



First report of the association of Genus *Chaetomium* with roots of *Cucurbita maxima*

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Abstract

Chaetomium sp. is a fungal genus in the Ascomycota that has a world-wide distribution throughout tropic and temperate regions. Species of *Chaetomium* are often associated with and classified as both seed-borne and soil-borne fungi, which inhabit a variety of terrestrial ecosystems including; agricultural, forests, grasslands, wetlands, and coastal zones. *Chaetomium* sp. plays an important ecological role as a saprophyte, but also shows strong anti-microbial activities which allow its use as a biological control agent (BCA). *Chaetomium* sp. has been recorded on the seeds and within the rhizosphere of a variety of genera of the family Cucurbitaceae including; *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria*, and *Luffa*. Within the genus *Cucurbita*, *Chaetomium* sp. has been documented in association with *Cucurbita pepo* and *Cucurbita moschata*, however up till now there is no record of association of *Chaetomium* sp. with *Cucurbita maxima*. Sampling of *Cucurbita maxima* roots in the eastern United States resulted in the discovery of a novel relationship between *Cucurbita maxima* and *Chaetomium* sp. This manuscript presents the first report of existence of a relationship between *Chaetomium* sp. and *Cucurbita maxima*, with the aid of light microscope to image the perithecia and spores produced by this fungus.

Keywords: *Chaetomium* sp., *Cucurbita maxima*, Cucurbitaceae, Microscopy, Ascomycota, Fungi

1. Introduction

Chaetomium (Kunze) is a genus of fungi in the Ascomycota that inhabits terrestrial ecosystems, and has a world-wide distribution throughout tropic and temperate regions. Mahdy *et al.*, (1987); Selvi and Balagengatharathilagam, 2014; Gashgari *et al.*, (2016) reported that *Chaetomium* plays an important ecological role as a saprophyte and as a biological control agent. Various *Chaetomium* species are known to produce the alkaloid Echinulin, which has been

shown to be incredibly toxic to mammals, causing damage to the internal organs including the lung and liver (Barros-Filho *et al.*, 2012).

Cucurbita maxima (Duchesne) is a member of the Cucurbitaceae family, and has been used by human for more than 4000 years as a nutritional source, medicinal plant, and as a storage container (Zhang *et al.*, 2006; Hulse, 2018; Hulse and Braselton, 2019). C.

maxima seeds and fruit were disseminated to Europe sometime in the 16th century, and taken by the European explorers to the Indian sub-continent and Southeast-Asia. Moreover, *C. maxima* is used in traditional medicine, because it is reported to have anti-diabetic, anti-cancer, and anti-inflammatory properties. In addition, it is also used as a food source by a wide variety of world cultures, due to its high fiber content, diverse carbohydrates, β -carotene, vitamins, alkaloids, minerals, fatty acids, flavonoids, and polysaccharides (Saha *et al.*, 2011; Zhou *et al.*, 2014; Hulse and Braselton, 2019; Maurya *et al.*, 2019).

Since the early 1900's, *C. maxima* has been widely used as a model organism as it has larger phloem cells than most plants of an equivalent size, which makes it an ideal candidate for different studies involving nutrient movement, nutrient acquisition, cell signaling, in addition to other studies that involve the phloem tissues (Ferriol *et al.*, 2004, Hulse, 2018, Hulse and Braselton 2019). It has also been used as a model organism in the inter- and intraspecific grafting studies, because it has a strong affinity for surviving the grafting process, and grafts easily to the other members of the Cucurbitaceae. Ferriol *et al.*, (2004); Hulse and Braselton, (2019) pointed that currently *C. maxima* remains a model organism for molecular, nutritional studies, and will continue to be used in more studies because of its multi-million-dollar economic impact in the United States of America (USA).

The current research was designed in order to more fully understand the microbial communities associated with *C. maxima*, and to investigate the presence of a relationship between *Chaetomium* sp. and *C. maxima*. Verification of contact between both organisms will create a point of support for researchers to expand on this knowledge, and further explore the ecological role of *Chaetomium* sp. in *C. maxima*'s rhizosphere. Due to the health concerns associated with the alkaloids produced by *Chaetomium* sp., it would be vital to understand more about this novel relationship from the public health perspective. This

manuscript provides the first report and evidence of the existence of a relationship between *Chaetomium* sp. and *C. maxima*.

