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REVIEW

Bacterial wilt of ginger (*Zingiber officinale* Rosc.) incited by *Ralstonia pseudosolanacearum* - A review based on pathogen diversity, diagnostics and management

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Abstract

Ginger (*Zingiber officinale* Rosc.) is one of the most important spice crops cultivated in India and several other countries such as China, Nepal, Indonesia and Nigeria. Bacterial wilt of ginger, referred to as "ginger blast" or "Mahali"/ "green wilt" caused by *Ralstonia pseudosolanacearum* Safni et al. 2014 (formerly *Ralstonia solanacearum)*, is the most destructive pathogen of ginger reported from all the ginger growing countries. This bacterium has a wide host range and is notoriously known for its aggressiveness and ability to survive in soil for many years. Without understanding the symptomatology, epidemiology or genetic diversity of the pathogen, it is impossible to develop suitable diagnostics or management strategies against this pathogen. Common strategies employed for bacterial wilt management met with limited success and still it remains as an enigma. This literature review therefore mainly focused on the symptomatology, diversity and diagnosis of the pathogen and also on the management strategies adopted to mitigate the problem.

Keywords Bacterial wilt · *Calcium chloride* · Diversity · Diagnostics · Ginger · *Ralstonia pseudosolanacearum* · *Bacillus licheniformis*

Ginger belongs to the family Zingiberaceae, is a perennial herbaceous spice cultivated in India and outside for the spicy vegetable ginger and dried ginger ("chukku"). It is one of the most important spices in Ethiopia and up to 35% of the total arable lands were allotted for ginger production (Kifelew et al. 2015). The top ginger growing countries are India, China Nepal Indonesia and Nigeria (https://www.worldatlas.com/articles/the-leading-ginger producing-countries-in-the-world.html) where Nigeria stands first in area under ginger cultivation (56.23%) followed by India (23.6%), China (4.47%), Indonesia (3.37%) and Bangladesh (2.32%) (http://efreshglobal.com/eFresh/Content/Products.aspx?u=Ginger_pcer). However, India ranks first in production and contributes to about 32.75% of the world production followed by China (21.41%), Nigeria (12.54%) and Bangladesh (10.80%) (https://www.nabard.org/english/ginger.aspx) and in 2013, the global production was 2.1 million tons. In India the major ginger growing states are Kerala, Karnataka, Sikkim, Meghalaya, Himachal Pradesh, West Bengal, Assam and Arunachal Pradesh (Singh et al. 2012).

Ginger grows from sea level up to an altitude of 1500 m (Pruthy 1993) and requires a rainfall of 150 to 300 cm during the vegetation period (Nybe and Mini Raj 2005). It is a monsoon crop in south India, but often cultivated under irrigated conditions in north and central India. Vegetative propagation is the only mode of cultivation where seed rhizomes called "piece" or "sett" or "knob" or "cutting" are used. In India small rhizome bits of 14–56 g with two to three sprouts are used for planting (Kandiannan and Thankamani 2012) while in China and other countries 75 g is the planting unit (Nybe and Mini Raj 2005). Seed material of healthy rhizomes are often pre-treated with fungicides and air dried and stored in pits with sand or saw dust. Manuring with cattle manure at the time of planting and fertilizers are often applied in split doses according to the soil fertility and agro ecological conditions.

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Mulching with green leaves is applied for weed control, maintaining the moisture and also protecting the seed bed from rain splashes and increasing organic content to the soil (Nybe and Mini Raj 2005).

Ginger is affected by many pests and diseases. Major economic loss is due to soft rot, bacterial wilt, ginger yellows, *Phyllosticta* leaf spot and storage rots (Nada et al. 1996). Apart from that, ginger is infected by various viruses and insect pests. The major insect pests in the field and under storage are shoot borer (*Conogethes punctiferalis* Guen.) and rhizome scale (*Aspidiella bartii* Sign.) respectively (Devasahayam and Abdulla Koya 2005). Mosaic disease and chlorotic flek virus were also reported in ginger (Thomas 1986; Dohroo 2005). Other minor diseases like dry rot, caused by *Macrophomina phaseolina* (Sarma and Nambiar 1974), *Leptosphaeria* leaf spot caused by *Leptosphaeria zingiberi* (Dhar et al. 1981), basal rot caused by *Sclerotium rolfsii* (Haware and Joshi 1973) etc. were also observed in different ginger growing areas of India and other countries. Among the many diseases, bacterial wilt caused by *Ralstonia pseudosolanacearum* is the most devastating one for which all the control measures are failing.

