

RESEARCH PAPERS

Decline of jackfruit (*Artocarpus heterophyllus*) incited by *Phytophthora palmivora* in Vietnam

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Summary. A new disease of jackfruit (*Artocarpus heterophyllus* Lam.) was observed in the south-eastern region of South Vietnam. Symptoms included root rot, cankers and gummosis of trunks, chlorosis, wilt, blight of leaves, defoliation, fruit brown rot, and tree death. The disease was found in 10% of surveyed farms with an incidence varying from 2% to nearly 60% of the trees. A *Phytophthora* species, identified as *P. palmivora* (Butler) Butler, using the ITS1-5.8S-ITS2 region of the rDNA as a barcode gene and morphological and cultural features, was consistently isolated from symptomatic roots, fruits, trunk cankers and leaves. Koch's postulates were fulfilled using pathogenicity tests on seedlings, leaves and detached fruits of jackfruit. To our knowledge, this is the first report of *P. palmivora* on jackfruit in Vietnam.

Key words: Oomycetes, South Vietnam, ITS regions, A1 mating type, Koch's postulates.

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam., family *Moraceae*) is an important multi-purpose fruit crop in tropical and subtropical regions, providing food, timber, fuel, fodder, medicinal and industrial products. Native to the south-western rain forests of India, *A. heterophyllus* is now widely grown in many Asian countries, especially Bangladesh, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam, Malaysia, Indonesia, India and the Philippines (Elevitch and Manner, 2006). From Asia it spread to tropical African countries, including Zanzibar, Kenya, Uganda, Madagascar and Mauritius. From the mid-17th

century to the late 19th century, the species spread further to tropical and subtropical America and Australia (Haq, 2006).

In Vietnam, jackfruit is widespread but grown mainly in the south eastern region, traditionally in household gardens, forest plantations or small farms, usually intercropped with other fruit crops, such as mango, longan, durian, rambutan, guava, water apple, pineapple, breadfruit or industrial crops such as coffee, cashew nut, black pepper, rubber tree, or cocoa (Sidhu, 2012). Jackfruit can generate an income for small farmers through the sale of fruits and is considered a good feed resource for cattle and pigs (Elevitch and Manner, 2006). Areas under cultivation have been rapidly expanding in the last 10 years, and it is estimated to cover approx. 50,000 ha with an average fruit yield of about 30 tons ha⁻¹. The crops have

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developed from small-scale household jackfruit production to important cash crops (Mai Van Tri and Nguyen Van Hoa, 2014). Approximately 60% of the production is for processing and the rest is for fresh consumption. Dried jackfruit as chips is the major processed product for local markets and export (Mai Van Tri and Nguyen Van Hoa, 2014).

In 2012 and 2013, in four provinces of South Vietnam (Ba Ria-Vung Tau, Binh Duong, Binh Phuoc and Dong Nai) a decline disease was observed in approximately 10% of surveyed jackfruit plantings, with an incidence varying from 2% up to approx. 60% of the trees in each planting. Affected trees showed symptoms of leaf blight and chlorosis, wilting, defoliation, trunk cankers with resin exudates and root rot. Sometimes, symptoms of brown rot on fruits hanging from the trees or fallen to the ground were also observed. Leaf blight began as small, water soaked flecks that, in a few days, turned brown and expanded rapidly into large necrotic lesions. In wet conditions, white velvety mycelium was visible at the edges of lesions on leaves and fruits. Stem cankers appeared firstly as wet lesions on the bark surfaces, often close to the insertion of large branches, but more frequently at trunk bases. A reddish-brown resin oozed from cracks in the bark. The wood tissues under the lesions showed cream to reddish brown discolouration. The infected areas enlarged, girdling the stems and causing severe decline of the trees. Decline was always associated with the rot of fine roots. In some cases, the whole tree died. Symptoms of decline were severe mostly in areas prone to flooding or with poor drainage. Leaf blight and fruit brown rot were observed after periods of heavy rainfall and when the relative humidity was high.