2. Material and methods

2.1. Field survey

A survey of nine varieties of *C. maxima* was conducted in the eastern United States, and traditional staining techniques were used to confirm the presence or absence of associations between *Chaetomium* sp. and the host. In addition, the field survey was conducted in order to provide further exploration of the colonization mechanisms of *Chaetomium* sp. in *C. maxima*.

2.2. Samples collection

Five root samples of *C. maxima* were collected from two farms in South Eastern Ohio, USA; one farm was in West Virginia, while the other farm was in Maryland, USA. These farms were selected based upon their different agricultural practices that covered all current cultivation practices including; certified USDA Organic, uncertified organic, unsprayed, and conventional chemical treatments. The five root samples were collected randomly from three plants of each cultivar, and then these roots were used for staining and microscopic analysis. The different cultivars of *C. maxima* examined in this study include; Banana, Blue Hubbard, Burgess Buttercup, Dills Atlantic Giant, Rouge Vif d'Etampes, Red Kuri, Sweet Meat, Polar Bear, and Turk's Turban.

2.3. Root staining

Root staining was an optimized modification of the Brundrett method (Brundrett *et al.*, 1984), originally used for clearing and staining plant roots. Root samples were heated in 10% (w/v) aqueous potassium hydroxide (KOH) solution for 50 min. at 95°C. Excess KOH was decanted off, and then 5% hydrochloric acid (HCl) (v/v) was added to neutralize the pH. The samples were kept in 5% HCl for 5 min. at 20°C. After that, 1% (w/v) Trypan blue was added

to the Falcon tubes, boiled at 100°C for 5 min. on a water bath, and then immediately washed with dist. water. Samples were washed once with 50% lactic acid-glycerol (v/v), cut into 1 cm² sections and mounted on glass slides with fresh 50% lactic acid-glycerol (v/v), finally stored at 4°C until imaging.

2.4. Light microscopy

Root segments of *C. maxima* cv. Banana, Blue Hubbard, Burgess Buttercup, Dills Atlantic Giant, Rouge Vif d'Etampes, Red Kuri, Sweet Meat, Polar Bear, and Turk's Turban, were examined for distinct morphological features associated with the genus *Chaetomium*. Two, 1 cm² segments of the root samples were mounted on glass slides containing 50% lactic acid and 50% glycerol. These slides were then examined for several features including; perithecial shape, melanized septate setae, and ascus containing fusiform spores, in addition to other features proposed by Hanlin, (1990); Hanlin (1998). Finally, the samples were imaged using an Olympus AX - 70 light microscope.

3. Results

Cucurbita maxima roots were collected from two farms in south eastern Ohio, USA. Different varieties of *C. maxima* were sampled from each farm (Table 1).

Results of investigating the samples of *C. maxima* demonstrated the presence of *Chaetomium* sp. perithecia, establishing the existence a relationship between *Chaetomium* sp. and *C. maxima* cv. Rouge Vif D' Etampes, in samples collected from the eastern panhandle at Kensho Farms, West Virginia, USA (Fig. 1A-D). As stated before, Kensho farm is an uncertified organic farm that doesn't use synthetic chemicals to treat its plants.

Sampling from Downing Fruit Farm Ohio (conventional), Five Oaks Farm Ohio (USDA Organic), Ecology Research Center Ohio (unsprayed), Mountain Valley Farm Maryland (conventional), Or Family Farm (conventional), do not show the

existence of any relationship between *Chaetomium* sp. and *C. maxima*.

Of the nine varieties of *C. maxima* collected over the 3 states, only one variety (cv. Rouge Vif D' Etampes) presented a relationship with *Chaetomium* sp., thus suggesting the presence of an established inoculum source in the rhizosphere. The other varieties including; Blue Hubbard, Burgess Buttercup, Dills Atlantic Giant, Red Kuri, Sweet Meat, Polar Bear, and Turk's Turban, do not demonstrate the presence of *Chaetomium* sp. perithecia, or any other signs of a fungal relationship.

