

Brief Report

Mortierella elongata Increases Plant Biomass among Non-Leguminous Crop Species

Kaile Zhang ¹, Gregory Bonito ², Chih-Ming Hsu ¹, Khalid Hameed ³, Rytas Vilgalys ³ and Hui-Ling Liao ^{1,*}

¹ North Florida Research and Education Center, Soil and Water Science Department, University of Florida, 155 Research Road, Quincy, FL 32351, USA; kaile.zhang@ufl.edu (K.Z.); jimmyhsu75@gmail.com (C.-M.H.)

² Plant Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA; bonito@mail.msu.edu

³ Department of Biology, Duke University, Durham, NC 27708, USA; khalid.hameed@duke.edu (K.H.); fungi@duke.edu (R.V.)

* Correspondence: sunny.liao@ufl.edu

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Abstract: Recent studies have shown that *M. elongata* (*M. elongata*) isolated from *Populus* field sites has a dual endophyte–saprotroph lifestyle and is able to promote the growth of *Populus*. However, little is known about the host fidelity of *M. elongata* and whether *M. elongata* strains differ from one another in their ability to promote plant growth. Here, we compared the impacts of three *Populus*-associated *M. elongata* isolates (PMI 77, PMI 93, and PMI 624) on the growth of seven different crop species by measuring plant height, plant dry biomass, and leaf area. *M. elongata* isolates PMI 624 and PMI 93 increased the plant height, leaf area, and plant dry weight of *Citrullus lanatus*, *Zea mays*, *Solanum lycopersicum*, and *Cucurbita* to a much greater degree than PMI 77 (33.9% to 14.1%). No significant impacts were observed for any isolate on the growth of *Abelmoschus esculentus* or *Glycine max*. On the contrary, *Glycine max* significantly decreased in height by 30.6% after the inoculation of *M. elongata* PMI 77. In conclusion, this study demonstrates that *M. elongata* generally promoted metrics of the plant performance among a diverse set of importantly non-leguminous crop species. Future research on understanding the molecular mechanisms that underlie strain and host variability is warranted.

Keywords: *Mortierella elongata*; *Mortierella elongata* isolates; plant height; leaf area; plant biomass

1. Introduction

Mortierella elongata, a member of the early-diverging Mortierellomycota, is a cosmopolitan fungal species that is often isolated from soils or roots. [1,2]. The ecological function of *M. elongata* is still not well characterized, but this species appears to be commonly active in soils and rhizospheres [1,2]. Some studies have demonstrated that *Mortierella* spp. (e.g., *M. elongata*, *M. antarctica*, and *M. verticillata*) have the capacity to improve plant growth [1–4]. This important trait may be associated with the dual lifestyles (i.e., endophyte–saprotroph), and may involve several potential mechanisms. Firstly, *Mortierella* may interact with plants as synergistic agents to promote plant growth, as *Mortierella* can produce antibiotics and phytohormones (e.g., apocarotenoid, indoleacetic acid, and gibberellic acid) [2,3,5,6]. Secondly, *Mortierella* may provide nutrition (e.g., nitrogen (N) and phosphorus (P)) to its symbiotic plant partners, as was demonstrated in the interactions between *M. elongata* and the microalga *Nannochloropsis oceanica* [7]. Thirdly, *Mortierella* may function as a saprotroph that drives soil carbon (C) cycling [8–10], P dissolution and immobilization [11,12], lipid metabolism [13], and chitin degradation [13,14]. Finally, *Mortierella* may drive bioremediation processes that reduce the impact of environmental stresses on plant growth [15–17]. In nature, many lineages of fungi bridge the

saprotroph–endophyte divide, including both fungal generalists (e.g., *Phomopsis liquidambari* [18], *Serendipita indica* [19], *Phoma glomerata* [20], and *Hypoxyton anthochroum* [21]) and fungal specialists (e.g., *Fusarium solani* strain K associated with tomato [22], *Suillus* spp. and *Rhizopogon roseolus* with gymnosperm, and *Leccinum* spp. with angiosperm [23]). Some fungi with endophytic activities in one plant species may be pathogenic in another plant species [24]. Moreover, endophytic fungi can also become pathogens in certain growing phases of some hosts, or under particular environmental conditions [25].

Recent studies have shown that *M. elongata* is common in agricultural and forest ecosystems, indicating that it may play an important role in them [1,26]. For example, *M. elongata* accounted for over 15% of the total sequences in organic agricultural soils [1]. Further, *Mortierella* is dominant in soil and rhizosphere niches and does not show pathogenic tendencies. Thus, some *Mortierella* species may have the potential to provide beneficial activities across a diverse range of ecosystems [1,2]. However, studies to date have failed to characterize the host range of *Mortierella* isolates.

