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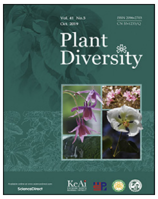

Root hemiparasitism in *Malania oleifera* (Olacaceae), a neglected aspect in research of the highly valued tree species

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ABSTRACT

Malania oleifera (Olacaceae) is a valued tree species, mostly because its seeds have high precious fatty acid content (particularly nervonic acid). However, seedling mortality rates are often high and regeneration of this tree has been problematic, which greatly hinders its utilization at a large scale. Cultivation difficulties of some tree species in the family Olacaceae have been attributed to their root hemiparasitic habit. Prompted by field observations and the taxonomic proximity of *M. oleifera* to root hemiparasites in Olacaceae, we hypothesized that tuberous structures observed on the roots of *M. oleifera* are parasitic organs known as haustoria. To test this hypothesis, we collected root samples from *M. oleifera* plants of various ages and growth conditions, investigated the morphological and anatomical features of tuberous structures and their connections to neighboring roots. Our analyses confirmed that *M. oleifera* are root hemiparasites. To the best of our knowledge, this is the first empirical report on root hemiparasitism in *M. oleifera*. Because life strategies of root hemiparasitic plants differ greatly from autotrophic plants, the root hemiparasitic habit needs to be taken into account for successful seedling regeneration of *M. oleifera*. This study establishes the foundation for investigations into a long-neglected but essential aspect in research of these highly valued tree species.

1. Introduction

Malania oleifera Chun & S.K. Lee is a tree species in the monotypic genus *Malania*, family Olacaceae (Qiu and Gilbert, 2003). It is endemic to very restricted areas in western Guangxi Province and southeastern Yunnan Province, China. The tree can grow up to 20 m in height and produces fruits containing a single large seed (ca. 9 g per fresh seed; Lü et al., 2016b). These fruits are highly sought after for medical purposes, as seeds of *M. oleifera* have high oil content (>60%) and the highest-known proportion of nervonic acid (>55%) (Ma et al., 2004; Tang et al., 2013). Unfortunately, *M. oleifera* trees are not common in the wild owing to habitat disturbances and overexploitation. *M. oleifera* has been listed in the IUCN Red List as a vulnerable species (Sun, 1998) and recently assigned as a plant species of extremely small population size with a high priority for conservation (Ma et al., 2013). Efforts have been taken to investigate causes of vulnerability in *M. oleifera* (Liang et al., 2003; Xiong et al., 2003; Wu et al., 2004; Lai et al., 2008; Xie et al., 2009), and to regenerate seedlings for conservation as well as utilization purposes (Yu, 2013; Lü et al., 2016a; Mao et al., 2018). However, regenerating seedlings remains difficult (Mao et al., 2018; Xu et al., 2018), and many aspects of *M. oleifera* biology are poorly understood. This greatly hinders the conservation and utilization of this valued tree.

Previous studies have reported that some species of Olacaceae are root hemiparasites (Kuijt, 1969; Werth et al., 1979; Pate et al.,

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1990), capable of extracting nutrients and water from host plant roots via swelling structures called haustoria while retaining their own photosynthetic capability. Because Olacaceae also consists of non-parasitic members (Qiu and Gilbert, 2003; Malécot and Nickrent, 2008), predicting the occurrence of parasitism in a species without reference or evidence (i.e., the competence to form haustoira; Kuijt, 1969) is challenging. Previous studies on *M. oleifera* have noted tuberous structures on their roots (Wu, 2002; Lai, 2006), which were assumed to have been microbial infections (Wu, 2002). Thus far, we have been unable to discover any empirical documentation of parasitism for *M. oleifera*. Based on taxonomic proximity of *M. oleifera* to other documented root hemiparasites in the family Olacaceae (Malécot and Nickrent, 2008), we hypothesized that those tuberous structures observed on *M. oleifera* roots may actually be their parasitic organs (i.e. haustoria). If that is the case, root hemiparasitism may at least partially account for the difficulty encountered cultivating *M. oleifera*. If *M. oleifera* is a root hemiparasite, specific care must be taken to successfully cultivate these plants, because life strategies of root hemiparasitic plants differ greatly from autotrophic plants.

