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A Review on Ginger Bacterial Wilt Disease and Its Breeding Approaches

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Abstract
Bacterial wilt disease a serious threat for ginger production in worldwide and caused by Ralstonia solanacearum biovar III race 4. Having knowledge on biology, ecology, epidemiology and identification of the pathogen and its interaction with host plants were helpful in designing cost effective breeding approaches and management strategies of the bacteria wilt disease in ginger. Since conventional crop improvement methods for bacterial wilt resistance are not suitable in ginger, due to lack of seed setting behavior leading to limited variability. Under these conditions, tissue culture (multiplication of diseases free ginger seed rhizome production scheme and utilization of tissue culture created variations) and biotechnological tools play very important role in large scale production of disease free planting material which can be introduced to newer areas. Therefore, germplasm introduction, selection or screening and the cloning of resistance genes (candidate gene) and subsequent transformation is the only solution to obtain disease resistant ginger cultivars.
Keywords: Ginger, Bacterial wilt, conventional crop improvement, Biotechnological tools.

1. Introduction

Ginger *(Zingiber officinale* Rosc.) 2n=2x=22 is a monocotyledonous plant belonging to the family Zingiberaceae is an important cash crop and one of the principal spice crop all over the country and world. Ginger is undeniably the most extensively cultivated spice (Lawrence, 1984). It is not known to occur in the truly wild state. It is believed to have originated in South East Asia and it has spread throughout the tropical and subtropical regions in both hemispheres. The main ginger growing countries are India, China, Jamaica, Taiwan, Sierra Leone, Nigeria, Fiji, Mauritius, Indonesia, Brazil, Costa Rica, Ghana, Japan, Malaysia, Bangladesh, Philippines, Sri Lanka, Solomon Islands, Thailand, Trinidad and Tobago, Uganda, Hawaii, Guatemala and many Pacific Ocean Islands.

It is prized for its flavour and medicinal properties. In addition to its use as spice and condiment, is also used to treat liver complaints, flatulence, anemia, rheumatism, piles and jaundice in Indian systems of medicines (Aired, Sridhar and Unani). Ginger oils and oleoresins also have a variety of uses. The essential oil is used in commercial flavorings. Ginger containing proteolytic enzymes that promote the digestive process and also enhance the action of the gall bladder and protects the liver against toxins (Yamahara *et al.,* 1990).

Bacterial wilt is a devastating disease destroying arable land globally at an astounding speed. The dreaded factor in disease spread is that once the soil has been infected with this pathogen, it is almost impossible to use the area again for any susceptible crops. The first bacterial wilt of ginger is reported from India, China, Japan, Indonesia, the Philippines, Hawaii and many other ginger growing countries. In India the disease is found in Kerala, Karnataka, Himachal Pradesh, Sikkim, West Bengal, Assam and other North Eastern States. In contrast to the report from Queensland, the strains causing bacterial wilt of ginger in India belong to biovars 3 that causes wilt in 5–7 days in 45-day-old ginger plants (Kumar and Sarma, 2004).

In Ethiopia it is one of the most important spices, largely for small scale farmer. At south part of the country in eight woredas 2.9 million quintal of fresh ginger was produced from an area of 18,240ha during 2006/2007 and average rhizome yield of 160 quintal per hectare (BOARD, 2008). From two woredas of Wolaita Zone (Hadaro tunto and Boloso Bombe) during the year 2008 alone, more than 1.2 million quintal of fresh ginger was produced from area of 8986 hectare. In the production area up to 85% of the farmers and 35% of the total arable land were allotted for ginger production (Endrias and Asfaw, 2011).

Eventhough, it was prized for its economic and medicinal importance, its quality, production and productivity hampered and the crop was completely wiped out by a sudden outbreak of bacterial wilt epidemics in 2011 and 2012 in Ethiopia. In Ethiopia, the bacterial disease has been reported on Potato, Tomato, Pepper, Enset, Banana and ornamentals but ginger bacterial wilt is not yet reported until 2012 and new to Ethiopia. Therefore, this review paper was designed to review the economic importance and description of the pathogen, epidemiology, identification mechanisms, host pathogen interaction mechanisms and breeding schemes for large scale production of disease free ginger rhizome yield.