Bacterial wilt of ginger

The first report of bacterial wilt in ginger in India was in 1941 by Thomas from the Malabar region in the Madras Presidency. A 100% bacterial wilt incidence was reported in Wayanad, Kerala (Mathew et al. 1979). The disease is caused by *Ralstonia pseudosolanacearum* race 4 biovar 3 strains. Bacterial wilt has been reported in other Zingiberaceae family members such as Alpinia [*Alpinia* spp. (Hayward, 1994b)], turmeric [*Curcuma longa* (Velupillai, 1986)], galanga [*Kaempferia galanga* (He, 1986)], Siam tulip (C. *alismatifolia),* and mioga [*Zingiber mioga* (Tsuchiya et al. 2004, 2005)]. The pathogen was isolated from yellow ginger (*Hedychium flavescens),* white ginger (*H. coronarium),* kahili ginger in Hawaii (Aragaki and Quinon 1965) and *Heliconia* sp. (*Zingiberales,* family Heliconiaceae) (Bhai et al. unpublished). In 2013, a sudden outburst of a disease in ginger suspected to be bacterial wilt occurred in south-western zones of SNNRPS, Ethiopia including Sheka, Kefa and Bench Magi and subsequently rapid spread occurred in central areas of the country including Wolaita, Kembata, Tembaro and Dawro leading to extensive crop loss irrespective of varietal, climatic and geographic variations (https://www.researchgate.net/publication/279442041_Ginger_Zingiber_Oficinale_Rosec). In Queensland, Australia, bacterial wilt in ginger was reported in 1955, following a large scale import of ginger seed rhizomes from the Guangzhou province of China. The disease was initially suspected to be caused by *Fusarium oxysporum* f. sp. *zingiberi* and later identified as *R. solanacearum* biovar 4, reclassified as *R. sequeirae* (Hayward and Pegg 2013). In China bacterial wilt is called "Green wilt disease" because the leaves of the infected plant remain green when the plant shows wilt symptoms.

Ralstonia solanacearum sensu lato

Ralstonia solanacearum sensu lato (now including *R. solanacearum and R. pseudosolanacearum)* is a soil borne, plant pathogenic, Gram negative, β-proteobacterium with a wide host range and geographical distribution. The host range of the pathogen has increased to over 450 plant species distributed in 54 botanical families holding many economically important crops including herbaceous plants, shrubs, and trees (Wicker et al. 2007; Hayward 1994a). *R. solanacearum sensu lato* is regarded as one of the most important bacterial plant pathogens because of its lethality, broad geographical distribution and long persistence in soil and water environments (Elphinstone 2005). It has been reported from tropical, subtropical and temperate areas throughout the world. It poses a serious threat to most cash and subsistence crops in the families Solanaceae, Musaceae and Zingiberaceae. The susceptible cash crops include potato, tomato, chilli, sweet pepper, tobacco, potato, groundnut, banana, eggplant, ginger, curcuma and Heliconia. The European Union Council Directive (EUCD) 2000/29/EC considers *R. solanacearum sensu lato* as a quarantine bacterium and phylotype II is one among the ten phytopathogenic organisms described in the "Agricultural Bioterrorism Protection Act" in the United States of America since 2002 (Lambert 2002). Different strategies are practiced for the management of the disease using chemicals and antibiotics, but have limitations. Breeding for resistant cultivars is the most successful, economical and environmentally benign method in disease control strategy, but in case of bacterial wilt, development of disease resistant lines met with limited success (Denny 2006). Cultural practices like use of disease free propagative tissues and good sanitation would help to reduce the yield loss due to bacterial wilt. In endemic areas, crop rotation is the alternative to reduce the pathogen inoculum, although the broad host range, including weeds, of *R. pseudosolanacearum*, limits its effectiveness and needs a larger number of years (≥ 6 years). In case of ginger, management strategies include selection of disease free rhizomes, crop rotation and phytosanitation (Hartman et al. 1993; Kumar and Hayward 2005).

R. solanacearum sensu lato exhibits a high degree of both phenotypic and genotypic diversity where the diverse genetic groups have been classified into five races based on host range (Buddenhagen et al. 1962). Apart from race classification, *R. solanacearum* has been classified into five biovars based on utilization and/or oxidation of hexose alcohols and disaccharides (Hayward 1964). Phylogenetic analysis classified the

