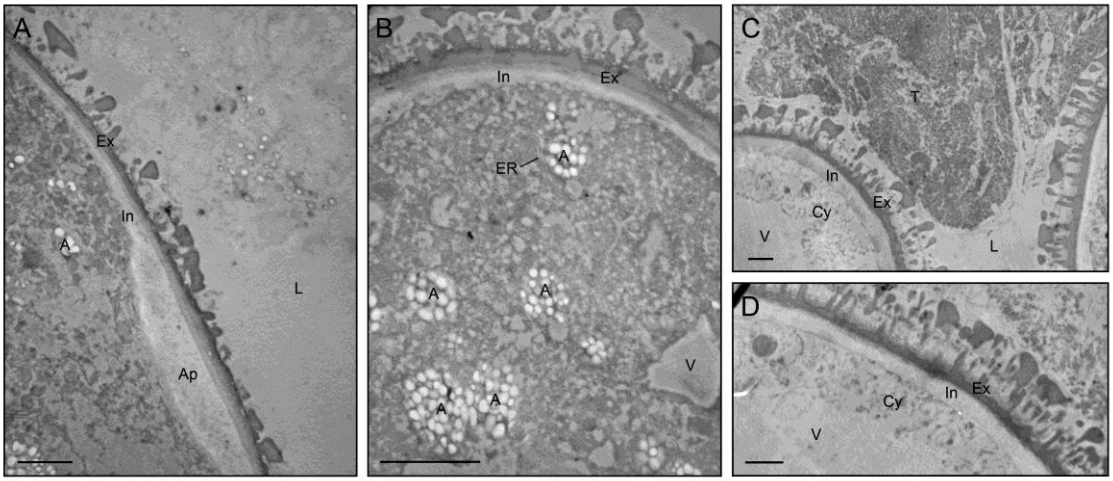

Figure 5. Ultrastructure of watermelon pollen grains. A-B) Ultrathin sections of maturing pollen grains from diploid watermelon plants observed with TEM. Sections were contrasted with uranyl acetate and lead citrate. C-D) Ultrathin sections of microspores from triploid watermelon plants. A, amyloplast; Ap, aperture; Cy, cytoplasm; ER, endoplasmic reticulum; Ex, exine; In, intine; L, anther locule; T, tapetum; MLC middle layer cell; V, vacuole. Bars= 2 µm.

CONCLUSIONS

1. **Pollen development within the flower of triploid watermelon plants is highly asynchronous.**
2. **Triploid plants-derived microspores/pollen grains are larger than those from diploid plants and show degradation symptoms and abnormal cell wall development.**

ACKNOWLEDGEMENTS

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MY OWN IDEAS

Trinidad María Caballero Sierra

First I want to thank all the researchers who have been with us, and to my Secondary School. This project has been a new experience because I had never used these kinds of microscopes. It was very nice to meet people who have devoted themselves to the investigation. They were explaining topics that we already learn in class; however, we were using this knowledge in a practical way. We have been using several microscopes that we had never seen, and for the first time we could use and handle them, thanks to the Experimental Center of the Zaidín. I didn't know very much on this topic, but now we known many things. What surprised me the most was to know that triploid watermelons do not have seeds, or just very little and small. I hope that with this project we will be able to improve the production in greenhouses. This project also served to reinforce our English skills. Finally and more importantly, I have meet new people and made very good friends.

Lidia Linares Parra

I can define this experience like unique, because working with plants in this way is not a game, nor easy. We have got the chance of sectioning, staining and observing the sections through different microscopes. The microscopes are impressive. With them, you can see the minimal details. Also we have seen the different stages of watermelon flowers. In the triploid watermelons (those without seeds), the pollen grains are alive during the early stages. However, pollen grains die when flower development progresses. Finally I would like to thank for this opportunity because it was wonderful to know the world of science, and to be able to work inside it.

Carlos Pérez Torres

Thanks to this fantastic project I have learn how to carry out a scientific research and the working methods of researchers. Our project dealt with triploid watermelons (seedless watermelons) and how to obtain them through the laboratory. We learn numerous working procedures, including sectioning, staining and dewaxing; moreover we have used different types of microscopes, which helped us to take pictures of flowers and different cell parts, using chemical products. Thanks for this experience, which helped me to know better the world of science.

Nieves Rivas Cañadas

To start with, I want to thank all people who have collaborated and worked in this project with us. This project helped us to know the world of science. Thanks to researches, we have carried our project "Seedless Watermelons". I think that all the instruments that are part of the CSIC's laboratories are incredible, particularly the microscopes. Also, we learnt to cut, stain, etc. flower samples. With the microscopes we could see the most complex details of different cells. One day we went to CSIC, we took pictures of the watermelon flowers and we managed to see the pollen grains very well. We have learnt a lot about cells, flowers, etc. Finally, I want to say that this is an amazing experience that all the people should have, and thank for this opportunity that we have had to know better the world of science, work with these incredible researches. Now, we like science a little bit more, because each day we can learn something new.