2. Importance of Bacterial wilt

The study of bacterial wilt is of economic importance in the whole world. In the developed world it forms a serious threat to the limited fertile arable land that is available. In the developing countries, the danger lies in the general food shortage that will be aggravated by the decline in crop production due to the presence of bacterial

wilt. Furthermore, it has the potential to lead to total crop loss when propagation material is contaminated thus posing a serious threat to the hungry. *Ralstonia solanacearum* has a wide host range, which makes it difficult to have a generalized estimate of the economic losses caused by the bacterial wilt disease. Direct yield losses vary widely according to host, cultivar, climate, soil type, cropping practices and pathogen strain. Therefore, the level of damage is commonly expressed on a crop-by-crop basis, and can range from minimal crop loss to very high economic damage (Elphinstone, 2005).

Habetewold et al., 2015) reported a survey and by laboratory work on the status of ginger bacterial wilt incidence in major growing areas of Ethiopia and identified the causal agent of ginger wilt. The diseases were found distributed in all ginger growing areas and the loss were estimated up to 100%. During the survey season the wilt incidence percentage was recorded maximum (93.5) in Sheka zone followed by Benchmaji zone (91.6) and Majang zone (65.7) while the lowest wilt incidence was recorded in Gamo Gofa zone (10.7). During 2014 survey season wilt incidence percentage was recorded maximum (98.9) in Benchmaji zone followed by Majang zone (98.8) and Sheka zone (97.4) while the lowest wilt incidence was recorded in Kefa zone (78.4). Therefore, this term paper was principally aimed to review bacterial wilt disease on ginger and breeding and manage metal options

2.2. The Pathogen Description

Ralstonia solanacearum previously known as *Pseudomonas solanacearum* (Yabuuchi *et at.,* 1995) is the causal agent of bacterial wilt. It belongs to the Proteobacteria, β subdivision, *Ralstonia* group and the genus *Ralstonia.* It is considered as one of the most important plant pathogenic bacteria due to its broad geographical distribution and great economic losses that occur globally due to its infection and spread (Hayward, 1991). This Gram-negative aerobic bacterium is rod-shaped with polar flagella (Holt, *et al.,* 1994).

The pathogen is a soil- and water-borne bacterium, Ralstonia solanacearum race 4. Synonyms for Ralstonia solanacearum include Pseudomonas solanacearum (Smith 1896) Smith 1914; Burkholderia solanacearum (Smith 1896) Yabuuchi et al. 1992; and others. The pathogen is widely distributed in areas where edible ginger has been grown previously. *Ralstonia solanacearum* belongs to rRNA homology group II (non-fluorescent) within the pseudomonads. *Ralstonia pickettii* (a saprophyte or human facultative pathogen), *Pseudomonas syzygii* (causing Sumatra disease of cloves), and the Blood Disease Bacterium (causing blood disease of banana in Indonesia) are closely related. These bacteria may cross-react in detection procedures based on serology or DNA. Sub-classification of *R. solanacearum* is based on genetic fingerprinting methods into Division I (biovars 3, 4, and 5 from Asia) and II (biovars 1, 2A, and 2T from South America). Additional, proposed taxonomic divisions are based on nucleic acid sequence analysis into “phyllovars” and “sequevars.” Clearly, this species is taxonomically intricate.

2.2.1 Morphological Aspect of the Bacteria

R. solanacearum is a strictly aerobic, gram negative, non-spore forming, non-capsulated, and nitrate-reducing, ammonia-forming, aerobic, rod-shaped bacterium (Stevenson et al., 2001). The bacterium does not hydrolyze starch, nor readily degrade gelatin. It is also sensitive to desiccation and is inhibited by low concentration of sodium chloride in broth culture. Optimum growth generally is between 28 to 32^{O} C except certain Race 3 strains pathogenic to potato that are able to grow at lower temperature” (Stevenson et al., 2001). On tetrazolium chloride (TZC) media the bacteria is fluidal, presents irregular shape and white with pink centered colonies (Kelman, 1954). In sterile distilled water the pathogen can be stored for years at room temperature; while long storage in liquid media and the lack of oxygen can induce the loss of virulence characterized by a morphological change of colonies. Virulent colonies appear fluidal, irregular in shape, white with pink center after 36 to 46 hr. of growth on TZC media. Virulent colonies are uniformly round, smaller, and dark red (Kelman, 1954).