Based on the broad incidence of *M. elongata* across geographic ranges and soil types, we hypothesize that three *M. elongata* strains (PMI 77, PMI 93, and PMI 624) are generalists and similarly impact phylogenetically diverse host species. Furthermore, we expect that plant growth-promoting ability will vary between isolates. In this study, we performed paired plant–fungal bioassays to determine the abilities of different host species and fungal isolates to increase plant performance.

2. Materials and Methods

2.1. Inoculum Preparation and Sample Inoculation

We selected the *M. elongata* isolates from different geographic and ecological habitats. Briefly, *M. elongata* PMI 93 was isolated from *Populus deltoides* in NC, USA, and may promote *Populus* growth by manipulating plant defense [2]. *M. elongata* PMI 77 was isolated from soil in NC and has the ability to provide C and N sources for host plants [7], it can also interact with bacteria to improve the growth and plant health of one another [13,27]. *M. elongata* PMI 624 was isolated from *P. trichocarpa* in CA [28]. Fungal mycelium of each strain was cultured on pure modified Melin Norkrans (1% agar media) at 25 °C for 3 days [2]. Sterile millet seeds were inoculated with *M. elongata* and used as a carrier for inoculating plants [2]. To accomplish this, the millet seeds were soaked in distilled water overnight. The culture bags were then closed tightly and autoclaved at 120 °C for 45 min. Each fungal isolate was then transferred to the sterile culture bags containing the sterile millet seeds, and was incubated at 25 °C for 1 month when the millet seed was completely colonized by fungal hyphae.

We used watermelon (*Citrullus lanatus*), corn (*Zea mays*), tomato (*Solanum lycopersicum*), squash (*Cucurbita*), bahiagrass (*Paspalum notatum*), okra (*Abelmoschus esculentus*), and soybean (*Glycine max*) as host plants. Before the plant seeds were transferred into double autoclaved potting mixes (Metro Mix 360) for germination, they were surface sterilized by soaking in H₂O₂ for 2 min and rinsed with sterile water three times [29,30]. After 10 days of germination (1st true leaf stage), the seedlings were transplanted into soil potting mixes that were gently mixed with prepared *M. elongata* inoculum at a concentration of 10% by volume. The uncolonized millet seed was autoclaved and used to inoculate negative control plants. Each treatment consisted of three biological replicates grown in pots containing 64 cm³ of soil assembled in a randomized block design.

2.2. Samples Collection

The inoculated plants were harvested after 1 month of growth in greenhouse conditions. Collected plants were washed using deionized water. Leaf areas were measured for each plant using a Li-Cor LI-3100C area meter. Plant height was measured using a tape measure. Plant tissues were harvested and were oven-dried at 80 °C for 4 days to obtain dry crop biomass [31]. We used the median of the data set for the box plot. Levene's test was used to examine the homogeneity of variance within the treatments. A one-way analysis of variance (ANOVA) with a Tukey test for post-hoc comparisons were further used to analyze data variances ($P < 0.05$) in R (version 3.5) when variances within treatments were homogeneous.

3. Results

3.1. Plant Height

Compared to the un-inoculated control plants, *Mortierella*-inoculated *Citrullus lanatus* exhibited a greater plant height, increased by over 32.2%, see Figure 1. Inoculation of *M. elongata* isolates PMI 624 and PMI 93 significantly increased the plant height of *Zea mays* (26.7% and 12.0%) and *Solanum lycopersicum* (73.1% and 35.9%) relative to the control. The same trend only occurred in *M. elongata* PMI 93 inoculated *Cucurbita*, increasing the plant height by 62.5%. Inoculation of *M. elongata* isolates PMI 77 and PMI 624 had a substantial increase of 34.3% and 39.6% in the plant height of *Paspalum notatum*, respectively. There was no significant difference between inoculated treatments and control in *Abelmoschus esculentus*. The height of *Mortierella*-inoculated *Glycine max* was reduced by over 30.6% relative to controls.

3.2. Leaf Area

In comparison to the un-inoculated control plants, there was more than a 60% and 30% increase in leaf area in *Mortierella*-inoculated *Citrullus lanatus* and *Cucurbita* plants, respectively. *M. elongata* PMI 624 and PMI 93 inoculation resulted in 24.5% and 9.1% greater leaf area in *Zea mays*, respectively. However, *Mortierella* isolates had no significant effect on the leaf area of *Abelmoschus esculentus*.

