International Journal of Research in Engineering, Science and Management
Volume 7, Issue 7, July 2024
https://www.ijresm.com | ISSN (Online): 2581-5792

Occurrence and Distribution of Potato Bacterial Wilt Disease and Variability of its Causal Agent in Southern Highlands of Tanzania

Zera Mwankemwa*
Research Officer, Tanzania Official Seed Certification Institute, Morogoro, Tanzania

***Abstract*: A survey was carried out in three potato growing Districts of the Southern Highland Zone of Tanzania to determine incidence and severity of bacterial wilt of potato caused by *Ralstonia solanacearum* and characterizing the strains of the causing pathogen. Ten villages from each district and five fields from each village were surveyed. The fields and plants were randomly selected and bacterial wilt symptoms identified. Wilt incidence was recorded based on number of plants showing symptoms and expressed as percentage of the total number of plants observed. Disease severity was recorded by severity score as described by Horita and Tsuchiya (2001). Asymptomatic and symptomatic tubers samples were collected. Asymptomatic for the detection of latent infection whereas symptomatic for characterization of *R. solanacearum* respectively. The results showed the highest incidence in Mbeya Rural (27.7%) followed by Rungwe (26.7%) and lastly Njombe (19.4%). The highest and lowest bacterial wilt severities recorded were 4.0 and 2.3 both in Mbeya rural fields. Asymptomatic tubers were (18.8%) latently infected after being tested by NCM-ELISA. Molecular characterization confirmed the isolates to be *R. solanacearum* by species specific and grouped to phylotype 2 by phylotype specific PCR respectively. Upon isolation in TZC medium twenty of thirty isolates were virulent whereas ten were avirulent. Pathogenicity test showed the isolates to be highly virulent on potato and tomato and slightly virulent on eggplant. No symptoms in tobacco and pepper were produced and therefore grouped as race 3. The isolates were grouped to biovar 2 as they oxidized the tested carbohydrates but not the sugar alcohols. The disease was observed to occur in all three surveyed districts. Use of certified and disease-free seeds, area for seed production, development of resistant varieties and training on symptoms and bacterial wilt control were recommended.**

***Keywords*: bacterial wilt, disease, distribution, detection, occurrence, variability.**

1. Introduction

Potato (*Solanum tuberosum* L.) is the main root and tuber crop and the third most important food crop in the world after rice and wheat. It is grown in over 125 countries and is consumed by over a billion people (CIP, 2008). Tanzania produces about 504,000 tons annually and most of which comes from the Southern Highlands of Tanzania (URT, 2007). Potatoes in Tanzania are essentially a food crop with growing domestic urban demand. Ninety percent of the national crop is grown in the Southern Highlands and predominantly around Mbeya and Njombe. Most of the crop produced in Tanzania is consumed as food at household level and through food service in outlets such as restaurants, street and food vendors. The crop can serve both as food as well as income to the rural population (Kadigi *et al.,* 2012).

Studies on potato have shown that production and consumption of the crop is increasingly becoming popular (Anderson, 1996; Namwata *et al*., 2010). Round potato is grown mainly by small scale farmers as a cash and food crop and therefore plays an important role in food security and creating employment.

Despite its importance the crop has not achieved its full potential because of a number of production constraints; including low soil fertility, inadequate supply of certified seeds, use of low yielding varieties and infection by diseases. The common diseases include late blight, bacterial wilt and viral infections (Kaguongo *et al*., 2008).

Bacterial wilt disease caused by *Ralstonia solanacearum* (Smith, 1896) is the second most important potato disease in tropical and sub-tropical regions of the world after late blight (Champoiseau *et al*., 2010). The disease is also known to affects over 200 plant species from more than 50 families (Hayward, 1993). It is widely distributed in tropical, sub-tropical and warm temperate climates of the world. In addition to potatoes, the disease also affects over 200 plant species from more than 50 families (Hayward, 1991).

Bacterial wilt occurs in about 45 countries in the southern hemisphere. In Africa, it is found in Angola, Burkina Faso, Burundi, Cameroon, Congo, Ethiopia, Gabon, Gambia, Kenya, Madagascar, Malawi, Mauritius, Mozambique, Nigeria, Rwanda, Senegal, Seychelles, Sierra-Leone, Somalia, South-Africa, Swaziland, Tanzania, Tunisia, Uganda, Zaire, Zambia and Zimbabwe (EPPO, 2004).

2. Material and Methods

A. Incidence and severity of potato bacterial wilt disease in cultivars grown in Southern Highlands of Tanzania

1) Location and sampling

The survey was conducted in potato growing season in

*Corresponding author: zmwanks@yahoo.com

Mbeya rural, Njombe and Rungwe districts of Southern highlands of Tanzania. Sampling was done at random in 5 farms per village. Ten (10) villages were surveyed in each district, the distance between one field and another was 3-5 km. Most of the potato fields surveyed were at flowering. In each field 10 rows of about 100 plants were selected randomly, bacterial wilt symptoms were identified by visual observation of typical bacterial wilt disease symptoms such as wilting, vascular discoloration, bacterial streaming in glass of water and browning of the vascular bundles of the tuber. Bacterial wilt incidence was recorded based on number of plants showing symptoms and were expressed as % of the total number of plants observed. Disease severity was done by recording on severity score as described by Horita and Tsuchiya (2001) as 1= no symptoms, 2= top young leaves wilted, 3= two leaves wilted, 4= 4 or more leaves wilted and 5= plant died. During the survey field altitudes and their corresponding geographical position were recorded. Two (2) samples were collected in each farm, one comprised of 25 healthy looking tubers and the other of 4 diseased tubers, packed in paper bags and labeled in each farm for further tests.