All *Ralstonia* species share common physiological properties such as chemoorganotrophic nutrition, aerobic metabolism, absence of fermentation, and absence of photosynthesis, inability to fix nitrogen, and capacity for growth on a large amount of organic substrates.

The *Ralstonia* species can be similar in phylogeny and chemotaxonomic properties, and different in pathogenicity, host relationship and other phenotypic properties (Denny and Hayward, 2001).

2.2.2 Genomic Organization of the Bacteria

In genomic studies the bacterium is found with a bipartite genome structure. Salanoubat et al., (2002) presented the complete genome sequence and an analysis of strain GMI1000. The 5.8- megabase (Mb) genome is organized into two replicons: a 3.7-Mb chromosome and a 2.1-Mb mega plasmid. Both replicons have a mosaic structure providing evidence for the acquisition of genes through horizontal gene transfer. The genome encodes proteins associated with a role in pathogenicity. *R. solanacearum* is a b- 36 proteobacterium belonging to a group of bacteria whose genomic organization is not well characterized yet.

2.2.3 Pathogenicity

Molecular studies concerning bacterial pathogenicity’s factors towards plants have focused on a limited number of bacterial species that represent the taxonomic diversity of principal Gram negative plant pathogens causing

the most common diseases. This bacterium has an unusually wide host range, and offers a unique opportunity for the analysis of virulence factors (Salanouba et al., 2002). *R. solanacearum* has been studied intensively both biochemically and genetically, and has been recognized as a model system for the analysis of pathogenicity. Well adapted to life in soil and water in the absence of host plants, the pathogen provides a good system to investigate functions governing adaptation to ecological niche. Swimming motility also is thought to be the most important contribution to bacterial wilt virulence in the early stage of host plant invasion and colonization (Trans-Kersten et al., 2001).

From the study conducted by Salanoubat et al., in 2002, it is known that a large majority of the genes encoding pathogenicity functions are part of the core genome, being a status in agreement with their base composition and codon usage that fit the general pattern of characteristics of the species. This suggests that pathogenicity is an ancestral trait in *R. solanacearum* with genes that vary from strain to strain (Salanoubat et al., 2002). The phylogenetic analysis based on the distribution of the (type III secretory system) TTSS effector genes revealed an important degree of congruence with the rest of the genome (Guido et al., 2007). This distribution suggests the possibility for these genes to have two different origins: either they are ancestral or ancestrally acquired pathogenicity determinants that follow the same evolution pattern as other genes or they were independently acquired in the different phylotypes during the evolution and were never exchanged between phylotypes (Guido et al., 2007).

2.2.4 Pathogen Biology and Mode of Infection

R. solanacearum is a soil-borne pathogen that naturally infects roots. It exhibits a strong and tissuespecific tropism within the host, specially invading, and highly multiplying in, the xylem vessels. *R. solanacearum Race 3* may have originated in the temperate highland regions of Peru and Bolivia (Van der Wolf and Perombelon, 1997). The term race is based on host range, while biovar is based on biochemical tests. *R. solanacearum* Race 3 biovar 2 is adapted to lower temperatures than what is found for the other *R. solanacearum* races (Van der Wolf and Perombelon, 1997). Highly phytopathogenic, the bacterial cells penetrate the xylem vessels and spread throughout the plant establishing foci of infection (Timms-Wilson et al., 2001); virulence factors that the bacteria use in host colonization are the lytic enzymes, the extra cellular polysaccharides, endoglucanases and endopolygalacturonases are critical factors in colonization and cause the rot and disintegration of the tissue (Timms-Wilson et al., 2001). It is known according to Saile et al., (1997) and confirmed by Timms-Wilson et al., in 2001 that extracellular polysaccharide production contributes to the biomass of colonizing bacteria, causing a rapid wilting of infected plants. Wilting is generally followed by vascular discoloration of roots stems and tubers. When the level of the bacteria becomes high, white ooze can exudates from stem and tubers. According to Van Elsa et al., (2005), "*R. solanacearum* has sophisticated machinery for plant tissue's invasion and presents a capacity to grow at very low substrate concentration as well as to convert to a viable but no cultivable VBNC form at low temperature".