3.3. Plant Dry Weight

Mortierella-inoculated *Citrullus lanatus* grew approximately a 10% greater plant biomass than that of the control. *M. elongata* PMI 624 significantly increased the plant biomass of *Solanum lycopersicum*, *Cucurbita*, and *Paspalum notatum* by 17.8%, 22.1%, and 47.3%, respectively. The same trends on plant dry weight were observed for PMI 93 on *Solanum lycopersicum* (11.4%), *Cucurbita* (16.2%), and *Paspalum notatum* (29.2%). In contrast, none of the *M. elongata* isolates had significant and positive effects on the plant biomass of *Abelmoschus esculentus* or *Glycine max*. In fact, *M. elongata* PMI 77 showed a suppressive impact on *Glycine max* growth, decreasing plant biomass by approximately 7.0%.

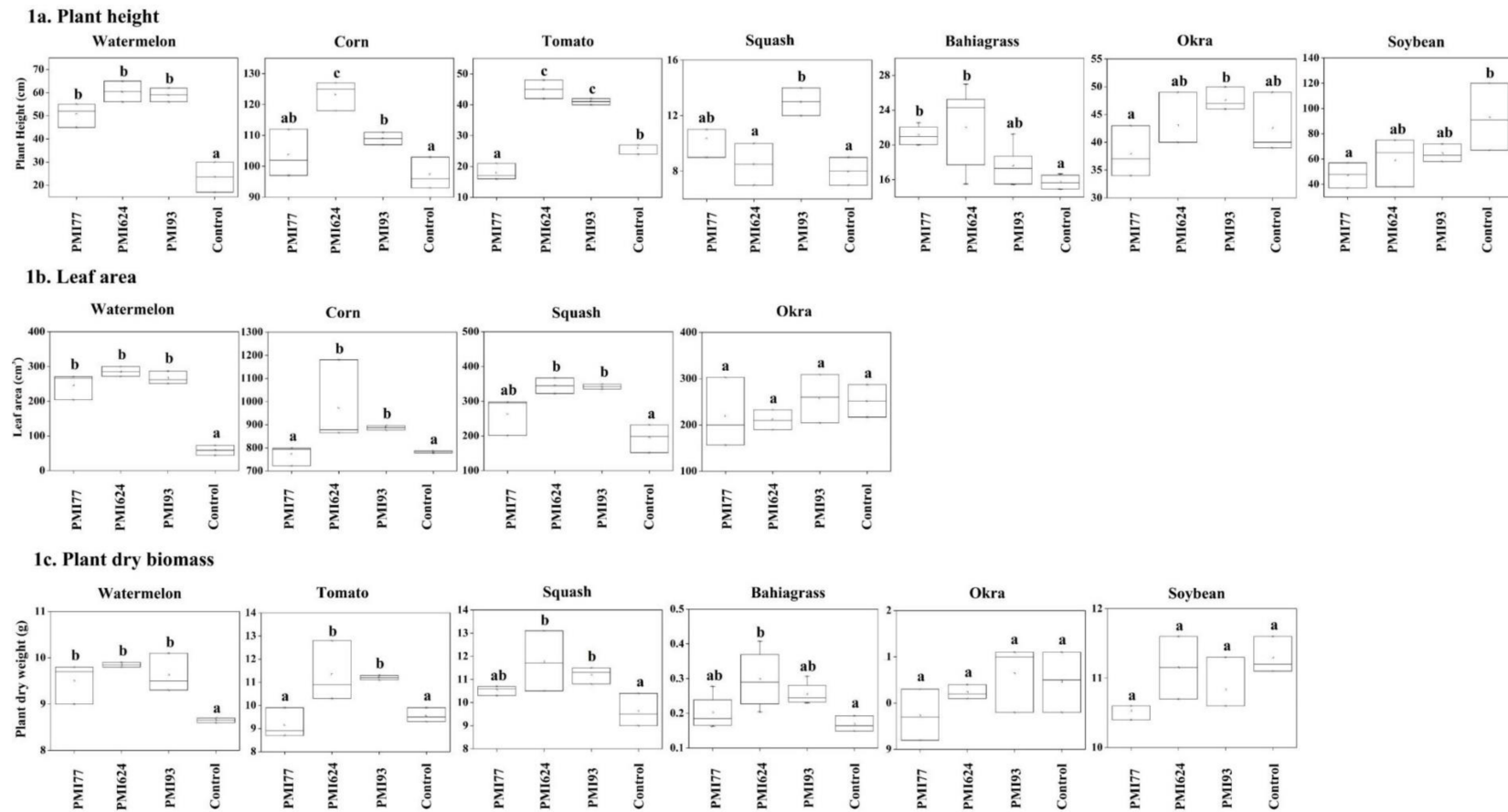


Figure 1. The impacts of *Mortierella* isolates on the growth of different plants shown by plant height (**1a**), leaf area (**1b**), and plant dry weight (**1c**). Vertical bars represent the standard errors of three replicates. Different lower-case letters represent significant differences across different isolates at $P < 0.05$.

