Zagazig J. Agric. Res., Vol. 42 No. (2) 2015
http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

ISOLATION AND IDENTIFICATION *Ralstonia solanacearum* THE CAUSAL ORGANISM OF POTATO BROWN ROT FROM DIFFERENT SOURCES AND REGIONS OF EGYPT

Rahma Abd-Elrahim[1*], M.M. Atia[1], M.R.A. Tohamy[1] and N.M. El-Sarkassy[2]

1. Plant Pathol. Dept., Fac. Agric., Zagazig Univ., Zagazig, Egypt

2. Agric. Bot. Dept., Fac. Agric., Zagazig Univ., Zagazig, Egypt

ABSTRACT

Bacterial wilt disease of solanaceae plants is an economic quarantine disease worldwide. Therefore, isolation and identification the causal organism from different sources and regions were carried out using rapid and accurate methods. Results revealed that, among 80 samples collected from different sources and regions, only 14 pathogenic isolates were obtained, five from tubers and three from each of weeds, water and soil. Ten from these fourteen isolates were isolated from El-Monufiya Governorate, four from El-Gharbia and none from El-Sharkia Governorates. Isolates number 4 and 5 of tuber, 9 of water, 1 of soil and 11 of weed showed the highest pathogenic capability on tomato seedlings. All pathogenic isolates were identified as *Ralstonia solanacearum* according to their biochemical and physiological properties including plating on King's B, TZC and SMSA media. *R. solanacearum* identification was also confirmed according to immunfluorescence antibody assay (IFAS), and Real-time (Taq-Man) PCR technique. Among the tested host plants and their cultivars using isolate T4 of tuber and W9 of water, only TY3018, Tymiland and 505 of tomato; Melda, Mekhaded and Destan of eggplant; Karm, Seuz and Maaz of pepper; Amaranths and Mallow, were susceptible, while cultivars of groundnuts; onion; broad bean and tobacco plants, were not. On the other hand, potato cultivars Pliny and Draga were the most susceptible ones, Kara was moderately resistant, while Mundial cultivar was considered as resistance one.

Key words: *Ralstonia solanacearum*, isolation, identification, immunofluorecence, real time PCR, host range.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth main food crop in the world after rice, maize and wheat. Brown rot of potato tubers or bacterial wilt of potato plants caused by *Ralstonia solanacearum* (Smith, 1896; Yabuuchi *et al.*, 1995) is one of the most important bacterial plant pathogens worldwide, because of its aggressiveness, wide spectrum host range, broad geographical distribution and long persistence in soil and water (Yabuuchi *et al.*, 1995; Tohamy *et al.*, 2007; Adriana *et al.*, 2011; Atia *et al.*, 2010). *R. solanacearum* race 3, biovar 2 (R3b2), was isolated for the first time in the Netherlands and caused an outbreak in the warm summer that appeared to be connected to use of contaminated irrigation water (Janse, 2012).

Ralstonia solanacearum is a soil-borne pathogen causing the widespread disease known as bacterial wilt. The pathogen is also the causal agent of Moko disease of banana and brown rot of potato (N'Guessan *et al.*, 2013). Tetrazolium chloride (TZC) agar medium was used to differentiate between virulent and avirulent isolates (French and Sequeira, 1970). *R. solanacearum* has been considered a species complex, which consists of a heterogeneous group of related but genetically distinct strains.

* **Corresponding author: Tel.** : +201007079493
E-mail address: usamaatia2@yahoo.com

These strains show some variability in host range and aggressiveness, but genetic change in plant may occur between them (Smith *et al.*, 1995 and Peeters *et al.*, 2013). Traditionally, *R. solanacearum* is classified into five races (r) on the basis of differences in host range and six biovars (bvs) on the basis of biochemical properties (Santana *et al.*, 2012). Recently using molecular methods, *R. solanacearum* has been classified into phylotypes based on the inter-genic transcribed sequence of the ribosomal RNA genes 16S and 23S and into sequevars based on the endoglucanase gene (egl) sequence (Sagar *et al.*, 2014).

A quick laboratory method to detect the pathogen in its latent infection has been serologically applied as indirect immunofluo-rescence antibody staining according to Janse (1988). A fluorogenic Taq-Man PCR assay was developed to detect *R. solanacearum* strains (Weller *et al.*, 2000). Li *et al.* (2014) reported that sensitivity and specificity of the conventional isolation, serological bioassay, real-time PCR and multiplex PCR were assessed for the detection of 25 *R. solanacearum* strains of biovar 1, 2 and 3 (Phylotypes I, II, and III) in spiked potato saps. The Taq-Man PCR assay, with an internal reaction control and confirmation by melting curve and electrophoretic analysis, achieved best sensitivity at 10^2-10^3CFU ml-1 for all eighteen strains of *R. solanacearum* R 3 bv 2. Selective enrichment on SMSA medium plates enhanced the detection sensitivity up to 10-100 CFU ml-1 for the conventional PCR-based assays.

The pathogenic bacterium has a wide host range of economically important crops such as tomato, potato, eggplant, chilli and non solanaceous crops such as banana and groundnut (Mondal *et al.*, 2011). The alternative weed hosts and non-host plants played an important role for the survival of *R. solanacearum* srains (Tohamy *et al.*, 2007). *Ralstonia solana*cearum biovar 3 has been described on some woody perennial hosts (Mayers and Hutton, 1987).

So, the aim of this research is to collect samples from different Egyptian sources and regions to isolate, identify and detect the causal agent of potato brown rot using: King's B and SMSA media, physiological and biochemical properties, immunofluorcence antibodies stain (IFAS) and DNA methods using Real-time (Taq-Man) PCR technique. In addition, host range of the detected isolates.