2.2.5 Pathogen Ecology

Race 1 (biovars 1, 3, 4), are pathogenic on potato and on a broad host range and restricted to tropical areas, while Race 3 that has a narrow host range (potato and tomato) and a lower optimum temperature occurs in cool upland areas of tropical regions and warm temperate areas; due to the low temperature optimum, Race 3 or biovar 2 is the causal organism of bacterial wilt on European potato crops (Wenneker et al., 1999).

2.3 Epidemiology

The detection of the *Ralstonia* inoculum source is a very important step to set up an efficient management of the disease, especially early detection in plants or weeds that had no symptoms and serve to spread the disease.

2.3.1 Pathogen Distribution

R. solanacearum is highly heterogeneous species containing hundreds of distinct strains differing in natural host range, geographic distribution, biochemical and genetic characteristics and is found worldwide (Stevenson et al., 2001; Grover et al., 2006). The disease is favored by high temperatures and generally limited to areas without frozen soils, being particularly severe in the tropical and subtropical areas (Agrios, 2005). Poussier et al., (1999) affirmed that *R. solanacearum* is one of the most important diseases in tropical, subtropical and warm temperate regions worldwide and also stated the possibility for the disease to occur in cool temperate areas. This diverse species differs in host range, geographical distribution, pathogenicity, epidemiological relationships, and physiological properties. Analysis of genetic diversity is important for understanding the distribution of strains worldwide and prevention of their further dissemination (Horita et al., 2001). The European strain that has been compared by Timms-Wilson et al., (2001), with strains from other races did show considerable genetic homogeneity with them; that could explain the occurrence of a new bacterial variety adapted to cooler temperatures. The bacteria possess a high degree of genetic variability. This genetic variability further translates to its versatility in exploiting various niches and hosts. In India where biovar 3 and 4 are present on ginger, biovar 3 is more frequent because of its versatility to adapt to varying environmental conditions and its ability to be less influenced by the vagaries of soil edaphic factors (Kumar et al., 2004)

A very high variability of the genome of *R. solanacearum* was observed in field and clonal isolates in the studies done by Grover et al., in 2006. This situation could be the reason of the importance of the host range and the difficulty in breeding sustainable resistance.

2.3.2 Plant Host

R. solanacearum have different host ranges and geographic distributions; race 1, 2, 3, 4 and 5 principally attacks bananas, ornamental planes, potato, Ginger and mulberries respectively (Kelman, 1997). *R. solanacearum* race 4 was found restricted to ginger (Paret, et al, 2010). According to Kumar and Sarma, (2004) race 4 didn't attack banana, potato, tomato, pepper and curcuma and cause wilt on ginger within 5 to 7 days, the causal pathogens were resembling as race 4 based on their specific pathogenicity to *Zingiberaceae* crops (Morita *et al.* 1996, Tsuchiya *et al.* 1999 &2005, Yano *et al.* 2005 & 2011).

2.3.3 Pathogen Survival

Although considered to be a soil borne vascular pathogen, the bacteria is reported to have a poor survival ability in soil, but it can survive on roots of alternate hosts, undecayed infected plant tissues, volunteers tubers from precedent crops or in deeper layer of soil where they do not confront the antagonism from others soil microorganisms (Wenneker et al., 1999). This bacterium may also survive by colonizing the rhizospheres of non-host plants (Wenneker et al., 1999). Several studies done by Wenneker et al., in 1999 to understand the epidemiology addressed the detection of natural infection of *R. solanacearum* of aquatic and riparian weeds.