The involvement of a *Phytophthora* species as causal agent of the disease was suspected on the basis of symptoms. As a consequence, analyses were focused on Oomycetes and the causal agent of jackfruit disease observed in South Vietnam was identified and characterized.

Materials and methods

Isolation and morphological characterization of isolates

Isolations were made from trees with symptoms of decline. Affected necrotic tissues were thoroughly washed with tap water to remove soil and plant debris. Small sections (2–5 mm) from symptomatic

fine roots and the edge of rotted tissues of stems, fruits and leaves were blotted dry on sterile paper towels and transferred onto BNPRAH selective medium for *Phytophthora* (Masago *et al.*, 1977) in Petri dishes, where they were incubated for 5 d at 24–25°C in the dark. Hyphal tips of *Phytophthora* colonies, after several transfers on BNPRAH medium, were sub-cultured on potato dextrose agar (PDA, Oxoid Ltd) to obtain pure cultures. Isolates were grown in Petri dishes (9 cm diam.) containing 20 mL of either PDA or V8 vegetable juice agar (V8A; Campbell) at 24±1°C for 14 d in darkness to determine the colony morphology. For cardinal growth temperature assays, a 5-mm diam. mycelial plugs were transferred to PDA and incubated at different temperatures (at 3°C intervals from 3°C to 39°C). Growth rates were determined on PDA at 27 ± 1°C for a maximum of 7 d. Mating type for isolates was determined on V8A in dual culture with A1 and A2 reference isolates of *Phytophthora palmivora* and *P. nicotianae*, as described by Brasier *et al.* (2003). Two representative isolates, MD5 obtained from fine roots and MD6 from leaves, were selected for DNA analysis and pathogenicity tests. Living cultures of isolates MD5 and MD6 are preserved both at the CABI Genetic Resource Collection, United Kingdom (IMI 503890 and IMI 503891) and in the collection of the Department of Agriculture, Food and Environment, University of Catania, Italy.

Sequencing and analysis of ITS regions

DNA was extracted from 10 mg of fresh mycelium of 7-d-old colonies of isolates MD5 and MD6 grown in Petri dishes on V8A at 24°C, using DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen GmbH). DNA samples were stored at -20°C prior to PCR amplification. The quality and quantity of extracted DNA samples were evaluated using a DNA Quant-it assay kit (Molecular Probes) and by electrophoresis in 1% agarose gels containing SYBR® Safe (Invitrogen Life Technologies) DNA gel stain. Amplification and sequencing of the internal transcribed spacer (ITS) region of rDNA were performed according to the protocol described by Cooke *et al.* (2000), using 20 ng of genomic DNA as a template and ITS6 and ITS4 primers. Amplicons were analyzed by electrophoresis and using a DNA Quant-it assay kit (Molecular Probes) as described above, purified using the ExoSAP-ITkit for PCR Product Cleanup (Affymetrix) and sequenced in

both directions using an external sequencing service (BMR-genomic). The identity of isolates was established according to the level of similarity of their ITS sequences with those of reliable reference-sequences.

Pathogenicity tests

Pathogenicity trials were performed on detached fruits as well as on leaves, stems and roots of potted 6-month-old *A. heterophyllus* plants using the isolates MD5 and MD6.

Detached immature jackfruit fruits (five fruits per isolate) were superficially disinfected with 1% NaClO, rinsed with sterile distilled water and dried with paper tissues. A hole (5 mm diam.) was made in each fruit with a cork borer and a disc of tissue from the fruit surface was removed; the wound was filled with a 5 mm agar plug containing actively growing mycelium, and was then sealed with adhesive tape. Sterile agar plugs were inserted into control fruits. The fruits were singly incubated in sealed plastic bags at room temperature and inspected daily for symptoms.