B. Characterizing races of Ralstonia solanacearum occurring in the Southern Highlands of Tanzania

1) Isolation of the bacteria and cultures

Infected potato tubers collected from different locations were washed with running tap water to remove soils and any dirty materials and then they were then dipped in 70% alcohol for 5 minutes for surface sterilization and were dried by tissue paper. The tubers were cut into half by a sterile knife, vascular ring was removed from the tuber by scoop and was then placed in test tubes containing 5 ml of sterile water. Bacteria were allowed to flow from the vascular bundles for 5 to 10 minutes. One loopful of bacterial suspension was streaked into 2, 3, 5 Triphenyl Tetrazolium Chloride (TZC) agar medium and incubated at 28°C for 48 h. Single colony of R. solanacearum showing virulent, fluidal, irregular and creamy white with pinkish at the centre was picked from the TZC Petri-plates and streaked to Casamino acid, Peptone and Glucose (CPG) medium and were incubated for 48 h. Virulent cultures were maintained in distilled water in screw taped tubes at room temperature after 48 hrs. of incubation

2) Pathogenicity test

Virulent strains were inoculated on 5 host plants namely, potato, tomato, eggplant, pepper and tobacco. Host plants were planted in pots containing soil, sand and compost mixture (1:1:1) treated with formalin, and kept in screen house until they attained a height of 15-20 cm. Five plants of each host were injected with 100 µl of bacterial suspension by inserting a sterile micropipette tip at the axil of fully expanded leaf from the top. The micropipette tips were left in a position until all inoculum was absorbed. Inoculated plants were observed daily for pathogenicity and severity. Disease severity was assessed by a scale of 1- 5 described by He *et al*. (1983) where 1= no symptom, 2= two leaves wilted, 3 = three leaves wilted, 4= four or more leaves wilted and 5= plant died.

C. Biochemical characterization of the pathogen

1) Gram staining

A loop full of bacterium was picked from maintained virulent cultures and was spread on a glass side and fixed by heating on a very slow flame. Aqueous crystal violet solution of 0.5 % was then spread over the smear for 30 seconds, and then washed with running tape water for a minute. Iodine (95%) was then flooded for a minute followed by rinsing with tap water. Then the slides were decolorized with 95% ethanol until colorless runoff. The slides were then counter stained with safranin for 10 seconds and washed with water. The slides were dried under the laminar flow cabinet and placed under the light microscope at 10X, 40X and 100X for observation using oil (Schaad, 1980).

2) Potassium hydroxide test

Bacteria were picked from petri-plates by wire loop and placed on glass side containing a drop of 3% KOH solution, stirred for 10 seconds and observed for the formation of slime threads (Suslow *et al.,* 1982)

3) Catalase oxidase test

A loop full of bacterial culture obtained from young agar cultures of 18-24 h were mixed with a 3% hydrogen peroxide (H_2O_2) on a glass slide to observe production of gas bubbles with a naked eye and under a dissecting magnification of 25X (Schaad, 1988).

4) Kovacs oxidase test

Oxidase reagent (1% tetra-methyl-p-phenyl diamine dihydrochloride) solution of 100ml was prepared and kept in rubber stopper dark bottle. A drop of reagent was added to a piece of filter paper placed within a glass Petri dish. Small quantity of inoculum was rubbed on the filter paper containing oxidase reagent solution. Bacteria were then observed for the development of purple color in 10-60 seconds.

5) Oxidation of glucose

Basal medium constituents were dissolved as described by Hayward (1964). The pH was adjusted to 7.0 to 7.1 and the medium was prepared and then for identification of acid production from sugars by aerobic, Gram-negative bacteria. The contents of the medium were Difco Bacto agar (0.3 g) which was mixed with 100 ml quantities of the basal medium. Glucose (1 g) was added into each Pyrex screw capped tubes containing 10 ml sterile distilled water. The solutions were heated to 100°C for 30 minutes for sterility. Semi solid basal medium in bottles were melted in microwave oven and cooled to 60- 70°C. 10 ml of 1% glucose was mixed in the basal media by rotation and 5 ml were dispensed into each sterile screw capped tubes. The medium was allowed to solidify at room temperature.

Inoculum from bacterial cultures was picked by sterile wire loop and was stab inoculated onto the tubes. Half of the tubes were sealed with 3 ml molten sterile

Difco Bacto agar cooled to 45°C. Sealed and unsealed tubes were then incubated at 28°C and examined after 1, 2 and 7 days for gradual pH change at the surface of the open tube.

D. Biovar Determination

Biovar test was done as follows; mineral medium was prepared in a litre of water as follows; $NH_4H_2PO_4$ 1.0 g, KCl

0.2 g, $MgSO_4.7H_20$ 0.2 g, Difco Bacto peptone 1.0 g, Agar 3.0 g, Bromothymol blue 80.00 mg. The pH was adjusted to 7.0-7.1 (an olivaceous green colour) by drop- wise addition of 40 % Sodium Hydroxide solution. The medium was heated to melt the agar, autoclaved at 121°C for 20 minutes and cooled to 60°C.