In this study, we collected root samples of *M. oleifera* from Guangnan County, Yunnan Province, China. Root hemiparasitic habit of *M. oleifera* was confirmed based on field observation and anatomical study of the tuberous structures, which turned out to be haustoria, the defining characteristic organs for parasitic plants (Kuijt, 1969). To the best of our knowledge, this is the first empirical evidence of root hemiparasitism in *M. oleifera*. These findings will directly contribute to both conservation biology and the sustainable utilization of this highly valued tree by providing guidance for regeneration efforts of *M. oleifera* seedlings.

2. Materials and methods

2.1. Root sampling

Root samples were collected from cultivated *M. oleifera* seedlings as well as wild old trees around Tianfang Village (23°52′17″N, 105°12′14″E; elev. 1327 m), Shuguang Town, Guangnan County, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, China. In total, 36 *M. oleifera* plants were sampled, including three cultivated one-year-old seedlings (Fig. 1A), 25 cultivated four-year-old juvenile trees (Fig. 1B), and eight wild, big trees (Fig. 1C). Root excavation involved careful removal of rocks and soil to expose the main roots, which were then traced to lateral roots. During excavation, care was taken to maintain intact connections between *M. oleifera* roots and those of neighboring plants. *M. oleifera* roots were easily identified because they are much lighter than the roots of other trees present. Rootlets with tuberous structures were cut off together with attached roots. Sampled rootlets were put into plastic bags with moisture soil immediately after excavation, then stored in 50% ethanol before further analysis.

2.2. Root sectioning and microscopic observation

Transverse sections of the tuberous structures observed on roots of *M. oleifera* were cut (ca. 10 μm thickness) with a portable sliding microtome (GSL1, Switzerland) and stained for microscopic observation following a modified protocol from Tennakoon and Cameron (2006). Briefly, samples were stained with Safranine (1% dissolved in reverse osmosis water, w/v) for 2–3 min and Fast Green (0.05% dissolved in 95% ethanol, w/v) for 20 s. Nuclei and lignified and suberised cell walls stain red, and cellulose stains blue-green. Stained sections were washed with 100% ethanol for 2 min and stored in glycerin until microscopic examination. Sections were mounted on a slide using glycerin and a glass cover slip. The slides were then examined using a stereomicroscope (Olympus SZX7, Japan). Photographs were taken with a digital microscope camera (Olympus DP74, Japan).

3. Results

All *M. oleifera* plants sampled in this study were found to form numerous haustoria attached to neighboring roots (Fig. 2A–D). Haustoria were observed on both fine rootlets (Fig. 2C) and thick roots (Fig. 2D). *M. oleifera* haustoria were found to encircle the host root when attached to a fine rootlet (Fig. 2C), while forming a bell-