MATERIALS AND METHODS

Samples Collection

Eighty samples of tubers, water, soil and weeds were collected from various localities of El-Salhia city and Isamilia cancal pivot Nos.10 and 20 in El-Sharkia Governorate. Also, El-Alamia and Kafer Yakope in El-Gharbia Governorate as well as Talia and El-Nagar in El-Monufiya were also investigated to isolate the causal organisms of potato brown rot disease.

Isolation and Purification the Causal Organism from Different Sources

Potato tubers

Infected potato tubers samples were collected from different Governorates *i.e.* El-Monufiya (Talia village), El-Gharbia (Kafr Yakope village) and El-Sharkia at El-Salhia city where potato plants were cultivated. Isolation process was carried out as described by Tohamy *et al.* (2007). Obtained isolates were used to inoculate plates of semi selective media of South Africa (SMSA) medium as described by Engelbrecht (1994) and modified by Elphinstone *et al.* (1996). Incubation was done at 28°C and observed daily for developing fluidal, slightly raised, irregular white or white with pinkish center colonies, typical for virulent colonies of *R. solanacearum*. Resulted virulent colonies were selected, picked up, inoculated on glucose nutrient agar medium and incubated for 48 hr., at 28°C for further studies.

Irrigation water

Four water samples (50ml/sample) using a sterile bottle from the central irrigation system pivot Nos. 10 and 20 at El-Salhia city were collected, 10 minutes after a brief course of irrigation. Samples from water streams at El-Nagar and Talia villages, as well as El-Alamia and Kafr Yakope villages, were also done at depth of 30-40 cm from the water surface. Samples collected from each site were centrifuged at 10,000 rpm for 15 minutes at 15°C. The supernatant was discarded and pellet was re-suspended in one ml phosphate buffer

(pH 7.2), vortex for homogenization and plated on plates containing SMSA medium.

Soil

Four samples (100 g soil/sample) from each location (pivot Nos.10, and 20 at El-Salhia city; Kafr Yakope village, El-Nagar and Talia villages were taken at a depth of 30 cm, using a sampling augar then mixed in the laboratory for homogenization. Isolation process was conducted on SMSA medium as previously mentioned according to the method described by Van Der Wolf *et al.* (1998).

Weed

The pathogen in symptomless weed plants (Amaranths, *Amaranthus viridis* L.) associated with potato crop in El-Monufiya Governorate, during the season 2011-2012, was monitored. Isolation process was carried out from the roots according to Tohamy *et al.* (2007). Resulted supernatant was plated on SMSA medium as described by Wenneker *et al.* (1999). In all the above mentioned isolation trials, typical appearance of *R. solanacearum* virulent colonies were picked up and used in pathogenicity tests.

Pathogenicity Test

Pathogenicity test was conducted in greenhouse of Plant Path. Dept., Fac. Agric., Zagazig Univ. The previously obtained fourteen bacterium isolates were confirmed by inoculation of two weeks old healthy seedlings of tomato plants cultivar GS12 (three leaves/ seedling stage) grown in 10 cm diameter plastic pots containing 1kg sandy-clay soil (1/1, *V/V*). Inoculation was carried out using 10^8cfu/ml by stem puncture technique described by Janse (1988). Control treatments were carried out using sterile water instead of bacteria. Inoculated tomato seedlings were observed daily to calculate the incubation period of the first sign of disease incidence. The percentage of infection was calculated as follows:

$$\text{Infection (\%)} = \frac{\text{No. of wilted plants}}{\text{No. of tested plants}} \times 100$$

Disease severity (DS) was determined 7 days after inoculation according to the following modified scale (Winstead and Kelman, 1952): 0 = no wilt symptoms; 1 = one or 2 wilted leave(s); 2 = three wilted leaves; 3 = all leaves wilted except the tip; 4 = whole plant wilted and 5 = death (collapse) of whole plant. The disease severity was calculated according to the following equation:

$$\text{Diseaseseverity\%} = \frac{\sum(\text{No.of wilted plants in each category} \times \text{wiltgrade})}{\text{Total No.of plants} \times \text{highest grade}} \times 100$$

The isolates in concern were classified accg to their DS into four severity groups: group 1: ≥ 90%, group 2: 70-90%, group 3:50-70%, group 4: <50%.

Re-isolation was carried out to complete Koch's postulates and the most pathogenic isolates were selected and take into consideration in the following tests. These isolates included soil isolates (S1, S2 and S3), tuber isolates (T4, T5, T6, T7 and T8), water isolates (W9, W10 and W11) and weed isolates (We12, We13 and We14).

Morphological, Physiological and Bio-chemical Properties of Bacterial Isolates

These properties including shape of cell, motility, gram stain reaction, sporulation, colony type, brown pigments, fluorescent pigments, oxidase test, catalase, arginine and dihydrolase tests, starch hydrolysis, gelatin liquefaction, nitrate reduction, growth on selective media (SMSA, King's B and TZC) and carbon source utilization, were carried out. All media used in these tests conducted according to the methods described by King *et al.* (1954), Schaad (1988) and Denny and Hayward (2001).

Immuno-fluorescence antibody stains (IFAS)

IFAS is a serological method for rapid detection and presumptive identification of bacteria. The polyclonal antibodies are produced in rabbits against living whole cells (Janse, 1988). All test procedure of the present investigation was conducted at Potato Brown Rot Control Project (PBRCP), Agric Res. Center, Giza, Egypt.

Quantitative, real-time, fluorogenic PCR (Taq-Man) assay

Taq-Man is a molecular detection method that combines polymerase chain reaction (PCR) with fluorescent detection of the amplicon (Weller *et al.*, 2000). The Quantitative, Real-

time, Fluorogenic PCR (Taq-Man) assay was conducted at PBRCP.

