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Occurrence of Bacterial Wilt Disease of Ginger Caused by *Ralstonia solanacearum* Species Complex in Myanmar

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A survey was carried out in November 2021 in Shan State in Myanmar to collect the bacterial wilt of ginger. Ginger plants showing leaf yellowing, and rhizome rotting, which are typical symptoms of bacterial wilt were found in the fields in Heho and Yatsauk townships. Bacterial slime oozed spontaneously from the cut surface of infected pseudostems when soaked in water, indicating the disease was caused by a bacterial pathogen. Isolation was performed using a 2–3–5 triphenyl tetrazolium chloride (TTC) agar medium. The colony morphologies were cream–white with pink center, irregular, and fluidal in most colonies, which are typical characteristics of the colony of *Ralstonia solanacearum* species complex. After repeating single colony isolation, the bacterial isolates were inoculated to ginger seedlings by leaf clipping. The yellowing lesions observed naturally infected ginger were reproduced on the leaves inoculated with the isolates, and the re-isolation was confirmed. Forty–five isolates of *R. solanacearum* species complex collected from two different areas were isolated and stocked in sterilized distilled water for further study. This is the first report of bacterial wilt of ginger caused by *R. solanacearum* species complex in Myanmar.

Key words: Bacterial wilt, Ginger, Myanmar, *Ralstonia solanacearum* species complex

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is usually cultivated by farmers and it offers major economic opportunities for many households in Shan State of Myanmar (Winrock International, 2016). Climatic conditions in Myanmar are favorable for ginger production, and ginger is in high demand in the world market. Therefore, ginger is one of the export crops that has the potential in production to expand its markets (Winrock International, 2016). Ginger is approximately grown on 4,985 hectares with an annual production of 66,085 tons in Myanmar (ILO, 2017). Although the production is only 3% of total world ginger production (Htwe, 2017), there are many opportunities and potential for the expansion of Myanmar ginger production. Since several fungal or bacterial pathogens cause serious disease to ginger and affect adversely the production, fundamental and applied research of these pathogens is necessary to increase ginger production.

Ralstonia solanacearum species complex is considered to be one of the most important plants pathogenic bacteria as it causes great economic losses worldwide (Hayward, 1991). The bacterium has an unusually wide host range; plant species susceptible to the pathogen have been observed to occur in over 50 plant families (Hayward, 2000). The host range includes solanaceous plants, leguminous plants, monocotyledons mainly banana, ginger, several trees, and shrub hosts. Satisfactory and long–term control strategy of bacterial wilt will be achieved by correct disease diagnosis by effectively combining different control strategies (Tsuchiya, 2014).

India and China, neighboring countries of Myanmar are the largest ginger producers–cum–exporters (Mulderij, 2017), and ginger farmers suffer bacterial wilt disease and become more serious these days in these countries. A systematic study on bacterial wilt of ginger in Myanmar was not currently occurred. Therefore, a detailed survey of bacterial wilt of ginger was performed for the first time in Myanmar.

MATERIALS AND METHODS

Disease surveying and sample collection

A detailed field survey of bacterial wilt of ginger was carried out in Heho and Yatsauk townships, Shan State in 2021 (Fig. 1). Ginger plants showing wilting and yellowing aboveground, which are typical symptoms of bacterial wilt were found and the diseased plants were collected (Fig. 2). Subsequently, laboratory studies were carried out at the Department of Plant Pathology, Yezin Agricultural University. Samples of the diseased plants were thoroughly washed with sterile distilled water. The pseudostems and rhizomes were cut and the surfaces were soaked in water to test bacterial oozing.

Isolation of pathogen

Isolation of the causal bacterium was done from infected rhizomes or pseudostems with brown discoloration. The rhizomes or pseudostems were surface sterilized with 70% ethanol and washed thoroughly with sterile water before isolation. Small pieces (about 3×3 mm) of the necrotic rhizome or pseudostems were excised out with a sterilized scalpel. Then the pieces were sur-

