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Research Article

First High-Resolution Scanning Electron Photomicrographs of *Cucurbita maxima* Pollen

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Abstract

Cucurbita maxima is a species of cucurbit native to Central America, that has been shown to have many medicinal uses including anti-diabetic, anti-cancer, and anti-inflammatory properties. This economically important member of the Cucurbitaceae is not thoroughly documented in the scientific literature, and this manuscript fills in a major gap that is missing by providing the first high resolution Scanning Electron Microscopy images of *Cucurbita maxima* pollen to date. The individual pollen grain is a monad with a polar spherical morphology, echinate projections, and is 100µm in diameter. Researchers conducting palynological or paleopalynology studies will be able to use these images as a literature reference for *Cucurbita maxima.*

Keywords: Cucurbita maxima; Cucurbitaceae; Pollen, Pumpkin; Gourd, Microscopy; Scanning Electron Microscopy; SEM, Palynology; Microscope; Squash; Morphology; Paleopalynology; Anthropology; Forensics

Introduction

The genus *Cucurbita* (2x = 2n = 40) includes 13 species, with *Cucurbita maxima* producing the most morphologically distinct fruit in the genus, and perhaps within the entire Cucurbitaceae family that contains roughly 800 species [1,2]. The Latin binomial name suggests vigorous growth of fruit, which is the most massive of any cucurbit. *C. maxima* produces the larges fruit of any plant in the world, some weighing over 1050 kg. *C. maxima* is an incredibly diverse species, and it is suggested by Ferriol and colleagues to have more cultivated forms than any other crop species [3]. The polymorphic nature of *C. maxima* can result in a misidentification of the species, and lead to confusion when using this cucurbit for botanical research. *C. maxima* originated in South American and is thought to be domesticated 4000 years ago [3]. Various forms of *C. maxima* were disseminated to Europe in the 16th century and taken by European explorers to the Indian sub-content and South-east-Asia [3].

C. maxima has many medicinal uses, including anti-diabetic, anti-oxidant, anticancer, and anti-inflammatory properties [4]. It is also a major food source for wide variety of world cultures, due to its; fiber content, carbohydrates, β-carotene, vitamins, alkaloids, minerals, fatty acids, flavonoid, and polysaccharide content [5]. Recently, it has been shown that polysaccharides derived from C. maxima, directly affect the hypoglycaemic and anti-oxidant properties of the fruit [5]. Studies have shown bioactivity of certain polysaccharides extracted from *C. maxima*, and much of this activity is derived from the molecules structure [5]. These polysaccharides can reduce potentially reduce diabetic affects in humans and needs further research in order to elucidate the entire benefits or medical properties of this fruit.

Cucurbita maxima has been a model organism for research for almost 100 years and will continued to be studied due to its economic importance. After conducting a through literature search, it has been determined that high-resolution images of *C. maxima* pollen are not available for reference in palynological studies. Partial, Low resolution images of *C. maxima* were provided from unfixed samples, but no complete high-resolution images from samples are available in the literature [6].

This publication will provide high resolution images of *Cucurbita maxima.* The individual pollen grain is a monad with apolar spherical morphology, echinate projections, and is 100µm in diameter. Researchers conducting palynological studies will be able to use these images as a literature reference for *Cucurbita maxima.*

Methods

Seed Germination

Seeds of *C. maxima* cv. Burgess Buttercup USDA Certified Organic were purchased from Seed Savers Exchange®. Ten seeds of each cultivar were placed in filter paper lined Petri-plates, moistened with de-ionized water. This was replicated 10 times, for a total of 100 seeds. Seeds were incubated at 22°C under 24 hours of florescent lights. Seedlings were transferred from filer paper to 3" peat pots, filled with moistened Farfard® 3B potting soil. Plants were grown on light carts or in light boxes under fluorescent lights at 22°C with a regime of 18 hours of light, and 6 hours of dark.

Pollen Sampling

Scanning electron microscopy specimen mounts with adhesive pads were used to acquire *Cucurbita maxima* pollen from plants grown in Miami Universities Greenhouse. Pollen was only sampled from *C. maxima* cv. Burgess Buttercup that were Certified Organic by the United States Department of Agriculture. The microscopy mounts were gently placed under the anthers of the plant, and the flower was gently tapped to release pollen onto specimen mounts. A total of 5 specimen stubs were prepared from 5 different plants.

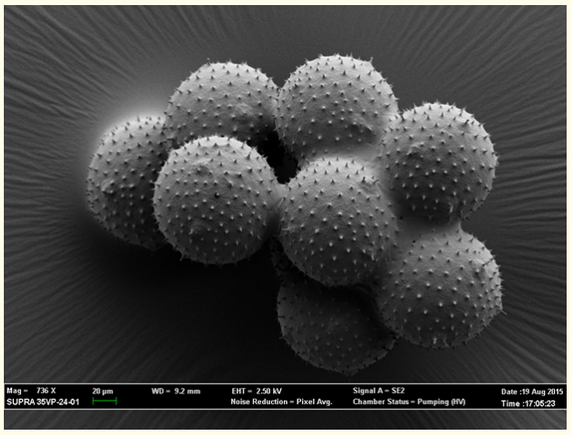
Sample Preparation

Scanning electron microcopy stubs were placed in a desiccator with Dry-Rite™ for 2 weeks prior to analysis. Once the samples were thoroughly dehydrated, they were sputter coated in a Den-