Ten millilitres (10 ml) of each 10 % lactose, maltose, cellobiose, mannitol, Sorbitol and dulcitol were added in sterilized screw caped tubes and they were heated to 100 °C for 30 minutes to sterilize the solutions. Bottles of semi-solid basal medium were melted in water bath and cooled to 70°C. Ten millilitres (10 ml) of carbohydrate solutions were added into 90 ml of media. About 200 µl of the melted medium was dispensed into the wells of microtitre plate. The media was then allowed to solidify under room temperature. Inoculum of each isolate was prepared by adding 2 loopful of bacteria from 48 h old TZC cultures to distilled water to make suspension containing about 10^8 c.f.u/ml, then 20 µl of bacterial suspension was added to the wells of the microtiter plate. Control was kept by adding 10 ml of distilled water instead of sugar solution. The plates were incubated at 28°C and examined 3-5 days for pH color change from olivaceous green to yellow. (Schaad, 1988).

E. Serological Detection of the Isolates

Samples of healthy tubers from the fields consisting of 25 tubers from each field were tested using procedures outlined in CIP, NCM-ELISA (Enzyme Linked Immunosorbent Assay on Nitrocellulose Membrane) protocol for detection of *R. solanacearum* in potato (Priou, 2001). The test consisted of sample preparation, loading a very small amount of plant extract (20 µl) on a nitrocellulose membrane (Dot blotting), blocking the area of the membrane that was free of samples, binding the samples with *R. solanacearum* (*Rs*) with specific rabbit antibodies, binding the *Rs*-antibodies complex with enzyme-labeled goat anti-rabbit antibodies and revealing the bound enzymes by adding the substrate leading to coloration reaction.

F. Molecular Characterization of the Isolates

Positive samples of tubers that were infected by *R. solanacearum* that were detected by NCM-ELISA were subjected to molecular characterization to species specific PCR to confirm if they were really *R. solanacearum*. The procedures used for molecular characterization are as indicated below;

1) DNA Extraction

Bacterial DNA was extracted as follows; two tubers per sample were washed under running water and air dried for an hour. The skin was removed at the heel end of the tuber with a clean disinfected vegetable knife so that the vascular tissues become visible. A small conical core (3-5 mm diameter) of vascular tissue at the heel end was carefully cut out. A sample of a heel end was crushed with the help of pestle and mortar and collected in a sterile Falcon tube containing 5 ml of double distilled water The content was allowed to stand for 30 min. The supernatant was then centrifuged for 2 min in 1.2 ml microfuge tube. The pellets were resuspended in a 500 µl tube by vortexing. Then 30 µl of Sodium Dodecyl Sulphate (SDS) and 3 µl of 20 mg/ml Proteinase K was added. The mixture was vortexed and incubated at 35°C for one hour. One hundred microlitre (100 µl) of 5M CTAB/NaCl solution was added and the mixture was incubated at 65°C for 10 min. Phenol, chloroform and isoamyl was added in the ratio (25:24:1) and the tubes were centrifuged for 5 min. The supernatants were then transferred to new microfuge tubes and 0.6 volume of Isopropanol was added. Centrifugation was again done for 5 min at 16000 g. The supernatant was then discarded and the DNA pellets were resuspended in 100 µl of 1X T.E. buffer. (Grover *et al*., 2012).

2) Species specific PCR

Species specific PCR was done by using a Random Amplified Polymorphic DNA (RAPD) Primer set AU 759/760. Master mix (readymade) from Thermo Scientific containing 0.4 Mm of dATP, dCTP, dGTP, dTTP, 4 Mm M_gCl_2 and 5 U/µl Taq Polymerase was used. Primer concentration in the master mix was 0.1 Mm. One µl of 10 ngµl^{-1} DNA The reaction volume was 25 µl.

The PCR conditions were set as one cycle of 94°C for 3 min., 53°C for 1 min. and 72°C for 1.30 min., followed by 30 cycles of 94°C for 15 s, 72°C for 15 s, one cycle of 72°C for 5 min and held at 4°C. Gel electrophoresis was done for 40 minutes in which 1.2% Agarose gel in TAE buffer stained with gel red was prepared and the samples were run against 1 kb DNA ladder for 45 min.

3) Phylotype determination

Phylo-typing was determined by Multiplex PCR (pmx PCR). The reaction involved four forward primers, one reverse primer and a species-specific primer. The four forward primers involved were Nmult21:1F, Nmult21:2F, Nmult23: AF, and Nmult22:1nF, one reverse primer used was Nmult 22: RR and species-specific primer pairs AU 759/760. The reaction mixture contained 6 p moles of the forward primers, 12 p moles of the reverse primer, 4 p moles of species-specific primer, 12.5 µl of Thermoscientific Taq Green Mastermix and 1 µl of 20 ngµl^{-} DNA templates making a total 25 µl volume of reaction mixture. The PCR conditions were set as one cycle of 96°C for 5 min., 59°C for 30 s and 72°C for 30 s. The final extension was done at 72°C for 10 min and samples were held at 4°C. For 45 minutes Agarose gel electrophoresis was done in which 1% of Agarose gel was prepared in TAE buffer stained with gel red and the samples were run against a 1 kb plus DNA ladder.