species into four phylotypes which displays the geographical origin of the strains. Phylotype I and II are composed of Asian and American strains, respectively, whereas phylotype III consists of African strains and phylotype IV of isolates from Indonesia, Japan and Australia (Fegan and Prior 2005). *R. solanacearum* is now described as a *Ralstonia solanacearum* species complex (RSSC) due to its extensive diversity including *Ralstonia syzygii* and the banana blood disease bacterium (*R. celebencis*) (Prior and Fegan, 2005). Recently a new classification was proposed by Safni et al. 2014. Based on polyphasic taxonomic studies using a large number of strains, Safni et al. (2014) proposed a taxonomic and nomenclatural revision of *R. solanacearum* species complex. By phylogenetic analysis of gene sequences of 16S- 23S rRNA ITS, 16S–23S rRNA intergenic spacer (ITS) region, partial endoglucanase (egl) gene and DNA-DNA hybridizations, the *R. solanacearum* species complex was delineated into three genospecies. Accordingly, the type strain of *R. solanacearum* is restricted to strains of phylotype II only. The second genospecies is subdivided into three distinct groups viz. *R. syzygii* subsp. *indonesiensis* subsp. nov., for strains of Sumatra disease of clove, *R. syzygii* subsp. *celebencis* subsp. nov. for the BDB strains and *R. solanacearum* phylotype IV strains isolated from different host plants mostly from Indonesia. The third genospecies is named R. *pseudosolanacearum* sp. nov. for the strains of *R. solanacearum* that belong to phylotypes I and III (Safni et al. 2014). The ginger bacterial wilt pathogen is grouped under the third genospecies *R. pseudosolanacearum.*

R. solanacearum sensu lato can survive free in the soil saprophytically for a long time but it requires moisture, conditions for the degradation of plant cell debris or the ability to colonize roots of non-hosts, including weeds, asymptomatically (Genin and Boucher 2004; Hayward 1991). It can also persist at low populations in naturally infested soil for years without host plants, and when hosts are planted, the population size can reach to the plant infection threshold within a season (Dittapongpitch and Surat 2003). Dissemination of the pathogen through infected planting material is a serious issue in case of ginger, curcuma, potato etc. In such cases detection, documentation and diagnosis of the pathogen become imperative for studying the epidemiology and to develop adequate control strategies. Without proper understanding of the genetic diversity of the pathogen, development of proper molecular diagnostics is not possible, which is very much lacking in the case *R. pseudosolanacearum* causing bacterial wilt of ginger.

Symptoms of ginger bacterial wilt

The typical symptom of bacterial wilt in ginger is downward drooping of the leaves, that at first remain green (Dake 1995), so called “green wilt” (Nelson 2013), followed by yellowing. Vascular browning advances and finally the plant collapses. Young shoots /tillers often become soft and rotten and break off easily from the underground rhizome at the soil level (Nelson 2013). Affected rhizomes will be dark in colour and on cutting milky white exudates ooze out from the cut surfaces (Pegg et al. 1974; Tsuchiya et al. 2005). The rotted rhizomes emit a foul smell characteristic of the disease (Kumar and Abraham 2008). High inoculum density of the bacterium inside the vascular tissue leads to blocking of upward translocation of water and nutrients that finally results in the death of the plant (Buddenhagen and Kelman 1964). In advanced stages of infection, the inner core of the rhizomes is rotted, leaving the outer epidermal layer intact and finally the plant collapses and the pathogen enters into the soil, thereby repeating the cycle (Kumar and Hayward 2005).

Global distribution of ginger bacterial wilt and the races/biovars of *R. pseudosolanacearum* involved

Bacterial wilt of ginger has been reported from Australia (Hayward et al. 1967), China (He et al. 1983), Ethiopia (Kifelew et al. 2015) India (Thomas 1941), Indonesia (Sitepu et al. 1977), Japan (Morita et al. 1996), Malaysia (Lum 1973), Mauritius (Orian 1953), Nigeria (Nnodu and Emehute 1988), South Korea (Choi and Han 1990), Thailand (Uematsu et al. 1981), the Philippines (Zehr 1969, 1970) and the USA (Hawaii) (Rosenberg 1962).

R. pseudosolanacearum strains isolated from naturally infected ginger belong race 4 (Denny 2006) and either biovar 3 or 4 with only one exceptional report from Malaysia by Abdullah (1982) where biovar 1 strains infecting ginger were reported (Hayward 1994a). In India the predominance of biovar 3 over biovar 4 was already reported (Kumar et al. 2004). In Kerala, 50–100% loss in ginger fields were found due to heavy rainfall, undulating topography to ease the movement of water from affected field to other area, close proximity of the fields, lack of internal quarantine measures and use of infected rhizomes for planting (Kumar and Abraham 2008).

