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Scot Marker as a Tool to Determine the Genetic Diversity of Bacterial Wilt Pathogen *Ralstonia solanacearum*

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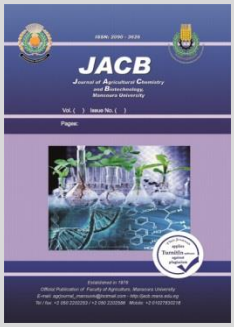

ABSTRACT

The causal organism of brown rot disease *Ralstonia solanacearum* is a common and dangerous pathogen affecting several economically important crop species. *R. solanacearum* has a wide range of occurrence and severity in potatoes and other nightshades such as eggplant, tomatoes and green peppers. The main goal of this study is to detect the genetic variation between 14 isolates of *R. solanacearum* from diverse geographic ecoregions of Egypt using the Start Codon Targeted Technique (SCoT). Ten SCoT primers were used to amplify SCoT markers, taking into account genetic diversity and phylogenetic distance between target isolates. The results showed 143 bands in a range of 130 to 2100 bp, of which 112 bands (76.40%) were polymorphic and 31 bands (23.60%) were monomorphic. Nei's genetic distance and UPGMA method divided the dendrogram of the fourteen isolates into two major groups. Isolates 1, 11, 12, 13 and 14 were grouped together in Group I, while Group II was divided into two main subgroups, in which isolates 5 and 6 were grouped in Subgroup I and isolates 2 and 3 in Subgroup II became. The third group was divided into three subgroups, with the first subgroup including Isolate 4 and Isolate 7. The second subgroup contained isolate 8 and isolate 9. Interestingly, isolate 10 was only grouped in the third subgroup and showed a diversity profile of unique and less related. to other isolates. Regarding disease diversity, this isolate may have different characteristics that will be considered in the future.

***Keywords*: *Ralstonia solanacearum*, SCoT markers, genetic diversity**

INTRODUCTION

Ralstonia solanacearum is a significant microorganism infectious agent worldwide, inflicting the wilt of many necessary crops, principally in the family Solanaceae family, in temperate, tropical and climatic zone areas in the world (Ahmed *et al*., 2013; Genin, 2010; and Mansfield *et al*., 2012). Various future crops develop bacterial wilt as a results of soil-borne pathogens colonizing (Ylem Nguyen and Ranamukhaarachchi, 2010). The pathogen has a significant host range, hundreds of species within fifty plant families (Cueva *et al*., 2013). *R. Solanacearum* can be wintered in debris of plants (Elphinstone, 2005).

Although cold climates, *R. Solanacearum* can survive for up to 40 years 20-25°C in water) but not suitable for cultivation, bacterial populations are exposed to harsh conditions see below (Temperature, PH, salinity, etc.). Moreover, It lives in cool conditions and can transition to a viable but non-culturable state (Coutinho, 2005). *R. solanacearum* was divided into five races depending on variations in host range (Coutinho, 2005 and Buddenhagen *et al*., 2001). Unlike other phytopathogenic bacteria, *R. solanacearum* shows no intergenic interfaces. In its place, these are defined by the virulence of each isolate on various plant hosts to better understand the genetic relationships of *R. solanacearum*. Recently, studies based on the DNA of Complexes of *R. Solanacearum* species were used.

Hayward and Hartman, 1994 work on Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), while Cueva, *et al*., 2013; Prasanna Kumar, *et al*., 2012 and Sumangala *et al*., 2012 reported about Randomly Amplified Polymorphic DNA (RAPD) and Start Codon Targeted (SCoT), all these techniques were applied to study the genotypic diversity of *R. solanacearum* strains. SCoT is one of the most powerful approaches in genetic research, analyzing genetic variability in plants, fungi and bacteria and mapping first linkage maps of species and specific pathogens. The SCoT approach of has been successfully used to analyze genetic variation (Ranamukhaarachi and Nguyen, 2010; Cueva, 2013; Prasanna Kumar, *et al.*, 2012; and Sumangala, *et al.*, 2012).

The cultivation of resistant cultivars was the main control method. However, pathogen density, pathogen strain and various soil variables have a significant impact on the maintenance of bacterial wilt resistance in potatoes and tomatoes. Various Egyptian research groups have worked to develop cultivars resistant to bacterial wilt, but few species have shown sustained resistance. The grafted commercial varieties introduction into wilt-prone and wilt-resistant rootstocks has proven to be an effective control strategy (Black, et al., 2003 and Rashid, *et al.*, 2000). However, grafting is popular with farmers because, as several AVRDC studies show, grafted seedlings are more expensive with less yield as compared with embedded sprouts. Soil amendment, crop rotation, biological control and field cleaning are generally ineffective as a management strategies. This is because there is no single control approach that provides effective long-term control of disease.