4. Discussion

There is still limited understanding of the ecology and function of *M. elongata* in soils and agricultural ecosystems, but other studies have found this fungal species to respond favorably to organic amendments and to promote plant growth and soil enzyme activities [1]. In this study, we demonstrated the potential of *M. elongata* for plant growth promotion in diverse crops and grass. The different responses of plants in reaction to the three *M. elongata* isolates used in this study indicate that the plant-growth promotion ability of *M. elongata* may vary between isolates, as well as between different fungus–plant combinations. Specifically, *M. elongata* PMI 624 and PMI 93 appeared to have a greater potential to benefit the growth of tested plant species relative to *M. elongata* PMI 77. In particular, *M. elongata* PMI 624 and PMI 93 appear to function as host generalists. Our previous study showed that PMI 93 inoculation increased the biomass of inoculum by approximately 10% in the roots and 400% in the soil [2]. The hyphae of *M. elongata* was able to colonize corn roots around 2 weeks after inoculation [2]. The ability of plant growth promotion may be associated with the colonization rate and/or the abundance of *M. elongata* in the soil. Nevertheless, the mechanism of interaction and plant growth promotion is yet to be determined. These results are partially consistent with previous studies where *M. elongata* has played a critical role in the growth promotion of diverse crops in agroecosystems [1,3], suggesting that *M. elongata* is able to adapt to, and interact with, diverse host plants and may exert physiological and ecological benefits to them [32–34]. This also indicates that *M. elongata*, similar to other beneficial fungal endophytes, can perform an essential role in plant fitness (survival, growth, and/or fecundity) while interacting with host plants. For example, some fungal endophytes induce host plants to produce hormones or directly produce plant hormones themselves, which increases host biomass [35]. Endophytes can, to some extent, protect the host from attack by bacterial and fungal pathogens through the production of secondary metabolites and antimicrobial compounds, triggering systemic resistance, and competing with pathogens for resources or niche space [33]. By reducing herbivore fecundity and survival rates, and increasing their susceptibility to predation, fungal endophytes can also enhance plant protection from herbivores [33,36]. Given that root exudates produced by crop roots consist of carbon-enriched components (e.g., carbohydrates, amino acids, and organic acids), the rhizosphere is able to recruit an array of microorganisms, including mycorrhizal fungi and nitrogen-fixing bacteria that increase nutrient availability for the host [7,12,37]. However, it is unknown whether root exudates produced by different crop species might favor different *M. elongata* isolates. Additionally, it is still poorly understood whether these aforementioned benefits have a single or combined effect on the plant growth under different *M. elongata* isolates.

In contrast to the other isolates studied, *M. elongata* PMI 77 only promoted the growth of *Citrullus lanatus* and *Paspalum notatum*. Interestingly, *M. elongata* PMI 77 had a suppressive effect on *Abelmoschus esculentus* and *Glycine max*. There is likely to be a lack of *Rhizobium* N-fixing symbionts in the rhizosphere region of *Glycine max* that may have affected their growth. Further, free-living pathogens can be mechanically chauffeured from the soil and can colonize the crop root in the seedling stage when crops are vulnerable to pathogens [34,38]. A recent study has shown that there is a possibility to decrease plant metabolism while endophytes offer benefits to the host [39]. However, it is difficult to identify the drivers for the suppressive effect of *M. elongata* PMI 77 on the growth of *Abelmoschus esculentus* and *Glycine max*. Future studies are warranted that take into account the molecular mechanisms that underlie strain and host variability.

In conclusion, our study showed that multiple isolates of *M. elongata* demonstrated plant growth promotion in diverse plant species. However, *Abelmoschus esculentus* and *Glycine max* appeared to have a negative response towards the inoculation of *M. elongata* isolates. Our results also indicate that different *M. elongata* isolates have different degrees of plant-growth promotion activities, which appears to vary between crop species. Specifically, *M. elongata* PMI 93 and PMI 624 were likely to act as fungal generalists in that they promoted the growth of many crop species compared to *M. elongata* PMI 77. Additional studies will be needed to quantify the positive and/or negative responses of specific species of plants to *M. elongata* along with involved mechanisms, and to discriminate *Mortierella*

generalists in agroecosystems. Their potential for promoting plant growth can be targeted as a tool for sustainable agriculture.

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