3. Results and Discussion

A. Bacterial Wilt Disease Incidence and Severity

Bacterial wilt disease of potato was found in all districts surveyed but not in all fields (Fig. 2 and Table 1). Common bacterial wilt symptoms observed in the fields were wilting of one side of a leaf and/ or stem, general whole plant wilting and brown discoloration of the vascular system in young stems (Fig. 3a and b). The infested tubers released the bacteria on their eyes, and showed a browning and a death of the vascular ring and the immediate surrounding tissues when cut (Fig. 3c –f). Wilting was the most obvious symptom in the field. Such conditions are a result of restricted water movement due to the

formation of slime that surrounds the bacterial masses in the xylem vessels (Martin and French, 1985). The survey results showed that the highest (27.7%) bacterial wilt incidence was observed in Mbeya Rural followed by Rungwe (26.6%) and Njombe district (19.4%) (Table 1). In terms of villages, bacterial wilt incidence ranged from (0- 84 %). The highest (84 %) disease incidence was observed in Lukata B village in Rungwe where all 5 fields assessed had typical symptoms of bacterial wilt infection. The incidences of bacterial wilt disease in all other fields were consistently ≤ 60 % (apparent infection). The bacterial wilt incidences in different districts were: 0-84 % (n =50, Rungwe), 0-60 % (n = 50; Njombe), and 0 – 60 % (n = 50; Mbeya rural (Table 1). Bacterial wilt disease occurred in all districts. Statistical analysis revealed that the means of incidence of bacterial wilt disease in the districts surveyed were not statistically significant different (Table 1).

Table 1
Disease incidence and severity in Southern Highlands Zone Tanzania

District	Village	Disease incidence (%) per village	Disease severity per village
Rungwe	Ndaga	15.2	3.92
	Malangali	15.2	3.52
	Ishinga	11.6	2.74
	Isebelo	28.8	3.14
	Ntokela	19.2	3.44
	Swaya	20.4	2.46
	Mbeye one	33.6	3.52
	Lukata A	17.6	2.58
	Lukata b	84.0	3.70
	Ushirika	22.0	3.22
Mean		26.7	3.22
Njombe	Welela	9.6	2.14
	Mtwango	26.8	3.08
	Kichiwa	21.2	3.10
	Lunguya	16.0	2.78
	Ilunda	25.6	2.5
	Ulembwe	35.2	3.34
	Usalule	31.2	3.26
	Igeri	0.00	0.00
	Magoda	18.8	3.02
	Luponde	9.2	2.8
Mean		19.4	2.60
Mbeya Rural	Itizi	8.4	2.7
	Sanje	40.8	3.66
	Jojo	48	2.82
	Santilya	15.2	2.34
	Idimi	34.4	2.82
	Haporoto	32	3.3
	Ifiga	22	3.1
	Hatwelo	25.6	3.3
	Simambwe	26	2.9
	Usoha	24.4	4.0
Mean		27.7	3.10
	$LSD_{0.05}$	13.97	

The mean severity scores for bacterial wilt disease symptom based on the scale by Horita and Tsuchiya (2001) ranged from 2 to 4.8. The lowest mean severity score was 2.0 in all the districts. The results show that bacterial wilt is indeed present in the surveyed areas however with a district variation in disease incidence and severity. Differences of wilt incidence and severity is due to great diversity of host plants affected by the pathogen, phenotype, genotype, its wide geographical distribution and the range of environmental conditions conducive to bacterial wilt.

(Chalterfee *et al*., 1997). Severity of the disease depends upon soil temperature, moisture, soil type, host susceptibility and virulence of strains. (Momol, 2003**)**.

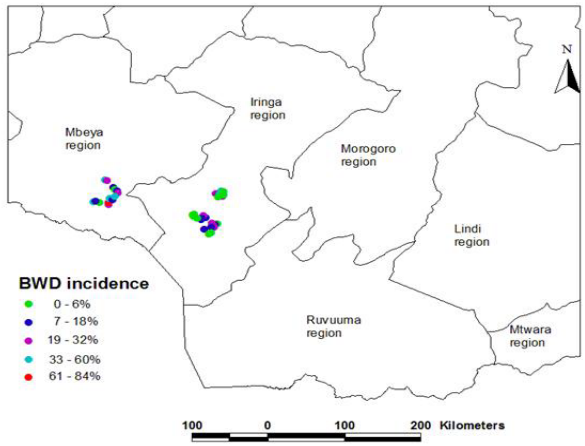

Fig. 1. A map of showing some regions of Tanzania indicating incidence and distribution of bacterial wilt disease in Mbeya and Njombe regions

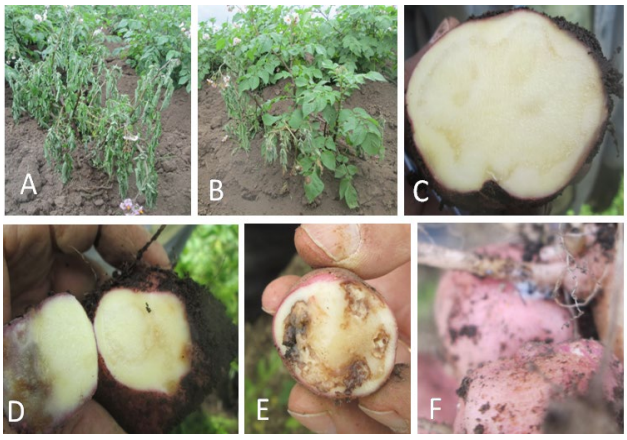

Fig. 2. Bacterial wilt and brown rot symptoms caused by *R. solanacearum* on potato plants in the Southern Highlands Zone of Tanzania: A: Wilting of the whole plant; B: Wilting of a few stems of the potato; C: Oozing of the vascular tissues and brown rot; D and E. Further rotting of tubers infected with *R. solanacearum* and F: Oozing on the eyes of potato tubers

