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First Report of Ginger (*Zingiber officinale*) Bacterial Wilt Disease in Ethiopia

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Abstract

Very recently a severe outbreak of ginger wilt disease was reported by agricultural bureau experts in Ethiopia, particularly in the Southern Nations, Nationalities and People and Gambela Regional States. Based on the report, continuous survey was conducted during the 2011/12, 2012/13 and 2013/14 to investigate the status of the disease, isolate and identify the causal pathogen. During the survey, about 1530 hectares of ginger plantation farms were visited and investigated. Disease incidence was estimated to 80-100%. In the field, disease symptoms like wilting of the lower leaves and a slight yellowing with upward progress of the wilt affecting the younger leaves followed by a complete yellowing and browning of the entire shoot was observed. Shoots become soft and completely rotted and break off easily from the underground rhizome. The entire shoot becomes flaccid and dries. When cross-section cut of a diseased rhizome was placed in a water-filled clear beaker, milky white bacterial streaming was evident. Isolations from collected diseased ginger plant samples on 2,3,5-triphenyl tetrazolium chloride (TZC) showed development of a bacterium characterized by fluidal, irregular and creamy white with pinkish red colonies. Using some biochemical tests and Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay, all bacterial isolates were identified as Ralstonia solanacearum biovar III indicating that the disease is ginger bacterial wilt disease. Ginger wilt disease by Ralstonia solanacearum is well known as a limiting factor in the production of ginger elsewhere in the world, but this is the first report of an occurrence of ginger bacterial wilt disease in Ethiopia.

Keywords: Ginger, *Ralstonia solanacearum,* Ethiopia.

Introduction

Ginger (*Zingiber officinale* Roscoe) is a slender herbaceous perennial herb belonging to *Zingiberaceae* family and propagated by rhizomes. The *Zingiberaceae* family is a tropical group consisting of more than 1200 plant species in 53 genera. The genus *Zingiber* includes about 85 species of aromatic herbs. Edible ginger (*Zingiber officinale*) is one of the earliest known oriental spices and is being cultivated widely both as a fresh vegetable and dried spice.

The when and how of ginger introduction to Ethiopia is not exactly known. It is assumed that, in the 13th Century, the Arabs brought ginger from India to East Africa and ginger has perhaps been known since then in Ethiopia[1]. In the contrary, another study reported that ginger cultivation and utilization was very little even around 1913[1]. However, ginger is now well known in Ethiopia as fresh or dry rhizomes for sale on most markets. It is mainly grown in the south and Southwestern part of the country with high land coverage and farmers percentage in the cultivation (Table-1 and 2).

In different parts of the world, like most cultivated crops, ginger is affected by biotic and abiotic factors. In most ginger producing countries like India, ginger is affected by different pathogens. Bacterial wilt (*Ralstonia solanacearum*), Pythium soft rot/rhizome rot, Leaf spot (*Phyllostica zingiberi*) and Nematodes were commonly known to cause disease on ginger crop. Ginger bacterial wilt disease caused by *Ralstonia solanacearum* is known as most limiting factor in Hawaii[3]. In Ethiopia, in the history of spice research achievements, no disease prevalence's have been recorded on ginger. But very recently, in 2011/12 and 2012/13 cropping season regional plant health clinics have reported that ginger crop is being infected by unusual disease. Based on such regional reports, survey was conducted during the 2011/12, 2012/13 and 2013/14 cropping seasons in the Southern Nations, Nationalities and People and Gambela Regional States to investigate the status and causal agent of the recently occurred ginger disease.

Materials and Methods

Sample collection: During the survey, about 1530 hectares of ginger plantation farms were visited, critically observed and disease symptoms were seriously noted. Based on the observations, a total of 260 representative diseased plant samples were randomly collected for further laboratory diagnosis and characterization.

Isolation: Bacterial suspension from crushed diseased rhizomes and bacterial exudates were streak-plated on pre-dried surfaces of 2,3,5-triphenyl tetrazolium chloride (TZC) plates[4]. In addition, the inside of infected rhizome was cut into small pieces and directly placed on pre-dried surfaces of TTC plates. All plates were incubated at 30 to 32°C for 48 h. The isolates were further purified by repeated plating for further biochemical tests.