2.3.4 Pathogen Spread

The pathogen is primarily rhizome-borne and it is believed to be transmitted to many ginger growing areas through latently infected rhizomes and secondary spread within the field and neighboring localities is through rain splashes and run-off water in the field. The pathogen furthermore is easily spread through irrigation water, contaminated tools and other farm implements, through seed or other planting materials and in some instances, by insects and by movement of infested soils (Denny, 2001). According to Deberdt et al., (1999) *R. solanacearum* and nematode populations usually coexists together in tropical and subtropical areas; the nematode feeding wounds produced on roots serve as entry for the bacteria thus the high correlation between the presence of root knot nematode and the level of bacterial infection. The presence of root knot nematodes increases more the disease compared to reniform nematode even in presence of tomato varieties that are normally resistant to bacterial wilt (Deberdt et al., 1999).

2.3. 5. The Disease Symptoms and Signs

R. solanacearum colonizes the xylem, the water-conducting elements of a ginger plant's vascular system, and causes wilt. The symptoms caused by this pathogen on ginger include the following:

- "Green wilt," the diagnostic symptom of the disease. This occurs early in the disease cycle and precedes leaf yellowing. Infected green ginger leaves roll and curl due to water stress caused by bacteria blocking the water-conducting vascular system of the ginger stems.
- Leaf yellowing and necrosis. Leaves of infected plants invariably turn yellow and then necrotic brown. The yellowing, however, should not be confused with another disease of ginger causing similar symptoms known as Fusarium yellows. Plants infected by the fungus *Fusarium oxysporum* f. sp. *zingiberi* do not wilt rapidly, as they do when affect by bacterial wilt. Instead, *Fusarium*-infected ginger plants are stunted and yellowed. The lower leaves dry out over an extended period of time. *E.E. Trujillo (1964) accurately described the progress of disease symptoms for bacterial wilt of ginger:* The first symptoms of wilt are a slight yellowing and wilting of the lower leaves. The wilt progresses upward, affecting the younger leaves, followed by a complete yellowing and browning of the entire shoot. Under conditions favorable for disease development, the entire shoot becomes flaccid and wilts with little or no visible yellowing. However, the plant dries very rapidly and the foliage becomes yellow-brown in 3 to 4 days. Young succulent shoots frequently become soft and completely rotted and these diseased shoots break off easily from the underground rhizome at the soil line. The underground parts are also completely infected. Grayish-brown discoloration of the rhizomes may be localized if the disease is at an early stage of infection, or discoloration may be general if the disease is in an advanced stage. A water-soaked appearance of the central part of the rhizome is common. In advanced infections, the entire rhizome becomes soft and rots. Bacterial wilt of ginger can be distinguished from other rhizome rots of ginger by the condition of the rhizome and the foliage. A better diagnostic feature is the extensive bacterial ooze that shows as slimy, creamy exudate on the surface of a cut made in the rhizome or on the above-ground stem of an infected plant. Disease "signs" refer to the observable presence of a plant pathogen in or on infected host tissues. Signs can be useful in diagnosing the pathogen and the disease. These are signs of bacterial wilt of ginger:
- Bacterial streaming, i.e., large populations of bacteria that exude from the cut surface of infected plant tissue when observed with a microscope or observed macroscopically when a diseased ginger rhizome is suspended in a glass or beaker of water.
- Bacterial ooze from infected tissues, especially from infected rhizomes. Ooze is the emission of

bacterial colonies from infected tissues, seen as moist, milky mounds collecting on the tissues' surfaces.

2.4. Pathogen Detection and Disease Diagnosis

The bacterial wilt pathogen, the disease, or both can be detected using a number of different laboratory methods. These methods test several different sources for the pathogen, including soil, water, and infected plant tissue. The tests may be performed either before or after planting.