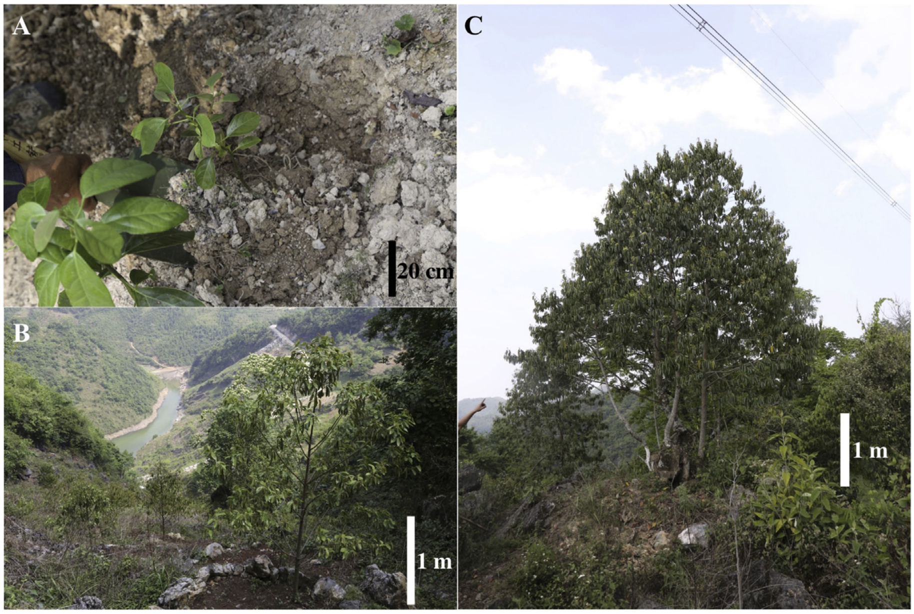

Fig. 1. Sampled *Malania oleifera* plants of various ages from different growth conditions. A. Cultivated one-year-old seedlings. B. Cultivated four-year-old juvenile plants. C. Wild, old tree with plenty of flowers.

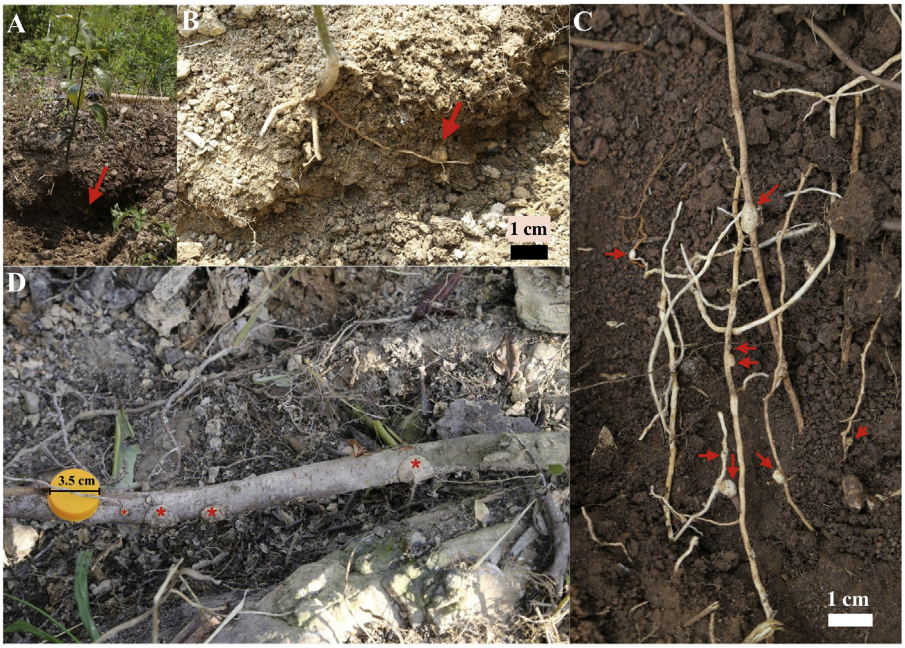

Fig. 2. Haustoria (indicated by arrows and asterisks) produced by *Malania oleifera* plants of various ages from different growth conditions. A. Cultivated one-year-old *M. oleifera* seedling forming haustoria. B. Close-up of a haustorium formed by the one-year-old seedling. C. Haustoria formed by a cultivated four-year-old *M. oleifera* plant. D. Haustoia produced by a wild, old flowering *M. oleifera* tree.

shaped structure when attached to a thick root (Fig. 2D). Most observed mature haustoria were bell-shaped, but newly initiated haustoria were oval or irregular tuberous swellings. Haustoria size varied, with diameters ranging from two millimetres to three centimeters. Developing and newly matured haustoria were white or light yellow with a fleshy texture (Fig. 2A–C), whereas aged haustoria turned brown or black and were more lignified (Fig. 2D).

M. oleifera produced haustoria laterally, in most cases by lateral roots (Fig. 3). One bell-shaped haustorium encircling a host root had an obvious merging line (Fig. 3A). On vigorous *M. oleifera* lateral roots, several haustoria formed in a row (Fig. 3B); in some cases, haustoria were closely adjacent to each other and ended up in a bead-like pattern (Fig. 3C). Haustoria were also found to form between rootlets of the same *M. oleifera* plant (auto-parasitism) (Fig. 3D).