There is no one-size-fits-all approach to disease management, so multiple modalities need to be integrated. Recently, biochemical analysis of *R. solanacearum* is applied by (Rahman, *et al.*, 2011 and Serag, *et al.*, 2020). The generation of resistant or tolerant potato cultivars through detection programs

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requires the population structure of this extremely diverse disease, even the potential to expect the biological, ecological, and epidemiological characteristics of *R. solanacearum* has been demonstrated, Clearly a better understanding is needed. Although the potential local population is also important, accurately assessing the genetic diversity of field bacterium populations is necessary for the development of effective diagnostic protocols and the use of resistant cultivars. Little work is achieved about the degree of genotypic diversity in this bacterial population. The SCoT marker was used because it can detect genome-wide mutations compared to other approaches. Several genera of bacteria have used their SCOT markers for strain identification, identification, and other genetic studies over the past two decades (Khoodoo, *et al.*, 2002). The research was achieved to help the potential of the SCoT as a marker to assess the diversity of *R. solanacearum*. which was sourced from farms in various regions of Egypt.

MATERIALS AND METHODS

1. Collection and analysis of samples

Studies were conducted to show the situation of bacterial abundance and severity in Egypt, particularly in the delta region, and to obtain isolates of *R. solanacearum*, the relevant driver of potato wilt. All early ten isolates were kindly provided by the Laboratory, Faculty of Agriculture, Moshtohor, University of Benha. Namely, three governorates with three main growing areas, Qalyubia (Moshtohor and Beltan), EL-Behira (Kom Hamada) and El-Gharbia (Kafer Elnasria) for potato stalks and tubers, and one main growing area, namely Giza, for potatoes. Three to four locations in each region and three farms in each cultivated area were screened to assess the incidence and bacterial wilt severity. Twelve samples of infected plants were gathered from each site evaluated and sent to a laboratory for isolation of multiple isolates of *R. solanacearum*.

2. Assessment of disease incidence and severity

The wilt status of potatoes was assessed based on the occurrence and severity of wilt. Wilt distribution information was collected at least three times from their three different farms in each growing area. Five infected plants were randomly selected from each farmer's field at each site to determine the degree of wilt in each growing area. Five matching plants were randomly selected from each farmer's fields at each field to detect the bacterial wilt severity score of each growing field. The total number of plants in each field is 100 plants. According to the severity scale previously issued by Ahmed *et al*., 2022, the severity of potato brown rot was assessed: 1 = no symptoms, 2 = upper young leaves wilted, 3 = two leaves wilted, 4 = four or more wilted leaves, and 5 = plant dieback.

A hypersensitivity reaction (HR) test was performed on the isolate using potato leaves. Bacterial suspensions were adjusted to an optical density of 0.2 at 600 nm, or approximately 108 colony forming units (CFU) per ml using a SPECTRONIC 20-D (Dhital, *et al.*, 2001).

It was done one side of the expanded tobacco leaf and it was treated with 1 milliliter of suspension of bacteria and the other side was washed with sterilized distilled water as a control. After bacterial invasion, observations were made daily for HR and leaved for five days. The host of *R. solanacearum* which is potato plants (*Solanum tuberosum* L. cv Spunta) was grown in sandy loam pots (25 cm) for 6-8 weeks at the Moshtohor Faculty of Agriculture, University of Benha, Egypt.

To inoculate three plants from each host with each bacterial strain, a sterile 100-microliter micropipette was given for insertion into the leaf axils of mature leaves. Each bacterial strain was inoculated into three plants from each host by using a sterile micropipette tip for insertion containing 100 µL from the axillary tip of a completely expanded leaf. The pipette tip remained in place until the inoculum was absorbed. Inoculated plants were monitored daily for signs of virulence and severity. Weekly assessments of disease severity were performed for 28 days applying the following scales and rating system. 1- indicates no symptoms, 2- indicates two wilted leaves, 3- indicates 3 wilted leaves, 4- indicates four or more wilted leaves, 5- indicates plant death (Oluoch *et al*., 2022).