2.4.1. Bioassay: - A bioassay consists of exposing ginger plants growing in pots to field soils to see if bacterial wilt disease symptoms develop. Bioassays are useful because they are reliable diagnostic tests, very sensitive, easy to set up, fast, because symptoms develop rapidly, and simple and comprise the following procedures

I. Collect samples of field soil from an area intended for ginger cultivation. Use appropriate soil-sampling methods.

II. Obtain young, tissue-cultured ginger plants. The advantage of using tissue-cultured plants is that they are free from pathogen and have no symptoms

2.4.2. Sign and Symptom Based: - Diagnosing the disease solely by its symptoms is not conclusive; other diseases can cause symptoms that closely resemble bacterial wilt. For a more dependable diagnosis, inspect the rhizomes for signs of the pathogen, bacterial ooze or bacterial streaming, together with the symptoms previously mentioned.

2.4.3. Immunological Tests: -These tests are based on serology, wherein antibodies designed to detect a specific antigen (i.e., the bacterial wilt pathogen) create a visible test reaction. The Immunostrip method (based on the Enzyme-Linked Immunosorbent Assay (ELISA) method) can detect *R. solanacearum* within 30 minutes. The Immunostrip user guide at https:// orders.agdia.com/Documents/m182.pdf describes the testing procedure.

2.4.4. Genetic based characterization /Identification (DNA tests): - Plant, soil or water samples suspected to contain *R. solanacearum* can be subjected to molecular (DNA) analysis for identification or detection purposes. Application of molecular techniques to determine presence or absence of *R. solanacearum*, particularly when there are no clear symptoms or isolation is not guaranteed, may involve working with a suspected material directly to extract DNA. The Polymerase Chain Reaction (PCR) is a test for bacterial wilt based on the selective amplification of the 16S ribosomal rRNA gene from *R. solanacearum* (Seal and Jackson 1991). If a fragment of target DNA is amplified and appears as a band on an electrophoresis gel, *R. solanacearum* was present in the extracted sample. There are also highly discriminatory PCR fingerprinting methods for *R. solanacearum*, ranging from amplification with 8–10 base pair primer (RAPD) to using longer primers that target repeated sequences such as tRNA gene consensus primer or bacterial repetitive elements (Seal *et al. 1992*). Cook *et al.* (1989) have assessed the diversity of the pathogen according to RFLP using *h*ypersensitive *r*esponse and *p*athogenicity (*hrp*) genes as probes. The RFLP technique revealed the presence of two major geographical origins of the strains, viz., American origin consisting of biovars 1 and 2, and Asian origin consisting of biovars 3, 4 and 5. Recently, a study by Poussier *et al.* 1999 using PCR– RFLP of the *Hrp* gene region, AFLP and 16s rRNA sequence analysis allowed identification of the African subdivision.

Versalovic and Lupski (1994) reported REP–PCR fingerprinting utilizes DNA primers complementary to the naturally occurring, highly conserved repetitive DNA sequence present in multiple copies in the genome of most Gram-negative and Gram-positive bacteria. In the study of the diversity of *R. solanacearum* causing bacterial wilt on ginger and other hosts in India, the use of REP-PCR (Repetitive Extragenic Palindromic-polymerize chain reaction) and RFLP-PCR (restriction fragment length polymorphism-polymerize chain reaction) as molecular tool could cluster the highly pathogenic isolate in a cluster at 100% similarity coefficient in conformity with their host origin and biovar (Kumar et al., 2004).