Host Range

Tomato (*Lycopersicon esculentum* L.) cultivars TY3018, Tymiland and 505; eggplant (*Solanum melongena* L.) cultivars Melda, Makhaded and Destan; pepper (*Capsicum annum* L.) cultivars Karm, Seuz and Maaz; groundnuts (*Arachis hypogąea* L.) cultivars Giza 6, Ismailia1 and Gregory; maize (*Zea mays* L.) cultivars 302, 321 and 323; onion (*Alium cepa* L.) cultivar Giza 20; broad been (*Vicia faba* L.) cultivar Giza 716; Amaranths (*Amaranthus viridis* L.); mallow (*Malva aegyptica* L.) and tobacco (*Nicotiana tabacum* L.) were used to determine the host range of *Ralstonia solanacearum*.

Aforementioned plant seeds and/or seedlings were planted in pots (10cm in diameter) containing 1Kg clay soil each under greenhouse conditions. The stem puncture technique injection was carried out as previously mentioned in pathogenicity test. Virulent isolates (T4 and W9) were selected where the highest severity of disease incidence through pathogenicity test. Five replicates were used for each host. Another five plants for each host plant were inoculated by sterilized distilled water with cutting roots with alcohol flaming knife as the control. Then, pots were inoculated with a rate of 50ml/pot (10^8cfu/ml).Un-inoculated controls were also considered. All inoculated and un-inoculated plants were observed daily for three weeks to induce wilt symptoms induction. The percentage of wilted plants was calculated as previously mentioned in pathogenicity test.

The tested tomato, eggplant and pepper cultivars were obtained from El-Salam nursery Abou-Hamade district, El-Sharkia Governorate and the tested groundnuts, maize, onion, broad bean, amaranths, mallow and tobacco were kindly provided by the Agricultural Research Center.

Reaction of Potato Cultivars to *Ralstonia solanacearum* Infection

This study was carried out in pot experiment under greenhouse conditions. Clay soil was used at a rate of 5.0 Kg/pots (30cm). Soil infestation was carried out with two virulent isolates (T4 and W9) were previously propagated in liquid King's B medium for five days at 28°C. The pots were infested at the rate of 200ml/pot (10^8cfu/ml). Seven days after infestation, each pot was planted with one healthy tuber of potato cultivars (pliny, draga, kara, alexant and mundial). The tested potato cultivars were kindly provided by PBRP. Five replicates were used for each potato cultivar. Un-infested controls were also considered (Tohamy *et al.*, 2007). Percentage of wilted plants, No. of tubers, No. of shoots and weight of potato tubers (g), were calculated.

Statistical Analysis

All data were subjected to statistical analysis proposed by Gomez and Gomez (1981), and means were compared using LSD as mentioned by Duncan (1954).

RESULTS AND DISCUSSION

Isolation, Purification and Characterization of *Ralstonia solanacearum* from Different Sources

Isolation

Eighty samples were collected, from tubers (30 samples), water (21 samples), soil (20 samples), and weeds (9 samples). According to the locality, 28 samples were collected from El-Monufiya, 18 from El-Gharbia and 34 from El-Sharkia Governorates (Table 1). Only fourteen samples were positive. Ten positive isolates were obtained from El-Monufiya Governorate, including two isolates from each of, soil (S) and weeds (We) while the reminders three were isolated from each of tubers (T) and water (W). Water isolated number W9, W10 and W11 were isolated from El-Nagar canal. Three tuber isolates (T4, T5 and T6), two soil isolates (S1and S2) and two weed isolates (We12 and We13) were isolated from Talia samples. Two tuber isolates (T7 and T8) were isolated from Kafer Yakope. One soil isolate (S3) and one weed isolate (We14) were isolated from El-Almia at El-Gharbia Governorate. Non *R. solanacearum* isolates could be detected from El-Sharkia Governorate samples. Several research reports indicated that occurrence of potato brown rot causal organism was detected from different district in Egypt (Sabet, 1961; Barakat, 1963; El-Goorani, 1967; Farag, 1970; Abd El-Fattah, 1971; Farag, 1976; Atia *et al.*, 2010). Samples collected from Ismalia canal and

Table1. Number of collected samples from different localities of three Governorates

Governorate	No. of samples	Source of samples			
		Soil (S)	Tuber(T)	Water (W)	Weed (We)
El-Sharkia					
Isamilia canal	**34**	*	*	11-	*
El-Salhia district		7-	16-	*	*
El-Gharbia					
El-Alamia	**18**	1+	7-	3-	1+
KafrYakope		*	2+	4-	-
El-Monufiya					
Talia	**28**	2+	3+	*	2+
El-Nagar		10-	2-	3+	6-
Total	**80**	20	30	21	9

*No samples collected from this site, - Negative reaction during isolation and + Number of positive reaction during isolation.

El-Salhia showed negative results because these districts are considered as pest free areas (PFA) and this confirm that this area is brown rot free disease and suitable for exportation to European markets.

Tuber samples collected from El-Monufiya and El-Gharbia Governorates used to isolate the pathogen showed positive brown rot infection by visual inspection exhibiting bacterial oozing out from the crossing section in vascular bundles. This sign of bacterial ooze in tuber are similar to the symptoms previously described by Kelman (1953).