3. Identification, and purification of the isolated *R. solanacearum*

Detection of the isolated and purified *R. solanacearum* were previously recorded by Rahman *et al*. (2011) and Ahmed *et al*. (2013). The mucus of bacteria was seeded onto nutrient agar from the cut end of each sample (NA). Incubation of the plates at 28°C was applied for a minimum of 24 hours. Each colony of each isolate of *R. solanacearum* was plated on triphenyltetrazolium chloride to purify the isolate (TTC or TZC). To validate *R. solanacearum* isolates, the virulence assay was performed using the soil inoculation method on 1-month-old seedlings from each host. Only one R colony was selected for each group of isolates, Solanacearum specimens showing virulence, irregularity, and a creamy white color with a pink center for virulence assays. A bacterial suspension of each isolate (approximately 108 cfu/ml) from a pool was injected into the leaves of 30–40-day old potato plants. The suspensions of *R. solanacearum* were injected into the intracellular spaces of the leaves using a hypodermic syringe. Daily observations of hypersensitivity reactions (HR) were made during the first 5 days after invasion. The isolates of *R. Solanacearum* were kept in 10% skim milk and at -20°C for future biochemical testing.

2.4. Extraction and quantification of genomic DNA

In 100 ml of LB medium, *R. solanacearum* was inoculated and incubated at 30 ºC overnight. Total DNA was extracted from *R. solanacearum*. Collect the cell pellet by centrifugation (8,000 rpm, 10 min, 4 °C), wash the pellet with sterile NaCl-saline (0.5%), harvest at 14,000 rpm for three minutes and wash for one minute with sterile distilled water. This step was repeated three times to reduce the amount of bacterial sugars that negatively affected DNA filtration. The precipitate was reconstituted with five ml of solution "A" containing 2 mg/ml lysozyme and at 37°C incubation applied for one hour. The solution was then treated with 0.5 mL of SDS (1%) and left at room temperature for 15 minutes. Equal volumes of phenol/chloroform and cell lysate were combined, and the mixture placed on ice for 5 minutes. Centrifugation at 10,000 rpm for 20 minutes at 4°C then removed residual proteins and the DNA-containing supernatant was treated once more with an equal volume of phenol/chloroform and left on ice for 5 minutes. Then for 20 minutes, centrifuge at 10,000 rpm at 4°C. To the supernatant containing DNA, 0.1 volume of 3 M sodium acetate (pH 5.0) and 2.5 volumes of absolute ethanol were added. The DNA was pelleted by centrifugation at 14,000 rpm for 20 minutes at 4°C. The supernatant was discarded, and the pellet was gently rinsed twice with 5 mL of 70% ethanol and allowed to dry for 15 minutes. Each pellet was dissolved in 100 microliter TE buffer. RNase was added (10 µl) at 37°C to

the foal and allowed to stand for two hours. This DNA will be used for all subsequent uses.

5. Estimated DNA concentration

2 µL of parental DNA sample with 3 microliters of DNA die and 3 µL of DNA marker (1K bp DNA ladder) were loaded on 1% agarose gel. Comparing the DNA fluorescence level sample with the various bands of DNA size markers is used to detect the DNA concentration.

2.6. Start Codon Target "SCoT" analysis

a) PCR-SCoT Reactions

Polymorphisms were detected using 10 SCoT primers, as shown in Table 1. 2.5 µl primers (10 pmol) were used to perform the amplification reactions were performed using, 2.5 µl template DNA (10 ng) in a 25 µl reaction volume include 12.5 µl master mix. 7.5 µl double-sterilized d.dH_2O according to Ibrahim, *et al*., (2019). In this study 14 isolates of *R. solanacearum* were screened using these SCoT primers.

Table 1. Primers SCoT name and Sequence.

Primer Name	Sequence
SCoT-01	5'-ACGACATGGCGACCACGC-3'
SCoT-02	5'-ACCATGGCTACCACCGGC-3'
SCoT-03	5'-ACGACATGGCGACCCACA-3'
SCoT-04	5'-ACCATGGCTACCACCGCA-3'
SCoT-05	5'-CAATGGCTACCACTAGCG-3'
SCoT-06	5'-CAATGGCTACCACTACAG-3'
SCoT-07	5'-ACAATGGCTACCACTGAC-3'
SCoT-09	5'-ACAATGGCTACCACTGCC-3'
SCoT-11	5'-ACAATGGCTACCACTACC-3'
SCoT-12	5'-CAACAATGGCTACCACCG-3'

b) Thermocycling Profile PCR

After an initial denaturation cycle lasting five minutes at 94° C, performing of PCR amplification on a Perkin-Elmer/Gene Amp® PCR system 9700 (PE Applied Biosystems) rated for forty cycles. Each cycle included 45 seconds for denaturation at 94°C, 50 seconds for annealing (annealing temperature was variable based on the TM of each primer sequence), and at 72°C, a one minute extension step. In the final cycle, the primer extension step was extended to seven minutes at 72°C.

c) Detection of products of PCR

The separation of PCR products was achieved by electrophoresis on 1.5% agarose gels with ethidium bromide (0.5 µg/ml) in 1X TBE buffer at 95 volts. Photographing of PCR products was done and seen under UV light using a gel documentation system (BIO-RAD 2000).