Table-1
Ginger area, production and productivity in southern Ethiopia

Administrative zone	Woreda	Area (ha)	Production (qt*)	Yield (qt/ha)
Hadiya	West Wadawacho	423	67680	160
Walaita	Boloso Bombe	7000	1120000	160
Kambata-Tambaro	Hadaro-Tunto, Qacha Bira	8223	1308652	159
Dawro	Gena Bosa	1500	225000	150
Kafa	Gimbo	55	8800	160
Shaka		379	60640	160
Bench Maji	Sheko	187	29920	160
Konta		473	75680	160
Total		**18240**	**2896372**	

Table-2
Extent of ginger cultivating weredas of SNNPRS

Woreda	Total area production (ha)	Ginger producing Farmers (%)	Total production (qt*)
Hadaro-Tunto	3986	80	637760
Boloso Bombe	5000	85	600000

Source [2] *qt= kuntal=100 kg

Biochemical Characterization: The purified bacterial isolates were further characterized using different biochemical tests.

Lipopolysaccharide (KOH) test: A bacterial colony was aseptically picked from the surface of a growing plate with an inoculating wire loop, placed and stirred for 5-10 seconds on glass slide in a drop of 3% KOH solution and formation of viscous and slime thread following the loop was observed[5].

Catalase test: The purified colonies were flooded with a 3% solution of hydrogen peroxide (H_2O_2) on a glass slide and observed for the production of gas bubbles with naked eye[6].

Levan production from sucrose: bacterial isolates were streaked on nutrient agar plates supplemented with 5% sucrose. Plates were then incubated at 28°C for 48 hrs. Separate, discrete colonies were observed for the Levan production with naked eye[7].

Cytochrome oxidase test: A small loopful of bacterial colony was rubbed on a filter paper with drops of 1X (W/V) aqueous N, N, N, N-tetramethyl-p-Phenylenediamine dihydrochloride solution. Then after, formation of a blue or deep purple color within 30 seconds was noted[6].

Starch hydrolysis: A loopful of bacterial suspension was streak plated on starch agar and incubated at 32°C for 48 h. Starch agar contained (g/l of distilled water) casein peptone, 5.0; meat extract, 3.0; NaCl, 5.0; soluble starch, 5.0; and agar, 12.0; pH, 7.0. The plate was flooded with dilute Lugol's iodine solution for 30 seconds. Clear zones around colonies indicated amylase enzyme production.

Serological diagnosis: Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay (NCM-ELISA) kit developed at International Potato Center (CIP), Lima, Peru, for detecting potato strain of *R. solanacearum* was found sensitive enough to detect *R. solanacearum* from ginger[8]. To serologically detect all the isolates, serological diagnosis was conducted using NCM-ELISA kit developed at International Potato Center (CIP), Lima, Peru following procedures stated in CIP-NCM-ELISA instruction manual[9]. The serological detection was performed using pure cultures inoculated into nutrient broth and incubated at 30 to 32°C for 24 h. The growth was checked just by looking for the turbidity/cloudiness and 20 µl of it was carefully added on the center of the membrane.

Biovar determination: The isolates were differentiated into biovars based on their ability to utilize disaccharides (sucrose,

lactose, and maltose) and sugar alcohols (manitol, sorbitol and dulcitol)[10]. The medium ($NH_4H_2PO_4$, 1.0 g, KCl, 0.2 g, $MgSO_4$.$7H_2O$, 0.2 g, peptone, 1.0 g, bromothymol blue, 0.03 g, Agar, 3.0 g) with its final pH adjusted to 7.0-7.1 (an olivaceous green color) was prepared by autoclaving at 121^0C for 20 minutes and cooled to 60°C. A ten percent solution of the disaccharides maltose, cellobiose, lactose, and the hexose alcohols mannitol, sorbitol and dulcitol was prepared in 10 ml amount. The disaccharides was sterilized by filtration using 0.22 mm micron Millipore membrane and the hexose alcohol was autoclaved at 110^0C for 20 minutes. A ten percent solution of each of the sugars was added (10 ml) and mixed in the basal medium cooled to 60^0C. Ten milliliter solution was poured into pre-sterilized labeled tubes, allowed to solidify at room temperature and then inoculated with the bacterial suspensions (10^8 cfu/ml) prepared from two-day-old cultures on TZC plates. Test tubes were set in duplicate, incubated at 30^0C and examined after 3, 7 and 14 days for change to orange color (acid pH) from the surface of the medium to downward.

Results and Discussion

Field observation: In the field, disease symptoms like wilting of the lower leaves and a slight yellowing was observed. The wilt progresses upward, affecting the younger leaves, followed by a complete yellowing and browning of the entire shoot, and the entire shoot becomes flaccid and dries concurrently (Figure-1). Shoots become soft and completely rotted and break off easily from the underground rhizome.

The underground rhizomes are also completely infected and produce petrifying smell shortly after about a day of harvesting. A rhizome cross-section cut, placed in a water-filled clear beaker with the end of the section just touching the water surface showed milky white bacterial streaming (Figure-2) distinguishing bacterial wilt from vascular wilts caused by fungal pathogens. These disease symptoms in the field and bacterial streaming from the cut surface of infected rhizome suspended in a beaker of water revealed that the disease is caused by bacteria.