The leaves of jackfruit plants (ten seedlings per isolate) were sprayed with 70% ethanol and allowed to air dry. A water suspension of each isolate was obtained by flooding a 12-d-old culture on V8A in a Petri dish with sterile distilled water (s.d.w.) and superficially scraping the mycelium with a sterile scalpel. The concentration was adjusted to 1×10^4 sporangia mL⁻¹ and the suspension was sprayed on the leaves. Ten seedlings sprayed with s.d.w. served as experimental controls. The plants were covered with large, transparent plastic bags and s.d.w. was nebulized into the bags to maintain high relative humidity.

In a third experiment, jackfruit plants (10 for each isolate) were wound-inoculated on the stems. A small strip of bark (3–4 mm in length) was cut aseptically from the stem of each plant with a scalpel and 3 mm agar plugs from the edge of actively growing cultures were placed into the wound. Sterile agar plugs were used for control seedlings. The bark strips were replaced on wounds and the stems were tightly sealed with Parafilm®.

In a fourth experiment, jackfruit plants (ten for each isolate) were transplanted into pots (12 cm diam.) containing a mixture of 1:1 (v:v) steam-sterilized sandy loam soil with 4% inoculum produced on autoclaved wheat kernels, according to Pane *et al.* (2009) and Faedda *et al.* (2013). Ten control plants were transplanted into pots containing non-infested

soil. All plants were then grown for 3 weeks and watered to field capacity once a week.

The last three experiments described above were carried out in a greenhouse, with natural light conditions and temperatures ranging from 24 to 28 °C. Plants were inspected daily for symptoms for 30 d after inoculation.

Results

Isolation and morphological and cultural characterization

The results of the survey are shown in Figure 1, where the proportion of symptomatic jackfruit trees relative to the number of trees surveyed in each province (Ba Ria-Vung Tau, Binh Duong, Binh Phuoc and Dong Nai) is outlined. The total numbers of surveyed trees and proportions of symptomatic trees per province was: 2126 trees in Ba Ria-Vung Tau province with 19% of symptomatic trees; 1540 in the Binh Duong province with 14.5% of symptomatic trees; 1892 in the Binh Phuoc province with 20.5% of symptomatic trees and 1962 in the Dong Nai province with 34% of symptomatic trees. A *Phytophthora* species was consistently recovered from all symptomatic trees on BNPRAH selective medium, with frequencies of 90% from fruits, 83% from leaves, 71% from roots and 54% from trunk bark samples. All isolates had identical morphological and cultural features and closely matched those of *P. palmivora* (E.J. Butler) E.J. Butler (Stamps *et al.*, 1990; Erwin and Ribeiro; 1996; Gallegly and Hong, 2008). On PDA, colonies had stoloniferous mycelium, whereas on V8A they showed stellate patterns. On V8A, all isolates produced elliptical to ovoid, conspicuously papillate sporangia with a mean length/breadth ratio of 1.8. Sporangia were caducous with short pedicels (mean pedicel length = 5 µm) and conspicuous basal plugs at the insertion of the pedicels. They were formed on sympodially branched sporangiophores. Minimum and maximum temperatures for growth on PDA were, respectively, 9°C and 36°C, with the optimum at 27°C and a growth rate of 7 mm d⁻¹ at this temperature. All isolates were heterothallic and were of A1 type, producing amphigynous antheridia, oogonia, and oospores after pairing with the A2 reference isolates of *P. nicotianae* and *P. palmivora*.

The origins of the isolates and the numbers of plantations where they were sourced are shown in Table 1.