Contradictory to the Indian scenario, in Australia rapid and a severe form of bacterial wilt was due to biovar 4. In Queensland, the wilt epidemics in ginger started in 1955, but the disease was only attributed to *R. pseudosolanacearum* in 1965 by Hayward and co -workers (Hayward et al. 1967). Ginger was not a commercial crop in Queensland till World War II and afterwards ginger seed material was imported to Queensland from China in 1954 and from there the bacterial wilt epidemics started in Queensland (Hayward and Pegg 2013). So there was an assumption that the source of pathogen inoculum was from the Chinese seed material. The host range studies by Pegg and Moffett 1971 showed that the biovar 4 strains causing rapid bacterial wilt epidemics in Queensland

can also infect other solanaceous crops such as tomato, potato, capsicum, eggplant, peanut, tobacco, *Solanum nigrum, Physalis minima, P. peruviana,* and *Solanm mauritianum.* So also the pathogen could be isolated from different weeds like *S. nigrum, Crassocephalum crepidioides, S. mauritianum, P. minima, P. peruviana* and *Ageratum houstonianum* from the ginger fields that may result in disease severity and pathogen survival without original hosts. Studies on the diversity of Chinese strains of *R. pseudosolanacearum* from ginger lead to a clear indication of the existence of a common population in the bacterial wilt outbreaks of China and Queensland by biovar 4 (Xu et al. 2009; Xu et al. 2011). Also these populations of ginger strains had the same genetic fingerprints, similar to isolates from solanaceous crops of nearby provinces in Queensland (Pegg and Moffett 1971). But after 1970, there was no such severe outbreak of bacterial wilt anymore in Queensland (Australia) due to the strict eradication strategies adopted to control the disease (Hayward 1994a; Hayward and Pegg 2013).

In countries like Japan and China *R. pseudosolanacearum,* infecting ginger was found to be caused by biovar 4, whereas in Thailand and Indonesia both biovar 3 and 4 were reported (He 1986; Titatarn 1986; Tsuchiya et al. 2005; Xu et al. 2009). Recent genetic diversity studies from Japan indicated the occurrence of biovar 3 in ginger (Waki et al. 2013). In Malaysia, cross inoculation studies with biovar 3 (Lum 1973) showed wilting symptoms as stunting and yellowing and took three weeks for the complete collapse of the plants. Ginger strains showed only weak infection towards tomato, tobacco and groundnut, whereas tomato strains can cause characteristic wilting symptoms in both solanaceous hosts and ginger. In Hawaii, ginger strains failed to wilt tomato, tobacco and groundnut and tomato strains failed to wilt ginger and tobacco (Quinon et al. 1964). Race 4 strains are very specific to ginger however; race 1 strains can cause slow wilting, while other races are not infective to ginger. Only limited reports are available for race 1 *R. pseudosolanacearum* infection in ginger (Hayward 1994b).

Morita et al. 1996 reported bacterial wilt in *Curcuma alismatifolia* (Zingiberaceae) from Japan and later in 1997 and 1999 ginger and mioga fields were reported to be affected by a bacterial wilt outbreak (Tsuchiya et al. 2005; Yano et al. 2005). The race 4 *R. pseudosolanacearum* strains from these isolations were found to be biovar 4. Genetic finger printing based on rep- PCR revealed that these Japanese strains of *R. pseudosolanacearum* contain two subclasses, viz. type I and II. The type I strains showed a genetic fingerprint similar to Australian and Chinese strains of *R. pseudosolanacearum.*

The type II strains were highly virulent on both zingiberaceous and solanaceous hosts, while type I strains were highly virulent on zingiberaceous hosts and weakly pathogenic on solanaceous crops (Horita et al. 2004).

In the Philippines, ginger isolates of *R. pseudosolanacearum* were found to be more virulent in ginger when compared to tomato (Zehr 1969, 1970). Host range studies from Thailand and China revealed that ginger is infected only by the virulent *R. pseudosolanacearum* strains isolated from ginger (Uematsu et al. 1983; He 1986). Biovar 4 strains of *R. pseudosolanacearum* from ginger, mioga and curcuma were able to infect both solanaceous crops and zingiberaceous crops, but the isolates from tomato and eggplant did not infect ginger and mioga (Tsuchiya et al. 2005). Similarly, *R. pseudosolanacearum* from tomato, potato, chromolaena and chilli were found to be non pathogenic on ginger (Kumar and Sarma 2004; Kumar and Hayward 2005) while *R. pseudosolanacearum* biovar 3 and 4 strains from zingiberaceous hosts could infect ginger and cause wilt. However, *R. pseudosolanacearum* strains from other plant families were unable to infect ginger (Waki et al. 2013). Biovar 3 strains causes wilting in ginger within 5 to 7 days upon stem inoculation and 7 to 10 days upon soil inoculation where a minimum CFU of 3×10^5 ml^{-1} is required to show the wilting symptoms (Kumar and Sarma 2004).

Source of pathogen inoculum for ginger bacterial wilt

The pathogen *R. pseudosolanacearum* is both found in plant propagative material (transplants, rhizomes, seed) and soil borne in nature (Dake 1995).