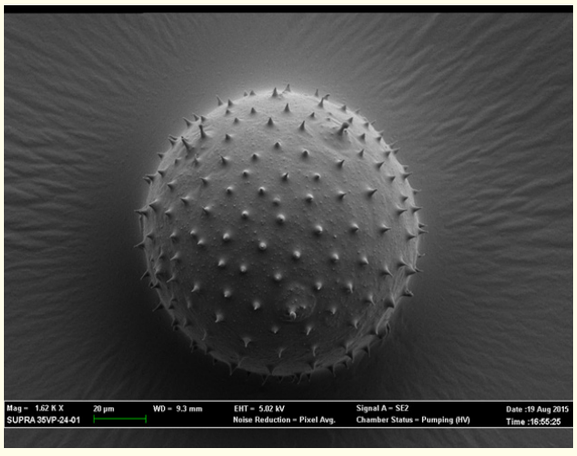
ton Desk II Cold Sputter Coater with pure gold, then imaged with a Zeiss Supra 35 VP FEG SEM at 2.5 kv and 5.02 kv (acceleration voltage).

Results

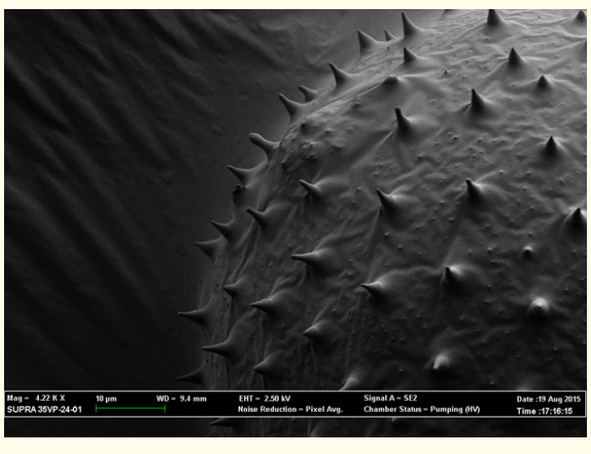
Figure 1A-1D are scanning electron photomicrographs of *Cucurbita maxima* pollen imaged at 736x, 1.62 kx, 4.22 kx, and 7.25 kx. Figure 1A provides an image of a cluster of pollen grains. Figure 1B provides a high-resolution image of an individual pollen grain is a monad with apolar spherical morphology, echinate projections measuring approximately 2µm in diameter, and is 100µm in diameter. Figure 1C and 1D show higher magnification of echinate projections measuring 5 - 6µm long and 2µm wide, as well as surface morphology of the pollen grain.



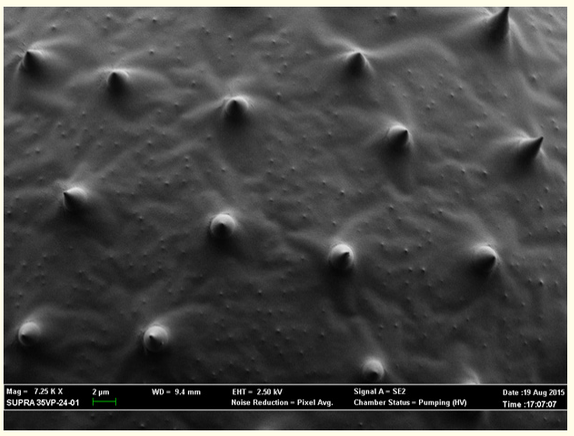
(A)



(B)



(C)



(D)

Figure 1 A-D: High Resolution scanning electron micrographs of Cucurbita maxima pollen observed under various magnification.

Discussion

Cucurbita maxima is an economically important crop that has been a model organism for decades due to its nutritional and health benefits. This diverse species of herbaceous plant is coveted for its massively sized fruit and extreme variation in phenotype. *Cucurbita maxima* has potential for anti-diabetes, anti-cancer, and anti-inflammatory medicines, and is currently a nutrition source for millions of people worldwide. With climate change making agriculture more unpredictable, the more critical it is for researchers to be studying economically important crops.

This manuscript provides high-resolution scanning electron photomicrographs to be used as a reference for researchers interested in pollen or are conducting palynological studies. Current researchers involved in paleopalynology will also be able to use these high-resolution images for a literature reference as well. Future research could examine differences, if any, in morphology at the variety level. Future research could also include spore dispersal studies, looking to determine current pollinators in the midst of a mass extinction of Hymenoptera. *Cucurbita maxima* offers many promising benefits and needs to be studied in more detail.

These images provided can be useful for forensic botanists as well as forensic anthropologists that need a reference for *Cucurbita maxima* pollen. Pollen grains are often recovered on crime-scenes, and these images provided in this manuscript could be useful for linking specific pollen to a specific plant.

Concussion

Cucurbita maxima is an economically important crop that has extensive nutritional and health benefits as well as is one of the most diverse species of herbaceous plant. Worldwide, *Cucurbita maxima* is coveted for its massively sized fruit and extreme variation in phenotype. This manuscript provides high-resolution scanning electron photomicrographs to be used as a reference for researchers interested in pollen or are conducting palynological studies. Current researchers involved in paleopalynology will also be able to use these high-resolution images for a literature reference as well.

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