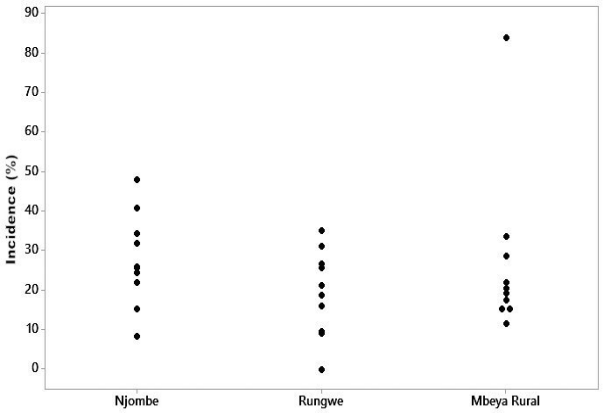

Fig. 3. Disease incidence (%) of potato bacterial wilt in Southern highlands of Tanzania. One dot is representing more than one score

B. Morphological characteristics of the isolates

1) Bacterial colony colour

When bacterial suspensions from potato tuber that had vivid symptoms of the disease were streaked to TZC medium large and elevated fluidal, colonies which were creamy with pinkish centre were observed indicating that they were virulent cultures. There were also colonies which were entirely white or with a pale red centre, smaller and non- fluidal which indicated that they were avirulent. These findings are in line with French *et al*. (1995) whom described virulent colonies as large, elevated, fluidal that are either white or with pale red centre and avirulent mutant colonies that are butyrous and deep red centers. The findings were also similar to Kelman (1954) who described virulent colonies as being pink or light red colour or characteristic red centre and whitish margin. Avirulent colonies were described as smaller, off white and non-fluidal.

Fig. 4. Virulent colony of R. solanacearum on TZC medium

2) Pathogenicity test

Using 18 bacterial isolates tested, N1, N4, MBY R2, MBY R4, R3, R4 and R10 were highly virulent on potato and tomato and they were slight virulent on eggplant after 3 weeks of inoculation. Strains N7, MBY R5, MBY R6 and R5 were highly virulent on potato and moderate to tomato and eggplant. Other strains N9, MBY R7, MBY R8, MBY R9, MBY R10, R2 and R7 were highly virulent on tomato and moderate to low on both potato and eggplant. None of the strains expressed wilting symptoms on tobacco and pepper. The strains were therefore grouped as Race 3. This race usually causes damage at lower temperature and attacks plants at higher altitudes (Oslon,2005). It is colder tolerant than race 1 and 2 in tropical highlands and temperate areas of East Africa (Oslon, 2005). *Ralstonia solanacearum* possesses hrp encoding the type III secretions system (T_3SS) and pathogenicity depends on interactions between the host plant and the type III effectors. According to Hichiki *et al*. (2007), once the pathogen is introduced, it invades intercellular spaces of roots through openings such as wounds. After invasion they accumulate around the stele before breaking into and filling the xylem vessels. Upon invasion of the xylem vessels, the bacteria grow and travel rapidly to the upper parts of the plants resulting in extensive wilting because of reduced sap flow caused by the presence of a large number of bacteria cells and Exopolysaccharides (EPS) slime produced by the bacteria in some xylem vessels.

Ralstonia solanacearum strains differ considerably in host range as well as aggressiveness to different hosts. Race 1 strains have a wide host range including numerous ornamentals and are present in most regions of the world. Race 2 is pathogenic to *Musa* spp and *Heliconia* spp. and occurs in tropical areas of the Central and South America, Hawaii and Philippines (Kelman, 1954; Bradbury, 1986; Elphistone, 2005). Race 3 strains are pathogenic mainly to potato and eventually infect tomato or other solanaceous host. Race 4 affects ginger (Pegg and Moffet, 1971) and race 5 is pathogenic to mullberry (Rodrigouz *et al*., 2012). Race 3 is an extremely destructive potato pathogen and it has been reported to cause Bacterial wilt disease in Highland tropics of Africa, Latin America and Asia. (Champoiseau, 2009). In Europe it disrupted seed potato production and caused serious quarantine related losses (Champoiseau, 2009). Measure to control this race is necessary in areas where its presence was not known like in the Southern Highlands of Tanzania so as to minimize the losses.

Table 2
Definition of races and biovars of *Ralstonia solanacearum* by host range

Race	Natural host	Biovars
1	Many solanaceae, some diploid bananas, numerous other crops weeds and many families	1,3 or 4
2	Triploid bananas, numerous other crops weeds and many families	1 or 3
3	Potato, Tomato and rarely a few other host	2
4	Mulberry	5

Source: EPPO, 2004

C. Biochemical Characterization of the Strains

1) Colour on TZC media

All eighteen (18) virulent potato strains from Southern highland zone of Tanzania produced fluidal and irregular creamy colonies with pinkish centre on TZC agar media. These results are similar to those of Dhital *et al.*, (2000) in which potato strains from Nepal were also fluidal and irregular with pinkish or light red centre on TZC Media.

2) Gram staining

All 18 isolates gave negative response when tested for Gram staining. Bacteria retained reddish pink colony colour when counter stained with safranin. This showed that they were Gram negative. In gram reaction as described by Schaad (1988) bacteria retaining reddish pink colony colour are Gram negative (G-ve) while Gram positive (G+ve) stain the blue violet colour.

3) KOH test

The isolates tested were negative on KOH loop test as they formed slime threads when the bacterial cultures (48 h) were mixed with 3 % KOH solution.

Gram negative bacteria have relatively fragile cell walls which are bounded by an outer membrane. The outer membrane is disrupted by exposing it to 3 % KOH solution which results in releasing slime threads which is actually the viscous DNA. On the other hand, the Gram-positive bacteria possess thick and more rigid cell wall which resists the disruptive effect of KOH. This test is useful supplement to the gram staining for the initial classification of anaerobic bacteria. Suslow *et al*., (1982)

reported that the KOH technique is easier and faster to distinguish Gram negative and positive bacteria than the traditional Gram stain in which dyes are employed.