Ginger rhizomes are normally cut into appropriate size and used as planting material and the pathogen in the soil can enter the rhizomes through the cut ends. So these rhizome pieces form the primary source of inoculum (Indrasenan et al. 1981). Infection can also occur through wounds in roots or rhizomes or at sites of secondary root emergence. After the entry, the bacterium colonizes the intercellular spaces of the root cortex and vascular parenchyma and produces extracellular enzymes that break down pectin in the cell wall and middle lamella and access the vascular system (Kumar and Hayward 2005; Kumar and Abraham 2008). Upon death of an infected plant, the bacterial cells up to a population level of 10^{10} reach the soil and remain as saprophytes till it infects a new host plant. The spread of pathogen occurs via soil, irrigation water or rain splash to the adjacent plant within a bed, as well as to other beds in the same field (Dake 1995). Weeds such as *Euphorbia hirta, Hedychium gardenarium*, *Chromolaena odorata*, *Glomus* sp. etc. in the ginger fields are reported as symptomless carriers of *R. pseudosolanacearum,* wherein the bacteria survive in the rhizosphere (Quinon et al. 1964; Ishii and Aragaki 1963; Zehr 1969; Moffett and Hayward 1980; Dake 1995).

The occurrence of bacterial wilt in fields where ginger is planted for the first time or the soil is fallowed for quite some time indicates the rhizome /seed borne nature of *R.*

pseudosolanacearum (Pegg et al. 1974; Kumar and Hayward 2005). It is assumed that only very low population survives in ginger without affecting the normal state of ginger. The pathogen remains latent in ginger rhizomes when it is under storage unless it initiates sprouting (Kumar and Hayward 2005).

Genetic diversity of ginger *R. pseudosolanacearum*

Based on the diversity studies by Cook et al. (1989), ginger strains were found to be of Asian division. Diversity of *R. pseudosolanacearum* race 4 biovar 3 and 4 strains from ginger and race 1 strains from other hosts in India was analyzed using REP–PCR and RFLP–PCR where they could cluster the highly pathogenic isolates in a cluster with 100% similarity coefficient in conformity with their host origin and biovar. High level of similarity (100%) among the ginger strains from geographically and chronologically separated isolations indicated that the isolates of biovar 3 of *R. pseudosolanacearum* were lineages of a single virulent strain and inter-state rhizome transmission could be one of the possible means of pathogen spread across the states (Kumar et al. 2004). Recently Waki et al. (2013) conducted a phylogenetic analysis of a collection of *R. pseudosolanacearum* from Zingiberaceae plants of Asia-Pacific region consisting of biovar 3 and 4 strains using the *egl* and *mutS* genes. The study included strains from all the major ginger growing countries like Japan, Thailand, China, Indonesia and Australia where ginger bacterial wilt persists. But unfortunately none of the Indian strains of ginger was included in this study. The strains were grouped into six sequevars up on *egl* gene based phylogenetic analysis and showed close correlation between the strains to either host or the country of isolation. Distinct diversity exists among the *R. pseudosolanacearum* strains from Zingiberaceous plants in the Asia-Pacific region and among the strains from different origins. This could be due to local or global dissemination through the rhizome planting materials (Waki et al. 2013).

Phylotyping using multiplex PCR revealed the predominance of phylotype I among the Indian isolates of ginger *R. solanacearum. A recN housekeeping* gene based phylogenetic analysis separating the strains based on their phylotypes/geographic origin clearly indicated the presence of a genetically distinct lineage of *R. pseudosolanacearum,* infecting ginger (Kumar et al. 2013). Within phylotype I, the highly pathogenic race 4 biovar 3 strains from ginger and small cardamom separately clustered from other strains (Kumar et al. 2014). Prameela et al. (2017), conducted a detailed investigation on the diversity of *R. pseudosolanacearum* race 4 biovar 3 strains infecting ginger from India. Strains collected from ginger and small cardamom (*Elettaria cardamomum* Maton) when tested for cross infectivity on ginger, tomato and eggplant clearly showed the specificity of race 4 biovar 3 strains to ginger. Based on *egl* gene sequence analysis for sequevars determination, the fast wilting lineage was found belonging to sequevar 17 (Prameela 2017).

Diagnostics for bacterial wilt

Detection of *R. pseudosolanacearum* from planting material and soil is important for the production of healthy crop. Tissue cultured ginger plants, red ginger or spiral ginger were once used as bio indicator plants for efficient detection of race 4 strains in the field soil (Paret et al. 2009) with a sensitivity of up to $\log_2$ CFU g^{-1}, but the assay took minimum 9–20 days. This bioassay was practiced in Hawaii also for the reliable detection of the pathogen from ginger fields (Nelson 2013). Bacterial ooze test or streaming test in water is another detection assay, when there are symptomatic plants in the field.