4. Discussion

Members of the Cucurbitaceae are grown around the world as materials to build musical instruments, water storage devices, as well as for food and nutritional resources. Moreover, in some locations in the world, cucurbits are used for their medical properties (Hulse, 2018; Hulse and Braselton, 2019). The species of *C. maxima* has been used as a model organism during the current research project, due to its long-term historical use within the agricultural research community (Hulse and Braselton, 2019). Microbial communities associated with *C. maxima* have not fully been documented, and this manuscript helps elucidate a critical component of the ecosystem that has been missing in the previous literatures (Hulse, 2018; Hulse and Braselton, 2019). *Cucurbita maxima* is an economically important species and its microbial communities should be studied in more details, because they can be beneficial not only to the plants such as in mycorrhizas, but also to find biological control agents (BCAs) against the potential pathogens (Hulse and Braselton, 2019). This plant belongs to a large family that contains about 800 species, and many of them are of significant economic importance to the farmers of Africa, Asia, Europe, as well as in North and South America.

Chaetomium sp. is an economically important Ascomycete fungus that has a world-wide distribution

Table 1: Presence or absence of *Chaetomium* spp. in varieties of *C. maxima* sampled from Maryland, Ohio, and West Virginia

| Farm Name | Horticultural practice | Varieties | <i>Chaetomium</i> present | State |
|---------------------------|-------------------------------|----------------------|--------------------------------------|--------------|
| Mountain Valley | Conventional | Burgess Buttercup | N | MD |
| | Conventional | Dills Atlantic Giant | N | MD |
| | Conventional | Rouge vif d'étampes | N | MD |
| Orr Family | Conventional | Burgess Buttercup | N | MD |
| | Conventional | Dills Atlantic Giant | N | MD |
| | Conventional | Polar Bear | N | MD |
| Downing Fruit Farm | Conventional Unsprayed | Banana | N | OH |
| | Conventional Unsprayed | Rouge vif d'étampes | N | OH |
| | Conventional Unsprayed | Turks Turbans | N | OH |
| Five Oaks | USDA Organic | Blue Hubbard | N | OH |
| | USDA Organic | Burgess Buttercup | N | OH |
| | USDA Organic | Red Curi | N | OH |
| | USDA Organic | Sweat Meat | N | OH |
| Kensho Family | Uncertified Organic | Blue Hubbard | N | WV |
| | Uncertified Organic | Burgess Buttercup | N | WV |
| | Uncertified Organic | Rouge vif d'étampes | Y | WV |
| | Uncertified Organic | Turks Turbans | N | WV |

where; Maryland (MD), Ohio (OH), West Virginia (WV). Presence or absences of *Chaetomium* spp. are demonstrated as Yes (Y), No (N)

