Influence of mycorrhizal fungi on orchid seedling growth

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Introduction

Seed germination and early physiological development of microscopic orchid seeds is dependent on mycorrhizal fungi in nature. The symbiotic relationship and partial to complete heterotrophy, however, continues even after plants become mature. Although approximately 70% of the estimated 30,000 species of Orchidaceae are native to the tropical zones of the planet and many are considered rare in their natural habitats, their biology and ecology is poorly understood. Further, in vitro propagation techniques have not been developed for many showy tropical taxa, which leads to plant poaching from natural populations. This study was designed to develop effective protocols for co-culture of plants and their mycorrhizal fungi to enable conservation activities that include introduction of both partners in the wild.

We tested the influence of several fungi isolated from roots of orchids on the growth of seedlings of a showy tropical orchid species, *Oncidium klotzschianum*. Five fungal isolates cultured either from the roots of *O. klotzschianum* or other orchids were used along with a treatment that excluded fungi. Orchid Mycorrhizal Fungi (OMF) are saprophytes that respond to in vitro culture in laboratory. Orchid seedlings were cultured separately and asymbiotically to obtain uniform seedlings for this study.

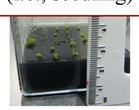
Methods

1. Isolation of fungi from roots of *Oncidium klotzschianum*:

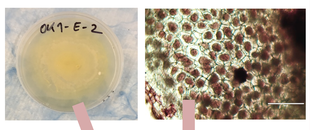
OMF Peloton inspection → Surface sterilization → Root mincing → Incubation in isolation medium → Selection of fungi → Sub-cultures of desired fungi

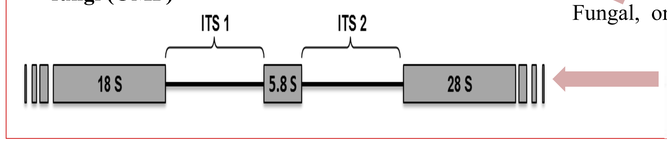
2. Co-culture of seedlings and fungi, and data collection:

Height and number of leaves for each experimental unit (i.e., seedling) → Inoculation with fungi → Incubation under a 12h photoperiod → Height and number of leaves on Day 25



3. PCR with ITS1-OF / ITS4-OF to amplify the fungal *nr*ITS region of orchid mycorrhizal fungi (OMF)





Results

Table 1. Results of a one-way ANOVA to test the influence of co-culture with fungi on growth of seedlings of a tropical orchid, *Oncidium klotzschianum*. Six fungal treatments were applied to plantlets, which were incubated for 25 days under a 12h photoperiod. Number of leaves **(a)**, the change in number of leaves **(b)**, and the height **(c)** were measured to estimate change in growth of plants in response to co-culture. A container with six seedlings was considered an experimental unit. $n = 12$ for each fungal treatment.

(a)

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	5	15.2305	3.0461	7.09	<.0001
Error	66	28.3456	0.4294		
Corrected Total	71	43.57			

(b)

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	5	17.6236	3.5247	29.16	<.0001
Error	57	6.8905	0.1208		
Corrected Total	62	24.51			

(c)

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	5	2.6727	0.5345	0.36	0.8739
Error	66	97.9538	1.4841		
Corrected Total	71	100.62			

Figure 1. Results of a one-way ANOVA to test the influence of co-culture witnumber of leaves of seedlings of *Oncidium klotzschianum* after 25 days. Means with different letters represent a statistical differences ($\alpha < 0.05$). $n = 12$ for each fungal treatment.

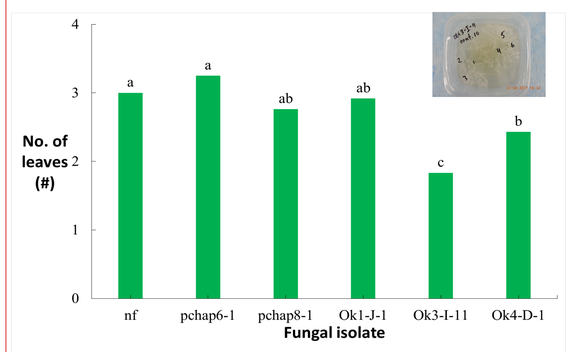


Figure 2. Results of a one-way ANOVA to test the influence of co-culture with fungi on the change in number of leaves of seedlings of *Oncidium klotzschianum* after 25 days. Means with different letters represent statistical differences ($\alpha < 0.05$). $n = 12$ for each fungal treatment.