ELISA based immunostrip method was described for the detection of *R. pseudosolanacearum* from infected tissues where the specific antibodies developed can detect the pathogen (Nelson 2013). NCM-ELISA kit developed in the International Potato Centre (CIP), Lima, Peru was tested for detecting *R. pseudosolanacearum* in ginger (Kumar et al. 2002) and found that the antibodies developed for potato were sensitive enough to detect the pathogen from other crops, including ginger, and the sensitivity was found to be 42 cells ml^{-1} of the ginger extract. PCR- based specific detection of *R. pseudosolanacearum* race 4 strains has been by developed by Horita et al. (2004) using a primer set derived from the polymorphic bands obtained from repetitive sequence based polymerase chain reaction. In this study *R. pseudosolanacearum* were divided into two subclasses, type I and II. Type I strains amplified a 165 bp amplicon and type II strains amplified a 125 bp amplicon from ginger isolates. Kumar and Abraham (2008) reported the use of PCR for indexing of ginger rhizomes and soil for *R. pseudosolanacearum.* They could detect the presence of the pathogen in apparently healthy rhizomes and soil in the vicinity of the field positive for the pathogen. But all these techniques for detection of *R. pseudosolanacearum* in ginger rhizomes are not used frequently for indexing the rhizomes or soil because they are expensive and laborious under the farming conditions of developing nations like Asia (Kumar and Hayward 2005).

For race 4 strains of ginger, a strain specific and sensitive detection methodology was developed using Real-Time Loop-Mediated Isothermal Amplification (Real-time LAMP) protocol (Prameela et al. 2017). The primers for the Real-time LAMP were designed from the *gyrB* gene and were found suitable for the detection of only *R. pseudosolanacearum* race 4 strains infecting ginger and not any other strains of *R. pseudosolanacearum* from solanaceous crops. The detection limit was 10^3 CFU/g of soil or rhizomes and the method is well suited to index both soil, water as well as rhizomes. The protocol

was also customized with soil supernatant and rhizome extract as template instead of genomic DNA, the extraction of which is cumbersome at the field level, for an easy and on-farm diagnosis.

Management of Bacterial wilt

Strategies adopted for bacterial wilt management include cultural, chemical, biological and integrated means. However, so far, no effective control method has been developed for the eradication of bacterial wilt disease in the field for any crop. Plant breeding, field sanitation, crop rotation, and use of bactericides have met with only limited success (Ciampi-Panno et al. 1989). Moreover due to highly diverse species ecology universal control measures are virtually impossible (Saddler 2005). Development of disease resistance is a widely accepted part of integrated disease management strategy, however breeding for resistance in most host plants is not effective in bacterial wilt due to lack of stability and/or durability (Hayward 1991; Boucher et al. 1992). Moreover the high variability of *R. solanacearum* strains and impact of environmental factors often confines the expression of resistance to specific regions only (Hayward 1991). Vascular harbouring of the pathogen, long term survival in soil, transmission through irrigation water and rhizomes and asymptomatic weeds account for the difficulty in controlling the disease at field level (Wang and Lin 2005; Yuliar et al. 2015). So different control methods were attempted for bacterial wilt management in ginger. The common strategies employed were selection of disease free rhizomes, selection of fields where no bacterial wilt had been recorded, cultural practices like raised ginger beds with deep drainage, pretreatment of rhizomes with plant protection chemicals and strict phytosanitory measures to avoid carryover of pathogen inoculum through field workers, tools used for bed making and earthing up or irrigating water. Removal of weed hosts from the fields and crop rotation with non host plants such as sweet potato and taro and also grain crops such as corn and rice are also strategies for the management of bacterial wilt of ginger (Dake 1995; Kumar and Hayward 2005; Nelson 2013). In Hawaii, Nelson (2013) advocated avoiding of planting ginger rhizomes in wet weather and spraying of 10% bleach solution to tractor wheels and tractor blades.

Resistance

Breeding for resistance is an important strategy in disease management, but none of the released varieties of ginger are resistant to *R. pseudosolanacearum.* This may be due to the lack of genetic variability among the ginger accessions since ginger is a vegetatively propagated crop (Prasath et al. 2011). The response of different species from the family Zingiberaceae viz. *Curcuma amada, C. longa, C. zedoria, C. aromatica, Kaempferia galanga, Elettaria cardamomum, Zingiber zerumbet,* and *Z. officinale* was tested towards *R. pseudosolanacearum* infection and it was that found Indian mango ginger and *C. amada* Roxb. were resistant (Kumar et al. 2006; Prasath et al. 2014). Prasath et al. (2015) developed mutants of ginger through induced mutation using gamma rays and upon screening realized six mutant genotypes imparting resistance to bacterial wilt; these mutants are now under field evaluation.