Pathogenicity test

Results in Table 2 demonstrate that isolates T4, W9 and T5 showed the highest severity of disease incidence revealing 100, 100 and 95.6, respectively relating to severity group 5 and 4. Isolate T6, T7, We12, S1, W10, We13, W11 and T8 revealing 89.20, 85.40, 85, 84.20, 79.40, 76.80, 76.60 and 75.2 disease severity, respectively. With the exception of isolate S1which related to the severity group 5, the other isolates related to severity group 4. On the other hand, S3, We14 and S2 isolates were the least disease severity revealing 42.8, 44.8 and 54, respectively, and related to severity group 2 and 3. Using tomato seedlings is easy test to induce wilt symptoms which usually appeared within a week (Elphinstone *et al.*, 1996). No clear correlation was observed between incubation period and disease severity.

Generally, soil isolates were less effective than other isolates. Tuber isolate (T4) revealed the highest value in disease severity group (5) and lower incubation period (3.4 days). Incubation period ranged between 3.4-6.8 days. Pathogenicity tests showed typical symptoms of bacterial wilt on tomato seedlings. Similar results indicated different in disease severity between isolates of *R. solanacearum* isolated from different sources. Tomato seedlings exhibiting wilt symptoms in pathogenicity test are resulted from the rapidly colonization of the pathogenic bacteria in intercellular spaces of the vascular bundles degrading the cell walls, resulting destruction of xylem tissues and production of tyloses that block the vascular system causing wilt in plants (Kang *et al.*, 1994).

According to Wallis and Truter (1978) results, the initial colonization of host tissue did not occur directly in the xylem vessels. Twenty four hours after inoculation, stimulation of tyloses was noted in invaded as well as non-invaded plant cells.

Table 2. Pathogenicity test measured as disease severity, incubation period and severity group for *Ralstonia solanacearum* isolates inoculated in tomato seedlings

Source	Isolate	Disease severity (%)	Average incubation period (day)	Severity group
Soil	S1	84.2	4.60	5
	S2	54.0	4.80	3
	S3	42.8	6.20	2
Tuber	T4	100.0	3.40	5
	T5	95.6	4.40	4
	T6	89.2	4.20	4
	T7	85.4	6.20	4
	T8	75.2	6.40	4
Water	W9	100	4.40	5
	W10	79.4	6.00	4
	W11	76.6	6.00	4
Weed	We12	85.0	5.00	4
	We13	76.8	5.80	4
	We14	44.8	6.80	2
LSD at 0.05		4.61	3.14	2.53

Identification

Morphological, Physiological and Biochemical Properties of the Isolated *Ralstonia solanacearum*

Table 3 revealed that all the fourteen isolates proved to be motile and short- rods gram-negative (G) bacteria. No fluorescent pigments could be detected with UV on Kong's B medium, but production of brown pigments on glucose nutrient agar medium was detected. All of isolates were positive concerning oxidase glucose during metabolism, while catalase was negative. Activity of arginine dihydrolase was negative and all isolates could not hydrolyze starch or gelatin liquefaction. Nitrate reduction was positive. As for the utilization of carbon sources concerning glucose, maltose, lactose and cellbiose, all the fourteen mentioned pathogenic isolates were utilized them. On the other hand, none of the examined isolates was able to utilize sorbitol, mannitol and dulcitol. According to the obtained results, these fourteen isolates related to, *Ralstonia solanacearum* race 3 biovar 2. In this peculiarity, Ramadan (2000) identified 24 *P. solanacearum* isolates as R3B2 using recent and accurate biochemical and seriological asssays for diagnosis the causal agent of brown rot in potato tubers.

Similarly Bulbul and Main (2001) isolated and identified isolates of *R. solanacearum* as race 3 biovar 2 from potato, tomato, chili, aubergine and groundnut.

Cultivation on SMSA, King's B and TZC media

The virulent colonies of *R. solanacearum* on SMSA medium are milky white, irregular and fluidal with blood having red coloration in the center. This typical colony characters of virulent isolates of *R. solanacearum*. No fluorescent pigments were detected with UV on King's B medium. On the other hand, these colonies are fluidal white with pink center on tetrazolium chloride (TZC) medium in the same way French and Sequeira (1970) carried out study to differentiate between virulence of *Ralstonia solanacearum* isolates.

Table 3. Morphological, Physiological and biochemical characters of the pathogenic isolates of *Ralstonia solanacearum*

Character	Bacterial isolate													
	Soil (S)			Tubers (T)					Water (W)			Weeds (We)		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Shape of cell	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporulation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fluorescent pigment	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolyze	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of:														
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-

R = short rod shape + = positive reaction - = negative reaction

Immuno-fluorescence antibody stain (IFAS)

Face contrast microscope revealed that cells of T4, T5 and W9 isolates were short rods stained evenly as bright green fluorescent. It is established that, the detection level of polyclonal antibody in IFAS is recorded of 10^4 cells/ml where the false reaction was limited to only 2-3%. Other levels give high false reaction. These results are in harmony with those reported by Janse (1988). IFAS is considered as one of the most sensitive serological tests for detecting bacteria (De Boer *et al.*, 1996). it is valid to confirm the presence of *R. solanacearum* but does not able to detect the races or biovars. On the other hand, it is also not completely reliable due to possible of the cross reaction with other bacteria (Janse 1988).

Quantitative, real-time, fluorogenic PCR (Taq-Man) assay

In this assay the isolates in concern did not show any pronounced variations in similarity. The probe used in Taq-man for detecting all biovars of *Ralstonia solanacearum* (RS) primers and probe detected that all biovars and races are of virulent *R. solanacearum*.

However, the probe used in Taq-man for detecting only biovar 2 of *Ralstonia solanacearum* bacterial wilt (B_2) primer and probe are specific for detection of the race 3 biovar 2 strain. Positive results were obtained in both assays with all 3 investigated isolates (T4, T5 and W9), indicating that all isolates were related to *R. solanacearum* race 3 biovar 2 (Fig, 1). Similar results were reported by Balabel (2006).