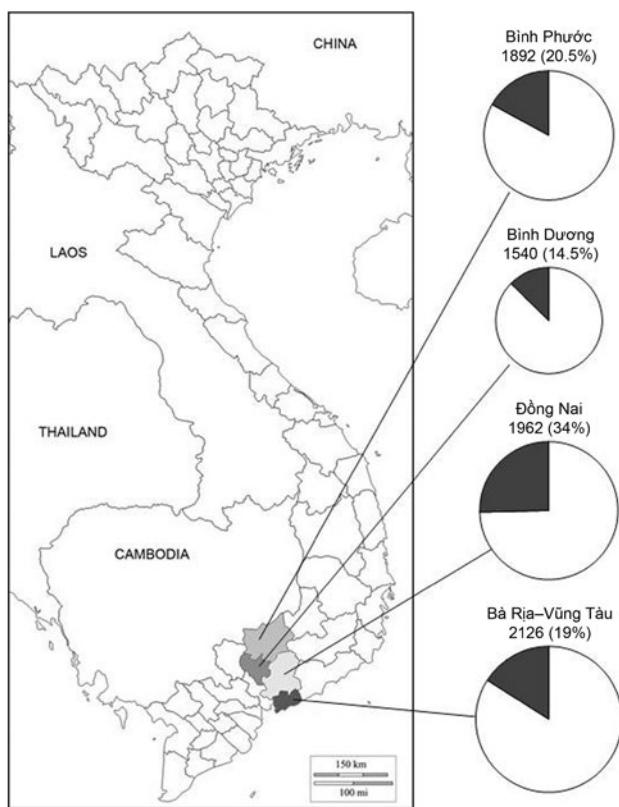


Figure 1. Diagrammatic representation of the incidence of the decline incited by *Phytophthora palmivora* in jackfruit plantings in four provinces of South Vietnam; the size of pies is proportional to the number of surveyed trees in each province. Figures above pies indicate the total number of jackfruit trees surveyed and the proportion (within brackets) of symptomatic trees in each province, respectively. Note that this is not a map of Vietnam, but only the indication of the provinces where the survey was carried out.

Table 1. Origin of *Phytophthora palmivora* isolates obtained in four provinces of South Vietnam from jackfruit trees with symptoms of decline, and number of plantings where they were sourced.

Provinces	Root	Trunk	Leaf	Fruit
Ba Ria Vung Tau	10	8	4	2
Binh Duong	7	4	-	-
Dong Nai	10	9	-	-
Binh Phuoc	8	7	2	2
Total	35	28	6	4

Molecular identification of isolates

The analysis of the ITS1-5.8S-ITS2 sequences from the representative isolates MD5 and MD6 (accession nos. KF823978 and KF823979) revealed 100% similarity with several reference isolates of *P. palmivora* (e.g., accession nos. HQ643305, GQ398157 and KC415917) and with a sequence (AF266781) deposited as *P. arecae* (Grünwald *et al.*, 2011; Robideau *et al.*, 2011). Other related species of clade 4 were clearly differentiated using the ITS regions as the barcode gene (Robideau *et al.*, 2011).

Pathogenicity tests

Water-soaked, brown lesions appeared on wound-inoculated fruits 2–3 d after inoculation. Seven days after inoculation, the mean diameter of lesions was 95 ± 5 mm, and white, velvety mycelium was visible on the fruit rind surrounding the lesions.

Water-soaked, small, brown (2–5 mm) lesions with indefinite margins developed on inoculated leaves within 4 d after inoculation. Lesions became necrotic in a few days, enlarged and coalesced into larger necrotic areas. White mycelium efflorescence was visible at the edges of lesions 7 d after inoculation. *Phytophthora palmivora* was re-isolated from symptomatic tissues to fulfil Koch's postulates. The leaves of plants sprayed with distilled water showed no symptoms.

Brown gum exudates appeared on the stems of wound-inoculated plants 7 d after inoculation. The mean length of gummy stem cankers 15 d after inoculation was 20 ± 2 mm. The plants developed leaf chlorosis and wilting and collapsed 30 d after inoculation. Control seedlings showed no symptoms. *Phytophthora palmivora* was re-isolated from all symptomatic plants.

All plants transplanted into pots filled with infested soil developed symptoms of decline within 1 week, and 60% collapsed by 30 d after transplanting. Non-inoculated plants remained healthy. *Phytophthora palmivora* was re-isolated from fine roots of inoculated plants.

In all experiments, no significant differences were observed between the two *P. palmivora* isolates used in pathogenicity tests.