Heat treatment

To prevent the bacterial wilt outbreaks in the field, the foremost importance should be given for selection of pathogen free rhizomes and soil (Pordesimo and Raymundo 1963; Supriadi 2000; Kumar and Hayward 2005). Soil solarization for 40 days before planting was suggested in reducing the pathogen load in the soil and further reduction in bacterial wilt incidence, increase in germination percentage and yield (Dake 1995; Kumar and Hayward 2005). As soil solarization is a hydrothermal process, it does not leave any toxic residues, moreover the process destroys most of the harmful organisms and even seeds of many weed hosts. In temperate regions polyethylene covers are used to trap sunlight and to raise the temperature (Kumar and Hayward 2005). Heat treatment is also an effective way to kill the pathogens inside the seeds. Exposure of infected ginger seed pieces to hot air at 75% RH, until their core temperature reaches 49–50 °C for 30 min, resulted in disease free rhizomes and the process did not adversely affected sprouting or subsequent growth of the plants. But prolonged exposure of the rhizomes to hot air would cause damage to the seed rhizomes (Kumar et al. 2005; Kumar and Hayward 2005). Tsang and Shintaku (1998) also recommended hot air with 75% RH for disinfection of ginger rhizomes. They also exposed ginger rhizomes as above and that resulted in minimal injury of rhizomes with more than 87% germination without any adverse effect on growth. Heat induction by dipping ginger seeds in hot water at 50 °C for 10 min is also a usual pre- planting procedure in Hawaii (Trujillo 1963; Nishina et al. 1992). Kumar et al. (2005) reported rhizome solarization as one of the effective and ecofriendly way of heat treatment of ginger seed rhizomes. Microwave exposure of infected rhizomes for 30 s at 45 °C was also found reducing the pathogen population. But longer exposure resulted in low germination of the ginger rhizomes (Kumar et al. 2005; Kumar and Hayward 2005).

Chemical control

Various antibiotics and chemicals were used for bacterial wilt management in ginger. As early as 1963, soil fumigation with methyl bromide at 3 lb/100 ft^2 was recommended against bacterial wilt of ginger (Ishii and Aragaki 1963). Consequent with the ban of methyl bromide, chloropicrin was evaluated for its

effectiveness to reduce bacterial wilt of ginger in China (Mao et al. 2014). Chloropicrin at the dose of 50 g m^{-2} covered with polyethylene film reduced *R. pseudosolanacearum* population and increased yield in ginger. Rhizome treatment and soil drenching with streptocycline 200 ppm, bleaching powder etc. were found effective in controlling bacterial wilt for the initial months of growth (Dake et al. 1988). Hartati and Supriadi (1994) experimentally proved the effect of rhizome treatment with both streptomycin and oxytetracycline for ginger rhizomes. Similar studies were attempted by Mulya et al. (1986) for effective bacterial wilt management in ginger. The bactericide Kekuling, applied as a wettable powder formulation to the rhizosphere of ginger five times during growth of the crop at dilutions of 1:500 to 1:1200, has been reported to provide good control of bacterial wilt in China (Zhang et al. 1993). Terramycin (oxytetracycline) at 500 ppm as rhizome protectant was found best in reducing the plant mortality (11.11%) and increasing yield (175 q ha^{-1}) (Ray et al. 2005). Streptocycline and chloramphenicol at 500 ppm were also found to be effective with plant mortality of 30.12% and 33.33% respectively. However, comparatively less mortality was observed with griseofulvin (plant mortality 45.58%, ledermycin (plant mortality 35.29%) and penicillin (plant mortality 38.05%) Copper oxychloride, carbendazim and Bordeaux mixture were also reported effective in reducing plant mortality in ginger but the effect was not as good as that of antibiotics (Ray et al. 2005). With all these, field control of bacterial wilt using commercial chemical compounds such as antibiotics, fertilizers, and fungicides has met with little success. A study for mitigating bacterial wilt of ginger was conducted at ICAR- Indian Institute of Spices Research, Kozhikode. In the study, a technology integrating soil solarization and soil amelioration with calcium chloride has been developed to manage BW efficiently and economically. $CaCl_2$ (2 to 4%) is found inhibitory to *R. pseudosolanacearum* under in vitro conditions and under challenge inoculation showed 98% to 100% reduction in BW with 3% and 4% $CaCl_2$, respectively (Suseela Bhai et al. 2019). Subsequent field evaluation using calcium chloride 3% (soil drenching at the time of planting, and at 30, 45 and 60 days interval) resulted in significant reduction in the population of *R. pseudosolanacearum* from 10^8 to 10^3. Further field demonstration in endemic areas of bacterial wilt viz. Manathavady and Kenichira (0.5 acres in each plots) in Wayanad district, Kerala, India also resulted in 100% disease suppression. The results obtained from the study would serve as a viable and effective integrated strategy for the management of bacterial wilt in ginger (Suseela Bhai et al. 2019).