4) Catalase oxidase test

The bacterial isolates tested produced gas bubbles when mixed with a drop of H_2O_2 on a glass slide, indicating that they might be *R. solanacearum*. Production of gas bubbles is a tendency of all Gram-negative bacteria, and it gives a clue for presence of aerobic and facultative anaerobic bacteria (Schaad, 1988). Catalase is a hemi-enzyme capable of decomposing hydrogen peroxide to water and oxygen gas (Klement *et al*., 1964).

The bacterial isolates tested produced gas bubbles when mixed with a drop of H_2O_2 on a glass slide, indicating that they might be *R. solanacearum* (Table 5). Production of gas bubbles is a tendency of all Gram negative bacteria, and it gives a clue for presence of aerobic and facultative anaerobic bacteria (Schaad, 1988). Catalase is a hemi-enzyme capable of decomposing hydrogen peroxide to water and oxygen gas (Klement *et al*., 1964).

5) Kovac's Oxidase Test

The tested isolates varied on the development of purple colour after being rubbed with Kovacs oxidase reagent. Thirteen (13) isolates produced the purple colour within 10 seconds whereas 3 isolates showed the purple colour in 60 seconds. Those which developed colour in 10 seconds were categorized as positive for this reaction whereas those which developed colour in 60 seconds were categorized as delayed positive isolates. Two isolates were negative on this test as they did not develop purple colour.

In Kovac's oxidase test bacterial isolates which give purple colour when mass of bacterial growth was rubbed on filter paper impregnated with oxidase reagent were categorized as positive whereas negative ones do not produce purple colour. This test is useful for differentiating aerobic and anaerobic bacteria (Kovacs, 1956) and is particularly important for differentiating Gram –negative bacteria. *R. solanacearum* gives a positive reaction.

6) Oxidation of glucose

Tested bacterial isolates were positive for oxidation of glucose. In positive isolates there was colour change from green to yellow at the surface of the open tubes. This indicated that there was gradual change of pH. There was no colour change in sealed tubes and therefore no pH change and thus glucose was not oxidized in sealed tubes.

Bacteria utilize glucose and other carbohydrates using certain metabolic pathways. Some are oxidative (respiratory) while others involve a fermentation reaction. Most bacterial plant pathogen of genus *Pseudomonas* and *Ralstonia* are oxidative as compared to genus *Erwinia* which are fermentative. Oxidative organisms can only metabolize glucose or other carbohydrates under aerobic condition, that is, oxygen is the ultimate hydrogen acceptor. Other organisms ferment glucose and the hydrogen acceptor is substance e.g. Sulphur. The oxidation fermentation process is used to differentiate species especially Gram-negative rods (Agrios, 2005).

D. Biovar Determined

Results of isolate tests with dextrose and hexose alcohols showed that they all belonged to Biovar 2 (Table 6). The dextrose (Carbohydrates) was utilized as there was colour change from olivaceous green to yellow. The colour change was observed in microtitre plates containing carbohydrates maltose, cellobiose and lactose. This indicated that there was utilization of carbohydrates. In the inoculated tubes containing hexose alcohols such as Dulcitol, Mannitol and Sorbitol no colour change was observed and it implied that they were not oxidized. These results agree with French *et al*. (1995) whom described Biovar 2 group as one which utilizes disaccharides but not oxidizing alcohols.

E. Serological Ddetection of the Isolates

Most of the sampled healthy-looking tubers were found not to be healthy after being tested serologically by NCM-ELISA (Table 3). Using 50 samples collected from each district 22 %, 36 % and 46 % of them from Njombe, Mbeya rural and Rungwe respectively were found to be latently infected. Different range of bacterial concentrations was observed in the positive samples. The concentration ranged from 10^7 to 10^8 cfu/ ml based on colour intensity of the positive and negative control strips of the NCM-ELISA kit**.** Results also indicated that out of 150 samples tested, 52 (34.6 %) were latently infected implying that the seed with symptomless infection will look absolutely healthy. But once planted, the plant will develop bacterial wilt and die. Even worse, it will also infect the soil with the bacteria where it is planted. Bacteria will also spread from the initial symptomless tuber to neighboring plants and infect them. Whether these plants will actually show symptoms depends on temperatures and soil humidity. They can also become symptomless carriers again, and spread the disease to yet another crop in a different field.

Table 3
Number of potato tuber samples infected by *Ralstonia solanacearum* as revealed by NCM-ELISA test

District	Number of samples	Number of samples with latent infection
Rungwe	50	23
Njombe	50	11
Mbeya Rural	50	18

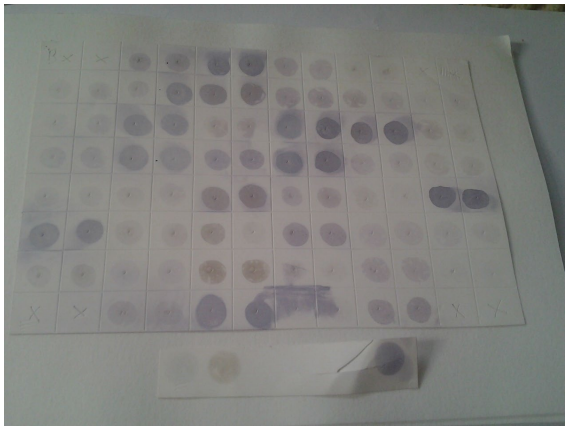

Fig. 5. NCM-ELISA membrane of tested potato samples with different concentrations, deep purple (10^8 bact /ml) and light purple (10^6 bact/ml)

F. Molecular Characterization of the Isolates

1) Species-specific PCR

Out of Fifteen (15) strains tested from each district; 7, 4 and 3 strains from Rungwe, Njombe and Mbeya Rural respectively were confirmed to be *R. solanacearum* using species specific PCR. The PCR produced a product of about 280 bp band was observed (Figures 6 and 7). The isolates that produced band size were selected for further characterization.