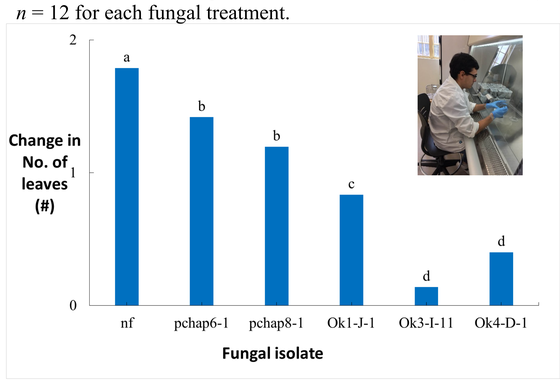


Figure 3. Results of a one-way ANOVA to test the influence of co-culture with fungi on the height of seedlings of *Oncidium klotzschianum* after 25 days. Means with different letters represent statistical differences ($\alpha < 0.05$). $n = 12$ for each fungal treatment.

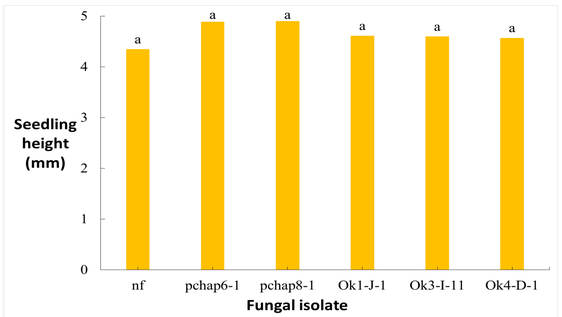
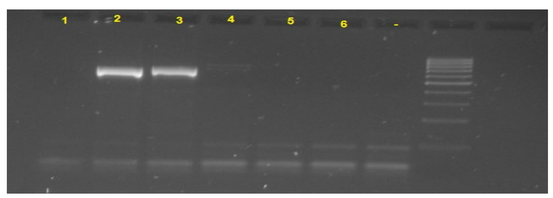


Figure 4. A gel image showing PCR amplification of the *nr*ITS region in two of the six fungal cultures obtained from roots of *Oncidium klotzschianum* and other tropical orchids.



Summary and Conclusions

- Number of leaves was the highest and similar for plants grown without fungi and those grown with pchap6-1, while the remaining treatments had fewer leaves (**Table 1a, Figure 1**).
- The largest change in the number of leaves was observed in un-inoculated ('nf') plants followed by seedlings inoculated with the two 'pchap' isolates (**Table 1b, Figure 2**).
- The parameter height did not show statistical differences (**Table 1c, Figure 3**) among any of the treatments.
- After conducting PCR to amplify fungal ITS region, DNA from two of the six fungal cultures we obtained from roots of tropical orchids amplified (**Figure 4**). This method followed by DNA sequencing will be used to identify all cultures obtained in our ongoing study.
- The short incubation period of 25 days may not have been sufficient for larger growth effects to become apparent because orchids are generally slow growing plants.
- The two OMF isolates 'pchap8-1' and 'pchap6-1' were not rejected by *Oncidium klotzschianum* even though they were isolated from a terrestrial orchid in the United States. This result is reasonable because OMF can have global and cosmopolitan distributions. It also shows that when growing orchids for conservation, fungi isolated from orchids other than the target species can be beneficial.
- It is possible that *Oncidium klotzschianum* may not be heavily dependent on OMF for seedling growth and development because un-inoculated seedlings and inoculated seedlings had similar growth (**Table 1, Figs. 1, 2, and 3**).
- It is also possible that other fungi (besides the most commonly known fungal families Ceratobasidiaceae, Tulasnellaceae, and Sebacinaceae) form OMF in orchids and have been previously ignored.
- DNA based identification of OMF is essential because fungal identification based only on morphological characteristics may not be correct.

Literature cited

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