Host range

Data in Table 4 revealed that the tested isolates were pathogenic for each of tomato, eggplant and pepper cultivars as well as amaranths and mallow. Results of percentage infection and disease severity of tomato cultivars revealed that TY3018 was the most susceptible while 505 one was the highest resistant cultivar and Tymiland was moderately for both tested isolates (T4 and W9) compared with control healthy plants.

Similary, Melda cultivar of eggplant was the most susceptible, Mekhaded was moderately and Destane was the highest resistant cultivar for the both tested isolates compared with healthy plants. But with lower values than tomato plants. As for pepper cultivars Karm was the most susceptible, while Seuz moderetaly and Maaz was the highest resistant.

While the infection percentage and disease severity of mallow were 81.00% and 78.00% caused by T4 isolate and 71.7% and 67.30% caused by W9 isolate, respectively. The infection and disease severity percentage of amaranths was 46.30% and 44.00% caused by T4 isolate and 37% and 35.00% caused by W9 isolate. It is worthy to mention that, bean, maize, onion, groundnut and tobacco cannot be infect by the two bacterial isolates used and no latent infection, were observed.

It is also worthy to notice that, the number of shoots in all resistant investigated cultivars are higher than of the susceptible ones.

In this respect, several hundred species, representing more than 50 plant families have been identified as hosts of *R. solanacearum*, including tomato, potato, pepper, eggplant, groundnut and banana as well as a number of ornamental plants, woody perenials and a large group of weed species (Buddenhagen and Kelman, 1964; Hayward, 2000; Tahat and Sijam, 2010).

Plants known as non- host range for the *R. solanacearum* pathogen such as peanut, maize, onion, bean and tobacco are very important in agriculture rotation. Such non-host range plants reduced the pathogen population in the soil rhizosphere and considered as important factors for disease control.

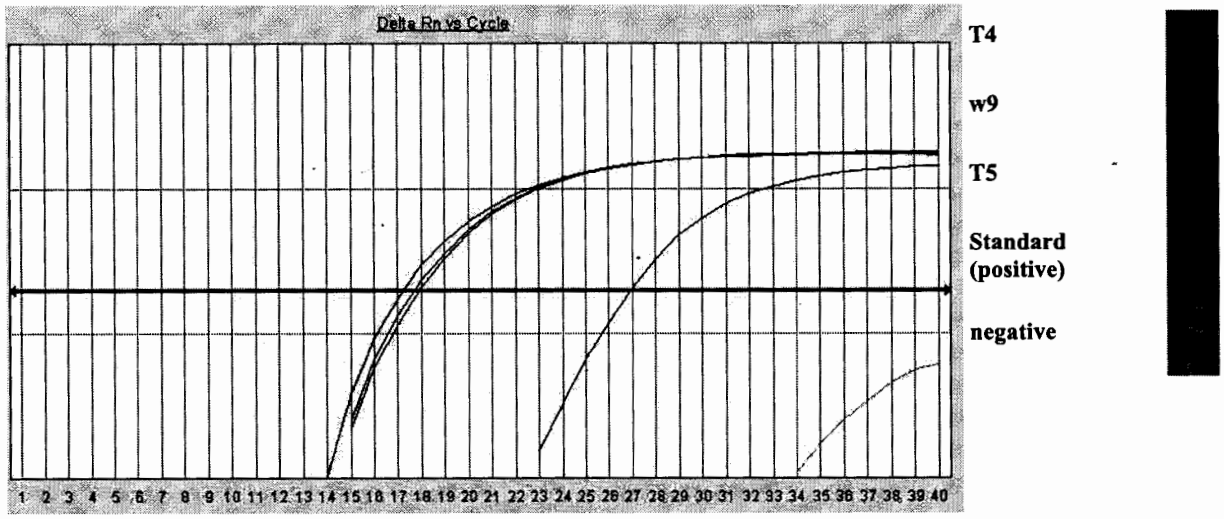

Fig. 1. Taq-Man assay of three virulent isolates of *R. solanacearum* recovered from infected potato tubers and water isolated in Egypt

Table 4. Host range of *Ralstonia solanacearum* (isolate T4 from tuber and W9 from water) determined as percentage infection, disease severity and number of shoots

Tested host	Cultivar	Infection (%)			Disease severity (%)			No. of shots		
		T4	W9	Healthy	T4	W9	Healthy	T4	W9	Healthy
Tomato	Ty3018	96.0	84.0	0.0	94.7	82.7	0.0	5.3	4.0	8.0
	Tymiland	78.0	60.0	0.0	74.7	58.7	0.0	5.0	6.0	9.3
	505	51.0	47.3	0.0	48.0	46.0	0.0	7.3	8.0	9.0
Eggplant	Melda	69.9	60.3	0.0	69.0	57.0	0.0	5.0	6.0	6.7
	Makhaded	48.7	43.7	0.0	47.7	41.0	0.0	6.0	5.7	7.0
	Destan	40.0	40.3	0.0	38.7	39.0	0.0	5.3	6.3	7.3
Pepper	Karm	40.0	38.0	0.0	38.3	36.7	0.0	7.0	7.0	9.0
	Seuz	20.3	19.0	0.0	19.3	16.7	0.0	8.7	10.0	11.0
	Maaz	16.7	16.7	0.0	16.0	13.3	0.0	10.0	10.7	12.0
Groundnut	Giza 6	0.0	0.0	0.0	0.0	0.0	0.0	8.0	7.7	8.3
	Ismailia 1	0.0	0.0	0.0	0.0	0.0	0.0	8.7	8.0	9.0
	Gregory	0.0	0.0	0.0	0.0	0.0	0.0	9.3	9.3	9.0
Maize	302	0.0	0.0	0.0	0.0	0.0	0.0	7.0	7.0	7.3
	321	0.0	0.0	0.0	0.0	0.0	0.0	7.7	7.0	7.3
	323	0.0	0.0	0.0	0.0	0.0	0.0	7.3	7.3	7.0
Onion	Giza 716	0.0	0.0	0.0	0.0	0.0	0.0	5.7	6.0	6.3
Bean	Giza 20	0.0	0.0	0.0	0.0	0.0	0.0	4.7	5.0	4.7
Amaranths		46.3	37.0	0.0	44.0	35.0	0.0	4.3	4.7	5.7
Mallow		81.0	71.7	0.0	78.0	67.3	0.0	4.7	5.3	5.3
Tobacco		0.0	0.0	0.0	0.0	0.00	0.0	5.3	6.0	7.0
LSD at 0.05		12.33	9.43	NS	8.74	7.55	NS	1.88	1.76	1.86