Transverse sections of mature haustoria revealed typical haustorial structures with clear xylem connections between rootlets of *M. oleifera* and those of a host (Fig. 4). Mature haustoria are composed of two regions: the haustorial cushion that is external to the host root and a central located vascular core that makes initial contact with the host root, penetrating the host tissue (Fig. 4A). The vascular core penetrates the host root cortex and differentiates into suckers that form direct contact with the host xylem (Fig. 4B). The host–parasite interface is almost entirely composed of parenchymatous tissue. No obvious phloem connections with the host were observed. The host cortex was broken and pushed apart after being penetrated. However, no collapsed host cells were observed.

M. oleifera had a wide range of hosts, forming haustoria on virtually all plant roots with which it came into contact. Host species frequently observed for *M. oleifera* included *Cunninghamia lanceolata*, *Keteleeria evelyniana*, *Myrica rubra*, *Albizia simeonis*, *Pistacia weinmannifolia*, *Imperata cylindrica*, *Erigeron canadensis*, *Artemisia argyi*, *Lophatherum gracile*, *Eupatorium adenophora*, *Potentilla chinensis*, *Bidens pilosa*, *Senecio scandens*, *Rostellularia procumbens*, *Eurya groffii*, *Fallopia multiflora*, and *Polygonum cuspidatum*. In general, haustoria formed on shrubs or trees were larger (one to three centimeters in longer diameter) than those on herbaceous plants (often less than one centimeter in longer diameter). Haustorial morphology and anatomical characters were not different between wild and cultivated *M. oleifera* plants.

4. Discussion

In this study, we confirmed hemiparasitism in *M. oleifera*. *M. oleifera* haustoria lack phloem connections with hosts, which is consistent with the majority of haustoria for root hemiparasites (Irving and Cameron, 2009). Our study of mature haustoria anatomy in *M. oleifera* showed that these haustoria are produced laterally, connecting directly to the xylem of compatible hosts. These attributes are typical of the sandalwoods (Santalales) (Werth et al., 1979; Pate et al., 1990; Tennakoon and Cameron, 2006). Previous studies on *Santalum album* (Sandalwood) haustoria have suggested that host penetration is mediated by cell wall-degrading enzymes, which are indicated by the absence of collapsed host cells (Tennakoon and Cameron, 2006). We found no evidence of collapsed host cells in host tissue penetrated by *M. oleifera* haustoria, suggesting that *M. oleifera* haustoria secrete cell wall-degrading enzymes.

How important root hemiparasitism is in the growth and development of *M. oleifera* is a question that cannot be definitely answered at this time, because root hemiparasitic plant dependence on a host varies greatly. Some root hemiparasites undergo a pre-parasitic phase lasting from several hours to several months before attaching to a host (Pate et al., 1990; Seel et al., 1993). Several facultative hemiparasites can complete their life history without a host (Press et al., 1993). Seedlings of *M. oleifera* have been noted to grow independently for several months, probably due to their large seeds, which have high nutrient reserves, and their slow growth rate at an early stage. The long period of independent growth along with hidden haustorial connections in soil may have partially led to the negligence of root hemiparasitic habit in *M. oleifera*.

Root hemiparasitism is an adaptive life strategy in the plant kingdom, which evolved to increase the efficiency of nutrient or water uptake under stressful conditions (Press et al., 1999).