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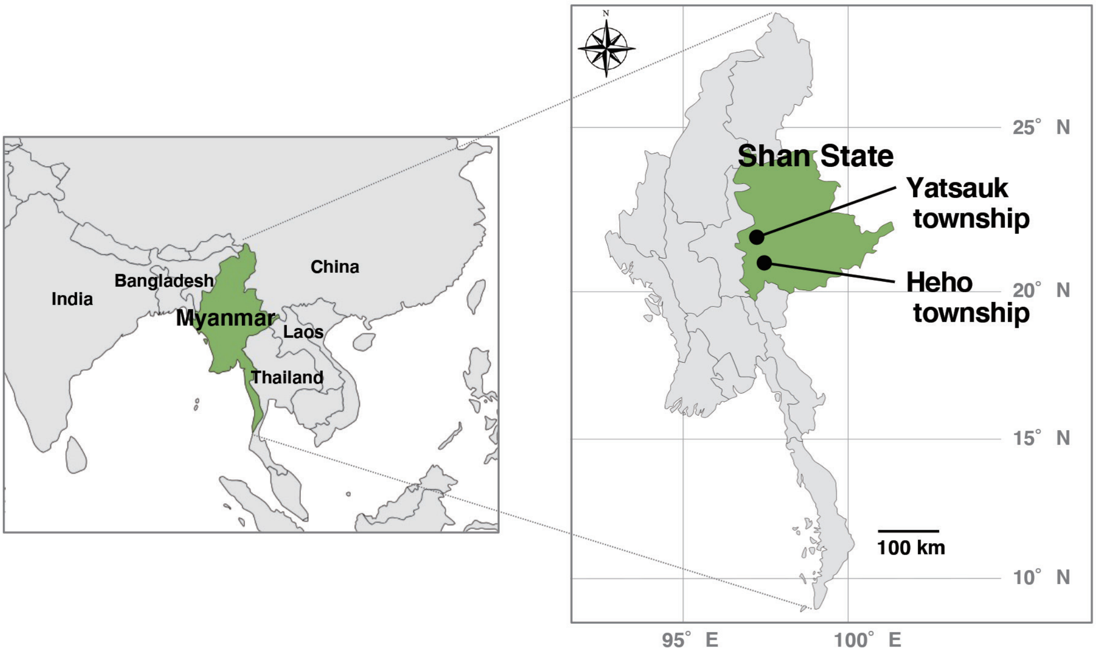

Fig. 1. The geographic location of Yatsauk and Heho townships, Shan State in Myanmar. A survey for ginger diseases was carried out in 2021.

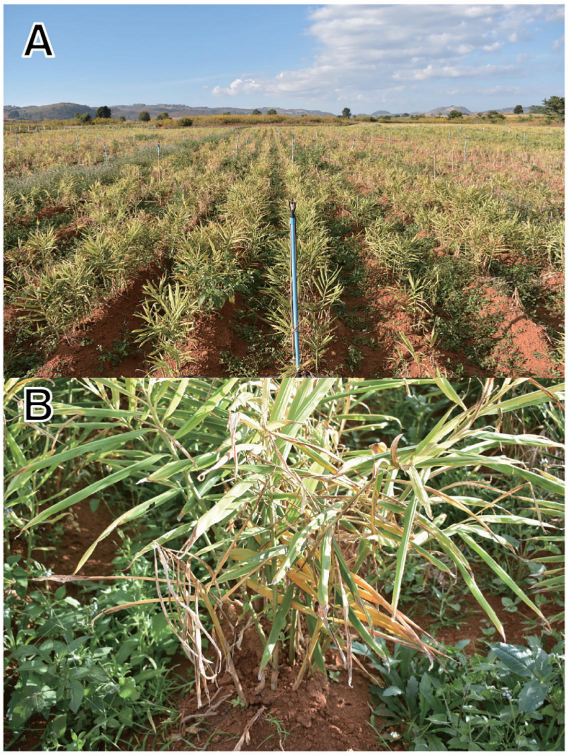

Fig. 2. The diseased ginger field at Heho township, Shan State, Myanmar (A), and ginger showing yellowing and wilting symptoms (B).

face sterilized with 70% ethanol for 1 min and with 1% sodium hypochlorite for 30 sec. Subsequently, the pieces were rinsed 3 times in sterile distilled water and dried between two sterilized filter papers. The pieces were then transferred individually into a few drops of sterile water on a sterilized slide glass and were left for one minute to allow bacterial ooze to come out in the water. Then, one loopful of bacterial suspension was streaked on 2–3–5 triphenyl tetrazolium chloride (TTC) agar media (Kelman, 1954). The inoculated culture media were incubated at 28°C for 72 h.

Pathogenicity test on ginger seedlings

About one–month–old ginger seedlings were used for the inoculation test. Bacterial isolates were cultured on TTC agar medium at 28°C for 48 h and bacterial colonies were suspended in sterile water to adjust to 0.3 OD at 600 nm using a spectrophotometer. Leaves of ginger were inoculated with the suspension by the leaf clipping procedure reported in our previous work (Iiyama *et al.*, 2021). The inoculated ginger plants were incubated at 30°C in a plant growth chamber and symptoms were checked daily.

When original symptoms were reproduced in the artificially inoculated ginger, re–isolation of the bacteria was performed in the same manner as described above.

RESULTS

Disease surveying, sample collection, and isolation of the pathogen

Ginger plants showing leaf yellowing and rhizome rotting were observed in the fields in Heho and Yatsauk townships in Shan State in Myanmar (Fig. 2). These were quite similar to the symptoms of bacterial wilt of ginger (Trujillo, 1964).