Potato Cultivars Response Against Bacterial Wilt Disease

Data in Table 5 represented the reaction of potato cultivars. Results indicate that, the tested cultivars were susceptible to the disease infection at different rates with both tested isolates at various values. In general, Pliny and Draga cultivars were the most susceptible ones scoring the highly disease severity percentage caused by both of tuber (T4) and/or water (W9) isolates.

Pliny and Draga cultivars, resulted the least number of shoots, tubers and weight of tubers when the soil was infested with T4 isolate. Higher values were recorded when the soil was infested with W9 isolate. On the other hand, Kara was moderately resistant, while Mundial cultivar was considered as resistant one scoring the least value of disease severity when soil was infested with T4 and W9 isolate. Number of shoots, tubers and weight of tubers were higher if compared with the susceptible cultivars.

The use of resistant potato cultivars played an important role in the integrated control of bacterial wilt. Unfortunately, the complexities of host pathogen environment interaction make breeding for resistance extremely different (Tung and Schmiediche, 1995), and no immunity has yet been identified in potato (Hayward, 1991). Tung and Schmiediche (1995) agreed on the dominanted character of resistance and suggested that only a few genes control it, in spite of the number of genes involved in this resistance that are still unknown.

Table 5. Reaction of certain potato cultivars with *Ralstonia solanacearum* bacterium (isolate T4 from tuber and isolate W9 from water) under greenhouse conditions as percentage disease severity and some growth properties of potato plants

Variety	Disease severity (%)			Plant growth parameter								
				Shoots (No.)			Tubers (No.)			Tuber weight (g/plant)		
	T4	W9	*Cont	T4	W9	Cont	T4	W9	Cont	T4	W9	Cont
Pliny	99.3	90.3	0.0	10.3	11.0	13.0	5.0	6.0	7.3	45.3	65.0	97.7
Draga	79.7	73.0	0.0	13.7	14.3	15.0	7.7	6.7	8.0	51.3	82.7	114.7
Kara	66.0	55.3	0.0	16.7	17.3	19.0	8.0	8.7	9.3	65.0	96.0	128.7
Alexant	38.3	29.7	0.0	18.3	19.0	20.7	8.7	9.0	10.0	82.3	104.3	139.0
Mundial	28.7	20.3	0.0	20.0	22.3	23.3	9.0	9.7	10.7	92.7	117.3	150.3
LSD at 0.05	5.61	3.135	NS	2.64	1.73	4.14	3.65	2.68	6.07	5.13	3.43	8.23

*Cont : Control

REFERENCES

Abd El-Fattah, A.A. (1971). Studies on brown rot disease of solanaceous plants. M.Sc. Thesis, Fac. Agric., Assiut Univ., Egypt, 119.

Adriana, G., P. Laure, R. Silvia, B. Christian and G. Stephane (2011). Detection and functional characterization of a large genomic deletion resulting in decreased pathogenicity in *Ralstonia solanacearum* race 3 biovar 2 strains. Environ. Microbiol., 13 (12): 3172-3185.

Atia, M.M.M., M.R.A. Tohamy, F.F. Gabriel and H.A. Mater (2010). Integrated management of potato brown rot. Proc- of the Sixth Inter. Conf. Sustain. Agric., Fayoum Univ., 275-294.

Balabel, N.M. (2006). Persistence of *Ralstonia solanacearum* (Syn. *Pseudomonas solanacearum*) in different habitats. Ph.D. Thesis, Fac. Agric., Ain Shams Univ., Egypt

Barakat, F.M. (1963). Studies in the bacterial wilt of potato caused by *Pseudomonas solanacearum* E.F. Smith. M. Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.

Buddenhagen, I. and A. Kelman (1964). Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathol., 2:203-230.

Bulbul, M.A. and I.H. Main (2001). Occurrence of rasces and biovars of *Ralstonia solanacearum* in Bangladesh. J. Plant Path., 17 (1-2): 5-12.

De Boer, S.H., D.A. Cuppels and R.D. Gitaitis (1996). Detection latent bacterial infections. Advances in Botanical Res., 23:27-47.

Denny, T.P. and A.C. Hayward (2001). *Ralstonia solanacearum* in Laboratory Guide for Identification of Plant Pathogenic Bacteria, Schaad, N.W.; Jones, J.B.Chun, W. (Eds.). 3rd Ed., APS Press, St. Paul, MN, 151-173.

Duncan, D.B. (1954). Multiple Range and Multiple F test. Biometrics, 11:1-42.

El-Goorani, M.A. (1967). Studies on potato tuber rots in U.A.R. Ph.D. Thesis, Fac. Agric., Alex.Univ., Egypt.