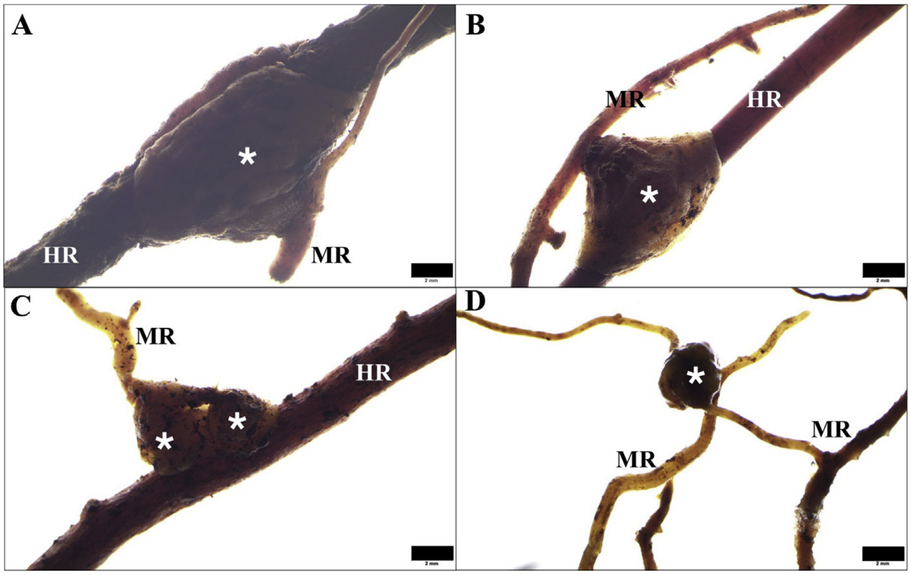

Fig. 3. Haustoria (indicated by asterisks) of *Malania oleifera* observed under a stereomicroscope. A. Typical shape of haustoria of *M. oleifera* attached to a host root. B. Rootlet of *M. oleifera* with one mature attached haustorium and several developing haustoria. C. Haustoria occurred beside each other in a bead-like pattern. D. One haustorium was observed on another rootlet of the same *M. oleifera* plant. MR, *M. oleifera* root; HR, host root. Scale bar = 2 mm.

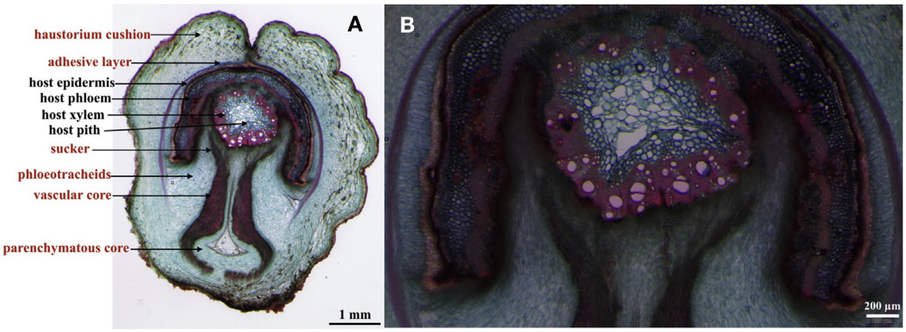

Fig. 4. Transverse section of a mature haustorium of *Malania oleifera* attached to an unknown host root. A. Overview of the haustorium transverse section (structures explained in red originate from *M. oleifera*). B. A close-up showing xylem connections between *M. oleifera* and its host.

Attachment to a compatible host often increases growth performance of the root hemiparasite (Irving and Cameron, 2009), which is the case even for facultative hemiparasites that do not require a host to complete their life cycle (Press et al., 1999). Apart from nutritive benefits, root hemiparasitism in some taxa has been reported to confer a competitive advantage over nearby plants (Veenendaal et al., 1996). Because life strategies of root hemiparasitic plants differ greatly from autotrophic plants, cultivation practices suitable for autotrophic plants may be problematic for root hemiparasites, which at least partially explains the low success rate in seedling regeneration for *M. oleifera*. Although *M. oleifera* has a wide range of hosts, it may have strong host preferences, as reported for other root hemiparasites (Irving and Cameron, 2009; Tennakoon and Cameron, 2006). Further investigations into root hemiparasitism of this tree may provide valuable insight for successful regeneration, including when young seedlings become parasitic, host preference and host switches between different developmental stages, and how we can manipulate host combinations for optimal *M. oleifera* growth.

5. Conclusions

This study provides the first empirical evidence of root hemiparasitism in *M. oleifera*. This tree exhibits typical morphological and anatomical haustorium attributes of root hemiparasititic plants. It produces lateral haustoria that connect to the xylem of a compatible host, ranging from herbaceous plants to large trees. In view of different life strategies of root hemiparasitic plants from free-living ones, root hemiparasitic habit should be taken into account in future efforts to regenerate *M. oleifera* seedlings for either conservation or utilization purposes.

Conflicts of interest

None.

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