Figure-1
Ginger bacterial wilt showing progressive disease symptoms, normal (a), wilting and yellowing (b) and complete death of the plant (c)

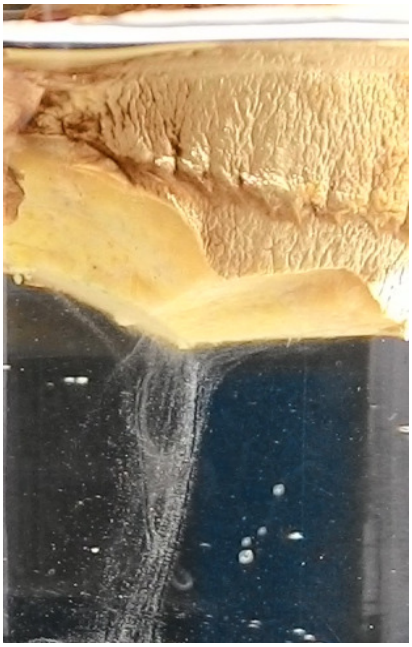

Figure-2
Milky white bacterial streaming from diseased rhizome suspended in water

Isolation: Isolations from collected diseased ginger plant samples on 2,3,5-triphenyl tetrazolium chloride (TZC) showed development of a bacterium characterized by fluidal, irregular and creamy white with pinkish red colonies (Figure-3).

Biochemical characterization of the isolates: Lipopolysaccharide (KOH) test: all the isolates revealed formation of viscous and slime thread when loop raised from the bacterial solution indicating that all isolates are gram negative.

Catalase test: all the isolates tested produced gas bubbles when mixed with a drop of H_2O_2 on glass slide.

Levan production from sucrose: all the tested isolates were negative for levan production.

Cytochrome oxidase test: The results of cytochrome oxidase test showed that all of the isolates were able to develop deep blue color with oxidase reagent.

Starch hydrolysis: all the isolates do not hydrolyze starch when streaked on starch agar.

All morphological and cultural characteristics of the isolates confirmed *Ralstonia solanacearum* and this was confirmed by serological test using NCM-ELISA.

Serological diagnosis: The results of the NCM-ELISA showed that all the isolates reacted positively confirming that the isolates are *R. solanacearum* (Figure-4).

Biovar Differentiation: The result of the biovar test showed that all the isolates oxidized disaccharides (sucrose, lactose, and maltose) and sugar alcohols (manitol, sorbitol and dulcitol) within 3-5 days indicating that all isolates belong to biovar III of *R. solanacearum.* It has been known that, biovar III of *R. solanacearum* oxidizes both disaccharides and hexose alcohols[8,,11,12] .

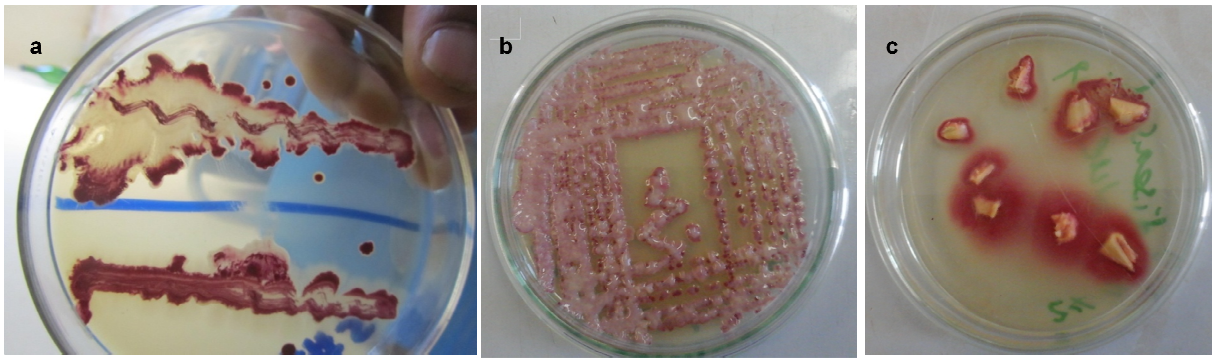

Figure-3
Pure culture on TZC agar isolated from diseased ginger (a, b) and pieces of infected rhizome on TTC plate (c)