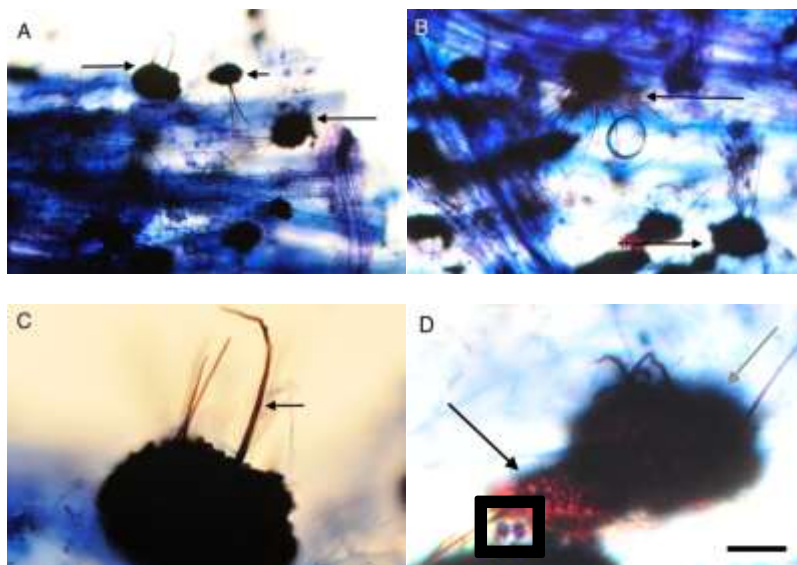


Fig. 1: *Chaetomium* sp. perithecia associated with *C. maxima* cv. Rouge Vif D' Etampes imaged after sampling events in West Virginia, United States of America. 1A-B: *Chaetomium* sp. perithecia (black arrows) in observed to be in association with *C. maxima* cv. Rouge Vif D' Etampes imaged with Bright Field Light Microscopy. 1C: *Chaetomium* sp. perithecia with septate setae (black arrows) observed to be in association with *C. maxima* cv. Rouge Vif D' Etampes imaged with Bright Field Light Microscopy. 1D: *Chaetomium* sp. perithecia (gray arrow) with ascospores (black arrow) and conidia (black box) observed to be in association with *C. maxima* cv. Rouge Vif D' Etampes imaged with Bright Field Light Microscopy. Magnification bars is 50 μ m.

in temperate regions. It has been shown to produce toxins that are fatal to mammals, and have antimicrobial properties. Previous studies of Suryanarayanan *et al.*, (2000); Avinash *et al.*, (2013); Rahim *et al.*, (2013) showed the existence of a relationship between *Chaetomium* sp. and other members of the Cucurbitaceae including; *C. pepo* and *C. moschata*, but never recorded in association with *C. maxima*. This indicates that the *Chaetomium* sp. reported in this study might not be described before in other studies. Future phylogenic studies using multiple molecular markers should be used to correctly identify the species of *Chaetomium* discovered during this study. The morphological characteristics alone cannot define a species with high certainty, due to complications in distinguishing between convergent evolution and true monophyletic clades.

Hulse and Braselton, (2019) reported that *C. maxima* has extreme phenotypic plasticity, which accounts for the hundreds or thousands of varieties of

this species cultivated throughout the world. Since a relationship has been recorded between *Chaetomium* sp. and *C. maxima* cv. Rouge Vif D' Etampes, future studies should be conducted to determine what other varieties of *C. maxima* are susceptible to colonization. With the important ecological and economic interests in *Chaetomium* sp., any information that can be collected on this novel relationship is beneficial to the agriculturalists.

It would also be advantageous to determine which other members of the Cucurbitaceae can be colonized by *Chaetomium* sp., in order to identify the hosts and the alternate hosts of this potential seed borne contaminate. Since Cucurbits are often intercropped, it is important not to cross contaminate seeds stock of Cucurbits that may be susceptible to infection by *Chaetomium* spp. Since several members of the genus *Chaetomium* are well known to produce toxic alkaloids, it is important to identify if the *Chaetomium* sp. recorded in this study (presented in Fig. 1A-D) is a

source of this alkaloid, and screen seeds that will be directly used for human consumption.

Growing *C. maxima* in the United States has a yearly economic impact of hundreds of millions of Dollars, and any information regarding its associations with economically important microbes should be documented. This study provides the first report and images documenting the existence of an association between *C. maxima* and *Chaetomium* sp., and it will hopefully lead researchers to better understand the microbial communities associated with the *C. maxima* agricultural ecosystems.

Conclusion

This study records the first report of *Chaetomium* sp. in association with the roots of *C. maxima*. Bright field light micrographs provided the evidence of existence of this relationship in the rhizosphere of *C. maxima* cv. Rouge Vif D' Etampes grown under uncertified organic conditions in the eastern panhandle of West Virginia, USA. Establishment of a relationship between *Chaetomium* sp. and the roots of *C. maxima* can provide basis for future research concerning the ecological role of *Chaetomium* sp. within the rhizosphere. The *Chaetomium* sp. discovered in this study should be identified using recent molecular techniques that utilize multiple markers, in order to accurately characterize this isolate. Future research should explore other varieties of *C. maxima* in order to determine if they harbor a relationship with *Chaetomium* sp. within their agricultural ecosystems.

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