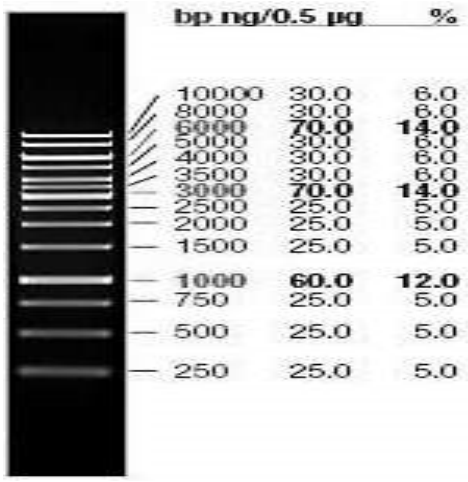

Fig. 6. Ladder map (I kb) as a reference used in the experiment to determine the size of the PCR product

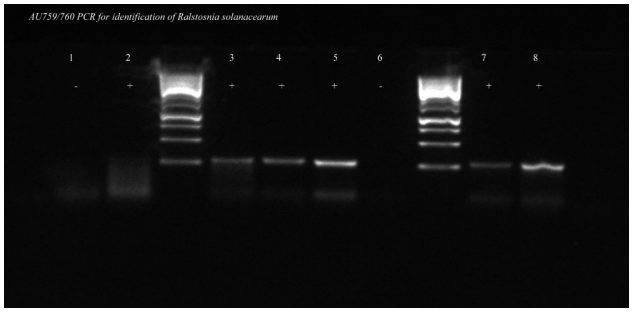

Fig. 7. An agarose gel image showing 280 bp PCR product using primer pair AU759/760 confirming bacteria in question as *R. solanacearum*. An arrow indicates 280 bp produced

G. Phylotype determination

Phylotype specific multiplex PCR revealed that *R. solanacearum* strains from Southern Highlands of Tanzania belonged to Phylotype III as 280 and 91 bp amplicon was observed in all the strains when Phylotype specific multiplex (Pmx- PCR) products of these strains were subjected to electrophoresis on 1.2% Agarose gel (Table 8 and Figure 8).

In the concept of integrated disease management, the use of resistant cultivars is of prime importance. A variety may be resistant to one phylotype but susceptible to the other and therefore it is very important to know the diversity of local strains of the pathogen (Sagar *et al*., 2014). Phylo-typing is needed for successful disease management and control strategies.

The results obtained correspond to those of Fegan and Prior (2005) whom described a phylotypic classification system consisting of four phylotypes, in which Phylotype 1 are those from Asia and are characterized by production of 280 and 144 bp amplicon, Phylotype II strains are from America and they produce 280 and 372 bp amplicons. Phylotype III are mainly from Africa and nearby islands such as Reunion and Madagascar which produce 280 and 91 bp and Phylotype IV strains which are from Indonesia, Japan and Australia which produce 280 and 213 bp amplicon. Phylotyping helps in the concept of integrated disease management where the use of resistant cultivar is of prime importance. A variety may be resistant to one phylotype but susceptible to another therefore it is very important to know the diversity of local strain of the pathogen (Sagar *et al*., 2014).

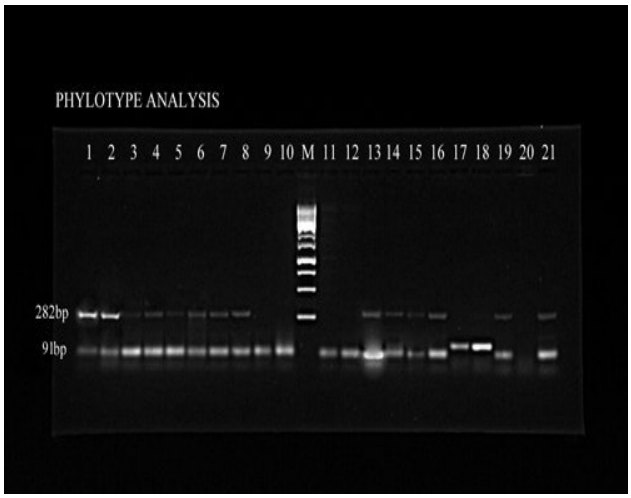

Fig. 8. Phylotype specific multiplex PCR of 20 isolates of R. solanacearum from the Southern highlands zone of Tanzania. Samples 17 and 18 were above 91 bp therefore, not R. solanacearum, lane M is 1kb ladder, and sample 21 was a positive control

4. Conclusion and Recommendations

A. Conclusion

Potato bacterial wilt disease was observed to occur in all three surveyed districts where major potato growing is taking place in the Southern Highland zone of Tanzania. Race 3, biovar II and phylotype III were prevalent in the study area.

B. Recommendations

So long as the disease is a seed borne, necessary measure such as use of disease-free seed tubers should be encouraged. In this study, potato seeds from Igeri in Njombe region are recommended for use by farmers in the southern of Tanzania as it was the location where no disease incidence was observed during the survey and which its samples were negative for serological test.

Use of certified seed potatoes at prices that small holder farmers can afford is recommended. To ensure quality seed potato availability there is a need of legalizing quality declared seed potato as in other crops.