When the pseudostems of the diseased ginger were cut and soaked in water, whitey ooze streamed out from the cut surface, indicating that a bacterial pathogen caused the disease (Fig. 3A). The colony morphologies were cream-white with pink center, irregular, and fluidal

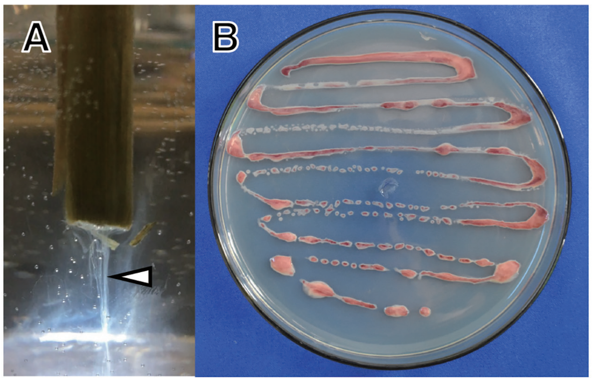

Fig. 3. Bacterial oozing out from the infected ginger pseudostem (A), and bacterial colonies isolated from the infected ginger on TTC medium (B). The arrow indicates the bacterial stream from the infected pseudostem. The bacterial colonies were cream–white with pink center, irregular, and fluidal, which morphologies are typical characteristics of *R. solanacearum* species complex.

(Fig. 3B), which are typical characteristics of the colonies of *R. solanacearum* species complex.

Single colony isolation was repeated, and resultant purified bacterial isolates were suspended in sterilized distilled water for long preservation. As a result, twenty–five isolates from Heho township, and twenty isolates from Yatsauk, Kalaw township were collected and preserved (Table 1).

Pathogenicity test on ginger seedlings

Yellowing lesions were developed from the inoculated site (clipping site) of ginger leaves. The lesions were gradually expanded to the direction of the leaf base. The inoculated ginger was completely wilted about three weeks after inoculation.

Bacterial ooze streamed out from the crosscut of the pseudostem of artificially inoculated ginger. When bacterial isolation was carried out from the ginger, the colony morphologies isolated were identical to that of inoculated bacteria.

DISCUSSION

Shan State is a major ginger cultivation area in Myanmar and is also the main wholesaler for domestic markets together with many potential markets for exports (ILO, 2017).

Bacterial isolates from ginger showing yellowing and wilting were identified as *R. solanacearum* species complex based on the symptoms of the diseased plants, and colony morphologies. This is the first report of bacterial wilt of ginger in Myanmar.

Bacterial wilt pathogen, *R. solanacearum* species complex was systematically and firstly studied in this research using fundamental studies in the fields of plant bacteriology although further studies on the distribution of ginger isolates in Myanmar will support the detailed information of the disease spread and prevention of the disease outbreak.

Genus *Zingiber* contains approximately 150 species, and the main centers of diversity are South China, Malaysia, Northeast India, Myanmar, and the Java–Sumatra region of Indonesia (Nair, 2013). Since particular strains of *R. solanacearum* species complex, known as race 4, infect *Zingiber* species, it is speculated that *R. solanacearum* species complex coevolved with *Zingiber* species. A detailed study for the genetic diversity of Myanmar isolates collected here will be necessary for understanding the evolutionary root of *R. solanacearum* species complex.

AUTHOR CONTRIBUTIONS

H. W. W. Kyaw designed the study, collected disease samples, isolated bacterial pathogens, performed the pathogenicity test and wrote the paper. T. A. A. Naing supervised the study, collected disease samples, isolated bacterial pathogens, and performed the pathogenicity test. K. Iiyama, S. Yonehara and N. Furuya designed the study, supervised the work, wrote the paper. All authors assisted in the editing of the manuscript and approved the final version.

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Table 1. List of *R. solanacearum* species complex isolated from the diseased ginger in Shan State, Myanmar in 2021

Isolates	Variety of ginger	Infected plant parts	Location[a]	Collection Date
H1–1	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H2	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H3	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H4	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H5–2	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H6–1	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H6–2	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H6–3	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H7–1	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H7–2	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H7–3	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H8	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H9	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H10	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H11	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H12–2	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H14	Kyauk Sein	Rhizome	Heho	Nov. 26th, 2021
H15	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H15–2	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H16	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H17	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H18	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H20	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H20–1	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
H20–2	Kyauk Sein	Pseudostem	Heho	Nov. 26th, 2021
GW1	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW2	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW3	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW4	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW5	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW6	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW7	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW8	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW9	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
GW10	Gyin War	Rhizome	Yatsauk	Nov. 26th, 2021
YS1	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS2	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS3	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS4	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS5	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS6	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS7	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS8	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS9	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021
YS10	Kyauk Sein	Rhizome	Yatsauk	Nov. 26th, 2021

[a] The latitude/longitude of Heho and Yatsauk are 20.7232° N / 96.8217° E and 21.2455° N / 96.8834° E, respectively.