2.5. Host Pathogen Interaction Mechanisms

The *R. solanacearum hrp* (hypersensitive response and pathogenicity) gene cluster is required for virulence on host species and induction of hypersensitive response in nonhosts (Genin *et al.,* 1992). The *hrpB* gene encodes for components of the type III secretion pathway (TTSP) (Van Gijsegem *et aI., 1995).* Inactivation of one of the more than 20 *hrp* genes causes almost complete loss of ability to cause disease and multiply in susceptible plants, as well as loss of the ability to cause hypersensitive defense response in resistant plants (Arlat *et al.,* 1992). Type **III** pathways have a central role in pathogenesis of many bacterial pathogens of plants and animals (Thomas and Finlay, 2003). In *R. solanacearum* Type III secretion requires the production of an Hrp pilus. The *hrpY* gene encodes this structural protein. There is speculation about the possibility of direct protein translocation across the cell wall (Genin and Boucher, 2002). Three other proteins have been shown to be secreted extracellularly, PopA, PopB and PopC, regulated by the HrpB regulator. Mutants of PopA, PopB and PopC, retain their virulence. This might be due to functional redundancy or that they are required to infect plants that have not yet been tested (Gueneron *et at.,* 2000). TTSP is regulated by at least two environmental factors in *R. solanacearum.* The first is detected when bacteria are grown in 'apoplast-mimicking' minimal medium. The second is a specific inducing

signal perceived in the presence of plant cells. *HrpB* and the *TTS* genes were induced in response to physical contact of bacteria with plant cells or cell wall fragments. This contact-dependent induction of *hrpB* gene expression requires the outer membrane protein PrhA, but not a functional TTSP. PrhA a ubiquitous and non-diffusible molecule, located in the *A. thaliana* cell wall, it could possibly aid in the translocation of effector proteins at the appropriate time and place, but this still needs to be investigated (Aldon *et al., 2000).*

2.6. Regulation of virulence and phenotypic conversion

The production of *R. solanacearum* virulence determinants is controlled by a complex regulatory network that responds to multiple signals (Schell, 2000). The central player of the regulatory network is PhcA, (putative outer membrane receptor) involved in the Phc cell density-sensing system. PhcA both activates a set of *hrp* genes and virulence genes *(EPS* biosynthesis, Pme and Egl exoproteins) and represses others (those involved III motility, polygalacturonase and siderophore production, *hrp* genes). The *hrp* and virulence genes are expressed differentially during exponential growth in batch cultures (Clough *et aI., 1996).* An endogenous signal molecule, 3-hydroxypalmitic acid methyl ester (3-0H PAME), controls the levels of active PhcA protein. Only when extracellular 3-0H PAME accumulates above 5 nM (this is at high cell density in the vascular system) repression of PhcA is relieved, resulting in activation/repression of the target genes (Flavier *et aI. ,* 1997). The above-mentioned model distinguished between early virulence factors, when PhcA is inactive in contrast to late virulence factors during high bacterial populations when PhcA is active. Spontaneous or induced mutations in *phcA* results in the pleiotrophic morphological changes called 'phenotype conversion' (PC). PC-type mutants correspond to the spontaneous avirulent mutants described as early as 1954, as a change in colony morphology from mucoid to non-mucoid, resulting in reduced virulence. Spontaneous PC-type mutants are most likely the result of distinct mutational events in *phcA,* some of them being reversible in the presence of a susceptible host. This was the first report illustrating the full cycle of phenotypic conversion/reversion in a plant pathogenic bacterium (Poussier *et aI.,* 2003). Denny *et aI.,* (1994), postulated that *R. solanaeearum* shifts between these two dramatically different physiological states, the PC-type being adapted for saprophytic survival while the wild type is specialized for pathogenesis. However, this phenotypic reversion remains controversial and disputed within the scientific community.

2.7. Breeding Approaches for Ginger Bacterial Wilt Resistance

The conventional breeding programmes in ginger are hampered by the absence of sexual reproduction and failure of seed setting and Ralstonia resistant cultivars so far developed in major producing countries were through introduction, selection and mutation.

2.7.1. Selection

For crop improvement, major emphasis was given for augmentation of germplasm from different localities and indigenous collections, their comparative evaluation and selection of superior types based on yield, quality traits and resistantance to different diseases (bacterial wilt). Need for improved screening techniques to evaluate promising and resistant germplasm of crop plants against bacterial wilt have been emphasized (Hayward, 1991). Such a screening procedure should be rapid, reliable and easy to carry out even in resource-poor laboratories (Mehan et al., 1995).