Developing resistant varieties to bacterial wilt of potato is also recommended so as to minimize its spread.

Training on symptoms and bacterial wilt control is recommended to farmers. It was observed that most potato farmers in the Southern Highland zone of Tanzania know the

disease but they find no single effective control method.

References

[1] Agrios, G.N., (2005). Plant Pathology. 5th Edn. Academic Press, New York, USA., ISBN-13: 9780120445653, Pages: 922: 635

[2] Anderson J.A (1996). Potato cultivation in the Uporoto Mountains, Tanzania. An analysis of the social nature of Agro-technological change. *African Affairs*, 95:85-106.

[3] Champoiseau, P.G. (2009). *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. http://www.apsnet.org/online/feature/Ralstonia

[4] Chatterjee, S., Mukherjee, N. and Khatun, D. C. (1997). *Status of bacterial wilt in West Bengal*. J. interacademicia. Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, India, 1(1): 97-99.

[5] CIP, (2008). Potato fact sheets. ftp://ftp.fao.org/docrep/fao/012/1127e/i1127e03.pdf

[6] EPPO, (2004). *Ralstonia solanacearum*. European and Mediterranean. *Plant Protection Organization Bulletin* 34: 173-178.

[7] Fegan, M and Prior. P. (2005). How complex is the *"Ralstonia solanacearum* species complex"? In C. Allen, P. Prior, & A. C. Hayward (Eds.) Bacterial wilt disease and the *Ralstonia solanacearum* species complex (pp. 449-461). American Phyto pathological Society, St. Paul, MN.

[8] French, E. R., Gutarra, L., Aley, P and Elphinstone, J. (1995). Culture media for *Pseudomonas solanacearum*: isolation, identification and maintenance. *Fitopatologia*, 30: 126-130.

[9] Grover, A., Swarup, K.; Azmi W, Khursana, S.M.P (2012) Rapid Method for Isolation of PCR Amplifiable Genomic DNA of *Ralstonia solanacearum* infested in Potato Tubers. *Advances in Microbiology* 2*:* 441-446.

[10] Hayward, A.C. (1993). *Biology* and Epidemiology of Bacterial Wilt caused by *Pseudomonas solanacerum*. *Annual review of Phytopathology* 29: 65-87

[11] Hikichi, Y., Yoshimochi, T., Tsujimoto, S., Shinohara, R., Nakaho, K., Kanda, A., Kiba, A and Ohnishi, K. (2007) "Global regulation of pathogenicity mechanism of *Ralstonia solanacearum*". *Plant Biotechnology*. p. 149–154.

[12] Kadigi, R. M. J; Mpogole, H and Mlambiti, M. E. (2012). Round Potato Production in Southern Highlands of Tanzania: Are smallholder farmers becoming commercial*. Journal of Agricultural Extension and Rural Development*. 14(4): 385-391.

[13] Kaguongo, W.P; Gildemacher, P; Demo, P; Wagoire, W; Andocide, J and Thiele, G (2008). Farmer practices and adoption of improved potato varieties in Kenya and Uganda, *Social Science working paper*. International Potato Centre (CIP) Lima, Peru.84pg.

[14] Kelman, A. (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*. 44:693–695.

[15] Klement, Z.G., Farkas, L and Lovrekovich, L. (1964). Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology*. 54: 474-477.

[16] Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature London, 178-703.

[17] Martin, C and French, E.R. (1985). Bacterial Wilt of Potato*. Ralstonia solanacearum technical information bulletin* no. 13.C/P.

[18] Momol, M.T., Pradhanang, P. M and Lopes, C. A. (2003). Bacterial wilt. In: K. L. Pernezny, P.D. Roberts, J.F. Murphy and N.P. Goldberg (eds.), Compendium of Pepper Diseases. *American Phyto pathological Society*, St. Paul, MN., pp. 7-8.

[19] Oslon, H. A. (2005). *Ralstonia solanacearum*. Soil borne pathogens www.cals.ncsu.edu/Ralstonia/Ralstonia/solanacearum

[20] Pegg, K. G., and Moffet, M. (1971). Host range of ginger strains of *Pseudomonas solanacearum* in *Queensland. Australian J. Experimental Ag Anim. Husbandry* 11: 696-698.

[21] Rodrigoez, L.M.R, Destefano, S.A.L, Da Silva, M.J, Costa, G.G.L and Maringoni, A.C (2012) Characterization of *Ralstonia solanacearum* strains from Brazil using molecular and pathogenicity tests. *Journal of plant pathology* 94(3): 505-516.

[22] Schaad, N.W. (1980). Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd edition, p: 164. The Bacteriology Committee of the American Phyto pathological Society.

[23] Sagar, V., Gurjar, M.S., Arjunan, J., Bakude, R.R., Chakrabarti, S.K., Arora, R.K and Sharma, S. S. (2014). Phylotype analysis of *Ralstonia solanacearum* strains causing potato bacterial wilt in Karnakata in India. *African journal of Microbiology Research* 8(12):1277-1281.

[24] Smith, E.F. (1896). A bacterial disease of the tomato, eggplant and Irish potato (*Bacillus solanacearum* nov. sp*.). Div. Veg. Phys. and Path. Bul*. 12. U. S. Dept. Agr., 1896:1.

[25] Suslow, T. V., Schroth, M. N. and Isaka, M. H. (1982). Application of rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology* 72: 917-918.

[26] United Republic of Tanzania (URT) (2007). Iringa Region Socio-Economic Profile. National Bureau of Statistics (NBS) and Iringa Regional Commissioner's Office.