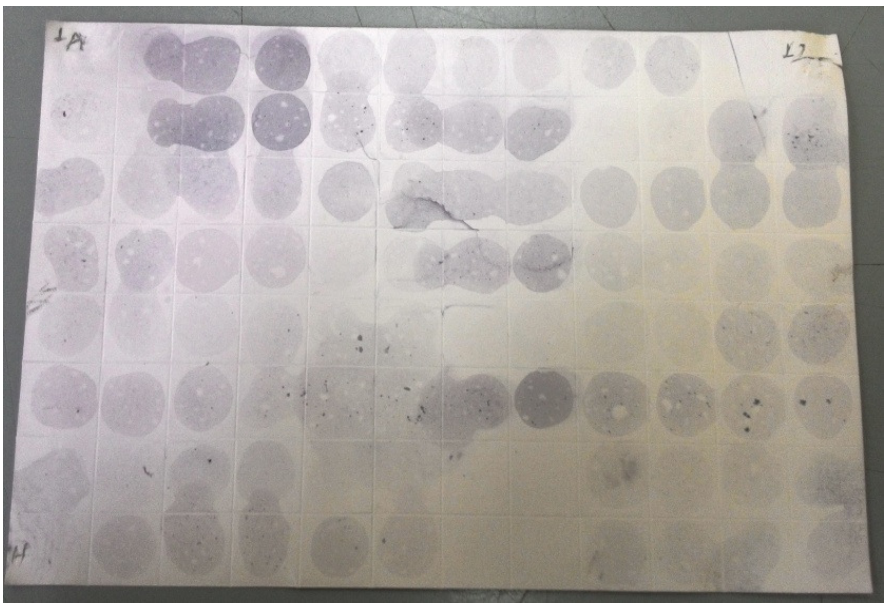

Figure-4
NCM-ELISA of different isolates from diseased ginger samples

Conclusion

In the present work, we have performed field survey, observation and laboratory diagnosis to investigate the status, isolate and identify the causal pathogen of ginger disease recently occurred in the country. Observations of disease symptoms in the field, bacterial streaming from the cut surface of infected rhizome suspended in a beaker of water, characteristic growth of fluidal, irregular and creamy white with pinkish red colonies on TZC medium revealed that the disease is bacterial wilt disease. Using some biochemical tests and Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay, all bacterial isolates were identified as *Ralstonia solanacearum* biovar III. Though no one is exactly sure how and when this disease is introduced into the country, ginger wilt disease by *Ralstonia solanacearum* is well known as a limiting factor in the production of ginger elsewhere in the world[13] . However, this is the first report of an occurrence of ginger bacterial wilt disease in Ethiopia.

Acknowledgment

NCM-ELISA kit supplied by CIP Ethiopia office is gratefully acknowledged.

References

1. Jansen P.C.M. (1981). Spices, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. Centre for Agricultural Publishing and Documentation Wageningen. (xii) + 327.

2. Endrias Geta and Asfaw Kifle. (2011). Production, processing and marketing of ginger in Southern Ethiopia. *Journal of Horticulture and Forestry*, 3(7), 207-213.

3. Paul Hepperly, Francis Zee, Russell Kai, Claire Arakawa, Mark Meisner, Bernard Kratky, Kert Hamamoto and Dwight Sato. (2004). Producing Bacterial Wilt–Free Ginger in Greenhouse Culture. *Soil and Crop Management,* SCM-8, 1-6.

4. Hugh R. and Leifson E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various negative bacteria. *J Bacteriol*. 66, 24-26.

5. Gregersen T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur. J. Appl. Microbiol*. 5, 123-127.

6. Bisen P.S. and Verma K. (1994). Hand book of Microbilogy. CBS Publisher and Distributors, New Delhi, India, 1-199. ISBN: 81-239-0269-7.

7. Schaad N.W. (1980). Laboratory guide for the identification of plant pathogenic bacteria. *Am. Phytopathol*. Soc. St. Paul. Minn. 28-45.

8. Kumar A., Sarma Y.R. and Priou S. (2002). Detection of Ralstonia solanacearum in ginger rhizomes using post-enrichment NCM-ELISA. *Journal of Spices and Aromatic Crops*, 11(1), 35-40.

9. International Potato Center (CIP). (2001). CIP-NCM-ELISA kit for the detection of *Ralstonia solanacearum* in potato. Instruction Manual.

10. He L.Y., Sequeira L. and Kelman A. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China, *Plant Disease, 67*, 1357-1361. http://dx.doi.org/10.1094/PD-67-1357

11. Kumar V., Singh B.M. and Sugha S.K. (1993). Variation in isolates of *Pseudomonas solanacearum* from Himachal Pradesh. *Indian. Journal of Mycology and Plant Pathology, 23*, 232-236.

12. Hayward A.C. (1964). Characteristics of *Pseudomonas solanacearum. J. Appl. Bacteriol*., 27, 265-277.

13. Sustainable Agriculture Research and Education (SARE) (2012). Control of Bacterial Wilt disease of Ginger through an Integrated Pest Management Program. 2012 Annual Report.