Discussion

The results of this study demonstrate that *P. palmivora* is the causal agent of the decline of jackfruit

trees observed in the south eastern region of Vietnam. The pathogen was accurately identified on the basis of morphological features and using the ITS1-5.8 S-ITS2 region of the rDNA as a barcode gene. ITS sequences of isolates from jackfruit sourced in south east Vietnam were identical to reference sequences of *P. palmivora* and *P. arecae* and were clearly differentiated from all other currently recognized *Phytophthora* species (Robideau *et al.*, 2011; Grünwald *et al.*, 2011). Furthermore, *P. arecae* has been shown to be synonymous with *P. palmivora* (Kroon *et al.*, 2012). All symptoms of the complex syndrome associated with the decline of jackfruit trees were reproduced in artificial inoculations using isolates of *P. palmivora* obtained from jackfruit trees with natural infections, and the pathogen was consistently re-isolated from symptomatic tissues, thus fulfilling Koch's postulates.

Phytophthora palmivora is believed to have originated in south-east Asia (Mchau and Coffey, 1994) but is now widespread in the Tropics and is spreading also in warm, temperate climate zone, including the Mediterranean region, on ornamental plants in nurseries (Cacciola *et al.*, 2002; Davino *et al.*, 2002; Moralejo *et al.*, 2009; Cacciola *et al.*, 2011; Dervis *et al.*, 2011) as well as on tree crops, including native Mediterranean species such as olive (Cacciola *et al.*, 2000; Lo Giudice *et al.*, 2010; Turkölmez *et al.*, 2014). It is a polyphagous pathogen, with the known host plants being in more than 160 genera in 60 families (Cline, 2008). The host range also includes *A. heterophyllus* and other related species of *Artocarpus*, such as breadfruit (*A. altilis*), cempedak (*A. integer*) and breadnut (*A. camansi*) (Chee, 1969; Gerlach and Salevao, 1984; Erwin and Ribeiro, 1996; Drenth and Guest, 2004). The list of more than a thousand known hosts of *P. palmivora* includes most of the plant species that are commonly intercropped with jackfruit in Vietnam. Very recently, a severe decline syndrome of jackfruit that emerged in the Philippines during the late 1990s was found to be caused by *P. palmivora* (Borines *et al.*, 2014). However, to our knowledge, the present paper is the first report of *P. palmivora* on jackfruit in Vietnam.

Phytophthora palmivora is a widespread pathogen in Vietnam, and has been reported as a common pathogen of durian (*Durio zibethinus*), one of the most appreciated and profitable fruit crops in this country (Drenth and Guest, 2004). On durian, *P. palmivora* causes symptoms similar to those observed on jackfruit, such as root and crown rot, stem cankers with gum exudates, fruit brown rot, leaf blight and whole tree decline.

Other hosts on which *P. palmivora* has been recorded from Vietnam include coconut (*Cocos nucifera*), cocoa (*Theobroma cacao*) and rubber (*Hevea brasiliensis*). On tree-hosts, *P. palmivora* is primarily a root pathogen. However, deciduous sporangia of this species can be disseminated by rain, wind and soil-splash and in wet, showery conditions these may cause aerial infections. Also insects, particularly ants, have been reported as vectors of *P. palmivora* in tropical areas (McGregor and Moxon, 1985; Holderness, 1992).

Several lines of evidence suggest the decline of jackfruit observed in southern Vietnam is the same disease described recently in the Philippines by Borines *et al.* (2014). Very probably, in the south eastern region of Vietnam, the disease has been present for a long time, but it has passed unnoticed because of the limited economic significance of jackfruit, which, until recently, has been cultivated only for domestic consumption or for local markets. The expansion of the cultivation of jackfruit, both as a fruit and a multi-purpose commercial crop, has determined the conditions for the emergence of this disease. The results of our survey indicate the complex of symptoms of jackfruit decline caused by *P. palmivora* pose a serious threat for jackfruit cultivation, with a significant impact both on yields and on the long-term viability of plantations. The precise identification of the causal agent of this disease constitutes a fundamental step for the development of appropriate and effective disease management strategies.

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