2.7.2. Mutation breeding

Hybridization in ginger is not feasible due to sterility and improbability of employing conventional breeding, an alternative to induce variability is through physical and chemical mutagens. The specific defects in the commercial cultivar or the incorporation of genetic variability and inducing resistance to common diseases in the materials can be accomplished by mutation breeding. The vegetative propagating nature of the crop may be an additional advantage for the propagation if desirable mutants are recovered (Bhanu 1994).

2.7.3. Biotechnological approaches

Biotechnology can play an important role in ginger improvement. Biotechnological techniques involve micro propagation, soma clonal variants, screening of germplasm against diseases and identification of genetically variable materials and genetic transformation.

Clonal multiplication of ginger from vegetative buds has been reported by many workers (Hosoki and Sagawa, 1977; Nadgauda *et al,* 1980; Nirmal Babu *et al.,* 1997; Sharma and Singh 1997; Rout *et al.,* 2001). Diseases of ginger are often spread through infected seed rhizomes. Tissue culture technique would help in the production of pathogen-free planting material of elite varieties. Field evaluation of tissue cultured plants indicated that it requires at least two crop seasons to develop rhizomes of normal size that can be used as seed rhizomes for commercial cultivation. Molecular characterization of micropropagated plants indicated genetic uniformity (Rout *et al.*1998) as well as certain polymorphism (Nirmal Babu *et al.* 2003). Regeneration of plantlets through callus phase has been reported from leaf, vegetative bud, ovary and anther explants (Nirmal Babu *et al*., 1992, 1995, 1996; Nirmal Babu 1997; Kacker *et al.,* 1993, Rout and Das 1997; Samsudeen 1996, Samsudeen *et al.,* 2001). This system could be used for inducing soma clonal variation as conventional breeding

is hampered by lack of seed set. A few promising high yielding lines with tolerance to bacterial wilt were identified from the soma clones (Samsudeen, 1996; Nirmal Babu *et al*., 1996, Nirmal Babu, 1997). RAPD characterization of these soma clones also indicated profile variations indicating genetic differences (Nirmal Babu *et al.,* 2003). Thus soma clonal variation is an important source of variability and can be exploited for crop improvement programmes in ginger. Germplasm screening of the cell or callus or cell culture and greenhouse/field screening for their insensitivity to toxic metabolites of pathogen. The regenerated plantlets are tested for their reaction with the actual pathogens. Such studies can be ameliorated with multiple resistances for fungal, bacterial nematodes, drought tolerance coupled with quality and productivity. Studies done in India also indicated that different in-vivo and in-vitro screening methods of ginger germplasms were reliable screening methods for bacterial wilt resistance (Kumar 2006). Kulkarni et al. (1984), reported isolation of Ralstonia - tolerant ginger by using culture filtrate as the selecting agent. Babu (1997) and Nirmal Babu et al. (1996a) observed variations in both micro propagated and callus regenerated plants with respect to their tolerance to Ralstonia solanacearum when it was inoculated with 2 rounds of the organism. Isolation of *R. solanacearum* lines were earlier reported in ginger (Kulkarni et al., 1987). In the absence of sexual reproduction candidate gene approach is more suitable for Zinger improvement.

3. Summary and Conclusion

Bacterial wilt disease a serious threat for ginger production and caused by *Ralstonia solanacearum biovar III race 4* and the diseases was found distributed in major ginger growing areas of Ethiopia. Detail knowledge on biology, ecology, epidemiology and identification /diagnosis procedures of the pathogen and host-pathogen interaction mechanisms were very important in order to design cost effective disease breeding and management strategies of the bacteria wilt disease in ginger. Conventional crop improvement methods for bacterial wilt resistance are not suitable in ginger, because of lack of seed set setting leading to limited variability. Under these conditions tissue culture (multiplication of diseases free ginger seed rhizome production scheme and utilization of tissue culture created variations) and biotechnological tools play very important role in large scale production of disease free planting material which can be introduced to newer areas. Hence, Germplasm introduction, selection or screening and the cloning of resistance genes (candidate gene) and subsequent transformation is the only solution to obtain disease resistant ginger cultivars.

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