Biological control

Biological control methods may either aid in the improvement of alternative management strategies or can be integrated with other control measures for effective management of the disease at the field level. Many attempts have been made to manage bacterial wilt of ginger using biocontrol methods. However, under field conditions effective and consistent wilt control still remains as an enigma.

Biofumigation

Essential oils of palmarosa (0.07%) and lemongrass (0.14%) were found effective in reducing the population of race 4 strain of *R. pseudosolanacearum* under in vitro and *in planta* condition (Paret et al. 2010a). The pathogen was not detected in *R. pseudosolanacearum* infested potting medium after treatment with palmarosa and lemongrass oils at 0.07 and 0.14% in any of the experiments. But application of these oils in the field is not feasible due to the huge cost involved. So burying of green manure crops such as mint, palmarosa, and lemongrass, having these oils months before planting has to be studied in the field (Paret et al. 2010a; Nelson 2013).

Bacteriophages

Bacteriophages were also exploited for the biological control of *R. pseudosolanacearum* infecting ginger. Tanaka et al. (1990) used bacteriophages for the control of bacterial wilt in tobacco, but the host range of this phage was too narrow. Several different types of phage were isolated and characterized that specifically infect *R. solanacearum* strains belonging to different races and/or biovars (Yamada et al. 2007). Prameela et al. (2012) also isolated bacteriophages infecting *R. pseudosolanacearum* from ginger rhizosphere soil and evaluated their host specificity in vitro, and found that the isolated phages were host specific and infected only *R. pseudosolanacearum* strains from the same geographical location from where the phages were isolated. Field evaluation studies were not conducted further since these phages were not a suitable strategy for a generalized adoption by all the ginger growing areas.

Biocontrol agents

Bacterial wilt management in ginger using antagonistic microorganisms are less practiced owing to the non-availability of efficient biocontrol agents. Asmaja (2003) reported antibacterial potential of *Trichoderma viridae* against *R. pseudosolanacearum* based on in vitro and greenhouse evaluation.

The plant endophytes include all the microorganisms inhabiting the internal tissues including plant apoplastic microbes which harbour the apoplastic fluid in the intercellular space. Bini et al. (2010) isolated endophytic bacteria from ginger rhizomes and screened for their inhibitory activity against *R. pseudosolanacearum in planta.* Among the endophytic

bacteria tested, five bacteria showed significant reduction in the disease and were identified as *Pseudomonas aeruginosa, Stenotrophomonas maltophila, Enterobacter* sp., *Klebsiella* sp. and *Acinetobacter calcoaceticus.* Exploitation of potential plant apoplastic microbes for the biocontrol of plant pathogens is a newly emerging area in plant disease management. Prameela et al. (2017) isolated 150 bacteria from apoplastic fluid of ginger pseudostem and leaves from different ginger growing tracts and ginger germplasm accessions using vacuum infiltration and centrifugation method, characterized and tested for their effect on *R. pseudosolanacearum* both in vitro and *in planta.* The bacteria were characterized morphologically and biochemically and found belonging to different families. The prominent families in which these bacteria belong to are *Bacillaceae* (38.66%), *Pseudomonadaceae* (22.66%) *Staphylococcaceae* (10%) and *Enterobacteriaceae* (7.33%). These bacteria were evaluated against *R. pseudosolanacearum,* by seed priming and soil drenching method, and found *Bacillus licheniformis* (IISRGAP 107- MTCC12725) as a very promising biocontrol agent against bacterial wilt with a disease reduction up to 67%. *In planta* evaluation under challenge inoculation also showed 71% reduction in bacterial wilt. Subsequent field evaluation involving soil solarization followed by soil application with *B. licheniformis* resulted in significant reduction in the population of *R. pseudosolanacearum* from 10^8 to 10^3. Further field evaluation in farmers' plots in bacterial wilt endemic areas Manathavady and Kenichira (0.5 acres in each plots) in Wayanad district, Kerala, India resulted in 100% disease suppression. This integrated management strategy integrating soil solarization along with soil amelioration with *B. licheniformis* is an effective biological strategy for managing bacterial wilt of ginger. The bacterium is now being promoted as a potential candidate to combat bacterial wilt in ginger (Suseela Bhai et al. 2019) and the product is named as "Bacillich".

Conclusion

Bacterial wilt in ginger caused by *R. pseudosolanacearum* is a very notorious disease for which management strategies are not or only partly successful. An integrated strategy involving cultural and biological /chemical input is required to manage the disease. A better option is selection of disease free rhizomes and soil. This is possible only through proper development or implementation of field diagnostics through simple and easily approachable strategies such as real time LAMP for indexing of soil and rhizomes. Such pathogen detected areas should be managed by an integrated disease management strategies involving soil treatment, rhizome priming and chemical /biocontrol agent treatments.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain any studies with human or animal subjects performed by the any of the authors.

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