Elphinstone, J.G., J. Hennessy, J.K. Wilson and D.E. Stead (1996). Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. Bulletin OEEP/EPPO, 26:663-678.

Farag, N.S. (1970). Studies on brown rot of potato in Egypt. M.Sc. Thesis, Fac. Agric., Ain Shams Univ., Egypt.

Farag, N.S. (1976). Interaction between some soil microflora and *Pseudomonas solanacearum*. Ph.D. Thesis, Fac. Agric., Ain Shams Univ., Egypt.

French, E.R. and L. Sequira (1970). Strains of *Pseudomonas solanacearum* from central and South America: A comparative study. Phytopathol., 60:506-512.

Gomez, K.A. and A.A. Gomez (1981). Statistical Producers for Agriculture Research. 2nd Ed. John Wiley Sons Ltd, New York.

Hayward, A.C. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathol., 29:65-87.

Hayward, A.C. (2000). *Ralstonia solanacearum*. Encyclopedia of Microbiology. Edited by J. Lederberg. Vol. 4. 2nd Ed. Academic Press, Elsevier Science, Amsterdam, Netherlands.

Janse, J.D. (1988). A detection method for *Pseudomonas solanacearum* in symptomless potato tubers and some data on its sensitivity and specificity. EPPO Bulletin, 18: 343-351.

Janse, J.D. (2012). Review on brown rot (*Ralstonia solanacearum* race 3, biovar 2, phylotype IIB) epidemiology and control in the Netherlands since 1995: a success story of integrated pest management. J. Plant Pathol., 94 (2):257-272.

Kang, Y., J. Huang, G. Mao, L.Y. He and M.A. Schell (1994). Dramatically reduced virulence of mutants of *Pseudomonas solanacearum* defective in export of extracellular proteins across the outer membrane. Mol. Plant-Microbe Interact., 7: 370-377.

Kelman, A. (1953). The bacterial wilt caused by *Pseudomonas solanacearum*: A literature review and bibliography. North Carolina Agric. Exp. Station Tech., 99: 194-194.

King, E.O., M.K. Ward and D.E. Raney (1954). Two simple media for the demonstration of pyocianin and fluoriscin. J. Lab. Clin. Med., 44: 301-307.

Li, X., J. Nie, D.L. Hammill, D. Smith, H. Xu, and S.H. De Boer (2014). A comprehensive comparison of assays for detection and identification of *Ralstonia solanacearum* race 3 biovar 2. J. Appl. Microbiol., 117 (4) : 1132-1143.

Mayers, P.E. and D.G. Hutton (1987). Bacterial wilt, a new disease of custard apple: Symptoms and Etiology. Ann. Appl. Biol., 111: 135-141.

Mondal, B., I. Bhattacharya and D.C. Khatua (2011). Crop and weed host of *Ralstonia solanacearum* in West Bengal. J. Crop and Weed, 7(2):195-199.

N'Guessan, C.A., S. Brisse, A.C. Roux-Nio, S. Poussier, D. Kone and E. Wicker (2013). Development of variable number of tandem repeats typing schemes for *Ralstonia solanacearum*, the agent of bacterial wilt, banana Moko disease and potato brown rot. J. Microbiol. Methods, 92 (3): 366-374.

Peeters, N., A. Guidot, F. Vailleau and M. Valls (2013). *Ralstonia solanacearum*, a widespread bacterial plant pathogen in the post-genomic. Molecular Plant Pathol., 14 (7): 651-662.

Ramadan, A.K.M. (2000). Serological, physiological and biochemical studies on *Burkholderia solanacearum* (*Pseudomonas solanacearum*) the bacterium causing potato brown rot disease. Ph.D. Thesis, Microbiol. Dept., Fac. Agric., Ain Shams Univ., Egypt.

Sabet, K.A. (1961). The occurrence of bacterial wilt of potatoes caused by *Pseudomonas solanacearum* (E.F.Smith) in Egypt. Tech. Bul., 1-10, Extensin Dept., Min. Agric. Egypt.

Sagar, V., A. Jeevalatha, M. Sarita, S.K. Chakrabarti, M.S. Gurjar, R.K. Arora, S. Sharma, R.R. Bakade and B.P. Singh (2014). Potato bacterial wilt in India caused by strains of phylotype I, II and IV of *Ralstonia solanacearum*. Plant Pathol., 138 (1):51-65.

Santana, B.G., C.A. Lopes, E. Alvarez, C.C. Barreto, C. Allen and B.F. Quirino (2012). Diversity of Brazilian biovar 2 strains of *Ralstonia solanacearum*. J. General Plant Pathol., 78 (3): 190-200.

Schaad, N.M. (1988). Laboratory Guide for Identification of Plant Pathogenic Bacteria 2nd Ed. The American Phytopathological Society, St. Paul. Minnasota, USA.

Smith, E.F. (1896). A bacterial disease of the tomato, eggplant and irish potato (*Bacillus solanacearum*). Bulletin, Division of Vegetable Physiology and Pathology, United States Dept. Agric., 12: 1-28.

Smith, J.J., L.C. Offord, M. Holderness and G.S. Saddler (1995).Genetic diversity of *Burkholderia solanacearum* (Synonym *Pseudomonas solanacearum*) race3 in Kenya. Appl. Environ. Microbial., 61:4263-4268.

Tahat, M.M. and K. Sijam (2010). *Ralstonia solanacearum*: The bacterial wilt causal agent. Asian J. Plant Sci., 9 (7): 385-393.

Tohamy, M.R.A., M.M.M. Atia, F.F. Gabriel and H.A. Mater. (2007). Identification of *Ralstonia solanacearum* isolated from potato tubers, weeds, water and soil in Egypt. Zagazig J. Agric. Res., 34 (3): 445-462.

Tung, P.X. and P. Schmiediche (1995). Breeding potato for resistance to bacterial wilt (*Pseudomonas solanacearum*): looking for stable resistance. In integrated management of bacterial wilt, 173-167. Proceedings of the International Workshop (eds. Hardy. B. and French, E.R.) Int. Potato Cent. (CIP) and Indian Council of Agric. Res., New Delhi, (India).

Van Der Wolf, J.M., P.J. Van Bekkum, J.D. VanElsas, E.H. Nuhuis, S.G.S. Vrtend and M.A. Rulssen (1998). Immunofluorescence colony staining and selective enrichment in liquid medium for study the population dynamics of *Ralstonia solanacearum* (race3) in soil. EPPO Bulletin, 28:71-79.

Wallis, F.M. and S.J. Truter (1978). Histopathology of tomato plants infected with *Pseudomonas solanacearum*, with emphasis on ultrastructure. Physiol. Plant Pathol., 13:307-317.

Weller, S.A., J.G. Elphinstone, N.C. Smith, N. Boonham and D.E. Stead (2000). Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (*Taq*Man) assay. Appl. and Environ. Microbiol., 66 (7): 2853-2858.

Wenneker, M., M.S.W. Verdel, R.M.W. Groeneveld, C. Kempenaar, A.R. van Beuningen and J.D. Janse (1999). *Ralstonia (Pseudomonas) solanacearum* race 3 (biovar II) in surface water and natural weed hosts: First report on stinging nettle (*Urticadioica*). European J. Plant Pathol., 105: 307-315.

Winstead, N.N. and A. Kelman (1952). Inoculation techniques for evaluation resistance *Ralstonia solanacearum*. Phytopathol., 42: 628-634.

Yabuuchi, E., Y. Kosako, I. Yano, H. Hotta and Y. Nishiuchi (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* Gen. Nov.: proposal of *Ralstonia pickettii* (Palleroni and Doudoroff 1973) Comb. Nov., *Ralstonia solanacearum* (Smith 1896) Comb. Nov. and *Ralstonia eutropha* (Davis 1969) Comb. Nov. Microbiol. and Immunol., 39: 897-904.

عزل وتعريف *Ralstonia solanacearum* المسببة للعفن البني في البطاطس من مصادر ومناطق مختلفه بمصر

رحمة عبدالرحيم[1] - محمود محمد محمد عطية[1] - محمد رضا أحمد تهامي[1] - ناصر محمد عبدالشافي السركسي[2]

١- قسم أمراض النبات – كلية الزراعة – جامعة الزقازيق – مصر

٢- قسم النبات الزراعي – كلية الزراعة – جامعة الزقازيق – مصر

يعتبر مرض العفن البني في درنات البطاطس أو الذبول البكتيري في نباتات العائلة الباذنجانية من أهم أمراض البطاطس التي يطبق عليها الحجر الزراعي على مستوى العالم ومصر، لذلك تم خلال هذه الدراسة عزل وتعريف مسبب المرض من عدة مناطق بمحافظات المنوفية والغربية والشرقية ومصادرمختلفة اشتملت علي درنات البطاطس والتربة والماء والحشائش وذلك باستخدام طرق سريعة ودقيقة، أوضحت النتائج أنه من بين ٨٠ عينة، تم عزل ١٤عزلة بكتيرية ممرضة من درنات البطاطس، والماء، والتربة والحشائش، تم الحصول على ١٠ عزلات من محافظة المنوفية و٤ عزلات من الغربية ولم يتم عزل المسبب المرضي من محافظة الشرقية وعلى الخصوص منطقة الصالحية، تم اختبار القدرة المرضية للعزلات المتحصل عليها بالحقن في بادرات الطماطم، وكانت العزلة رقم (١) المعزولة من عينات التربه وعزلتي رقم (٤، ٥) المعزولة من درنات البطاطس وعزلة رقم (٩) المعزولة من الماء وعزلة رقم (١١) المعزولة من الحشائش أكثر العزلات قدرة علي إحداث المرض علي بادرات الطماطم، أوضحت نتائج التعريف أن العزلات المتحصل عليها (١٤ عزلة) تتبع البكتيريا *Ralstonia solanacearum*، وذلك طبقا لخصائصها البيوكيميائية والفسيولوجية، وتنميتها على بيئة كنج B وعلى بيئة SMSA، تم تأكيد النتائج بإستخدام الأجسام المضادة المعلمة فلورسنتيا، وعن طريق تحليل الحمض النووي DNA وذلك باستخدام Real-time *(Taq-* Man) PCR وعند إختبار المدي العوائلي للبكتريا المسببة لمرض العفن البني، وجد أنها تسبب أعراض الذبول علي بادرات الطماطم والباذنجان والفلفل والخبيزة الأفرنجي، ولم تظهر أعراض الذبول علي نباتات الذرة والفول السوداني والبصل والفول البلدي والدخان، أوضحت النتائج أن جميع أصناف البطاطس تحت الإختبار (pliny, draga, kara, alexant and mundial) كانت حساسة للإصابة بدرجات مختلفة حيث ظهرت عليها أعراض الذبول البكتيري عند الحقن باستخدام عزلتين من البكتيريا أحدهما معزولة من الدرنات T4 والأخرى معزولة من الماء W9 .

المحكمـــون:

١- أ.د. ناجي ياسين عبدالغفار أستاذ الأمراض البكتيرية – كلية الزراعة – جامعة عين شمس.

٢- أ.د. دولت أنور عبدالقـــادر أستاذ أمراض النبات المتفرغ – كلية الزراعة – جامعة الزقازيق.