7. Evaluation and Data Analysis

The SCoT analysis only used distinct bands that could be visually classified as present (1) or absent (0) for the complete samples, and polymorphic bands and polymorphic bands were included in the final data set. Both monomorphic bands were included. A binary statistical matrix was then created. An unweighted pair-group approach with arithmetic mean was then used to determine the genotypic cube similarity matrix (UPGMA). This UPGMA was applied to obtain the dendrogram or phylogenetic tree according to PAST software version 1.91 (Hammer and Ryan 2001).

RESULTS AND DISCUSSION

Results

1. Differences in the intensity and wilt frequency in some fields of specific regions

There are three main regions (Qalyubia, El-Bahira, and El-Gharbia) for potato cultivation. The three areas were screened to determine the prevalence and severity of potato wilt disease. According to published reports, there were significant differences in the incidence of brown rot concerning the main growing fields tested Ghorai, *et al*., 2022 and Tessema, *et al.*, 2022.

2. Identifying the isolated isolates of of *R. solanacearum*

Fourteen *R. solanacearum* isolates were collected from samples of wilting potato stems and tubers maintained at different sites investigated. All the isolates of *R. solanacearum* collected from infected potato plants with bacterial wilt (stem cuttings and tubers) produced colonies with cream-colored on NA medium 24 hours from inoculation. Biochemical examination showed that all segregating groups were *R. solanacearum* (Ahmed, *et al.*, 2022 and Kemboi, et al., 2022). The results of the pathogenicity test revealed that all isolates of the *R. solanacearum* from potatoes can cause wilting symptoms in potato seedlings.

3. Biovar and race differentiation of *R. solanacearum* isolates

In situ collected isolates, biotypes and isolates were identified to assess the phenotype of *R. solanacearum*. The biotypes of the first 10 isolates from *R. solanacearum* from potato stems and tubers were detected using disaccharides and hexose alcohols (Tessema, *et al*., 2022).

Biotyping consequences display that each one *R. solanacearum*. *R. solanacearum* can ferment disaccharides (sucrose, lactose, maltose, etc.) and sugar alcohols (mannitol, sorbitol, dulcitol, etc.) in three-five days. The shadeation alternate signaled an oxidation reaction. The consequences confirmed a alternate in hue from blue to yellow, indicating that the bacterial isolates have been oxidizing carbohydrates. Therefore, all *R. solanacerum* isolates are from Biovar II, however the manage plates for numerous sugars and sugar alcohols have been unchanged. The cultivar dedication of *R. solanacearum* cannot be accomplished biochemically. *R. solanacearum* virulence research in distinct hosts have enabled the identification of these strains. Pathogenicity check consequences confirmed that every potato *R. solanacearum* isolate examined on this have a look at changed into capable of result in wilting signs and symptoms in inoculated potato plants. As a result, race three changed into contained in all *R. solanacearum* isolates that purpose potato wilting. Therefore, all isolates of *R. solanacearum* that purpose bacterial wilt in potatoes from 3 distinct developing areas are assigned to race three.

4. SCoT analysis of *R. solanacearum* isolates

To assess the genetic diversity analysis of *R. solanacearum* isolates which isolated from potatoes from different fields, a total of 14 isolates were analyzed, representing 3-4 isolates from each growing region. I chose isolate. Despite the small number of isolates for genetic diversity studies, this is the first time to determine whether SCOT markers are useful for the analysis of genetic diversity in *R. solanacearum* in Egypt. The first 10 primers evaluated showed more amplification results with higher intensity, less smear and higher resolution. (Figure 1 and SCoT-2). Ten primers produced different banding patterns, with band numbers ranging from 8 (SCoT-5) to 20 (SCoT-1). Primers amplified the great number of polymorphic bands. PCR amplicon sizes ranged from 130 to 2100 bp. Ten primers produced 14.3 evaluable bands per primer and 11.2 polymorphic SCoT markers per primer (Table 2). The primers of SCoT techniques produced 143 bands of which 112 were polymorphic (76.40%). Polymorphic information content (PIC) values varied from 0.34 to 0.37 with a mean of 0.36.

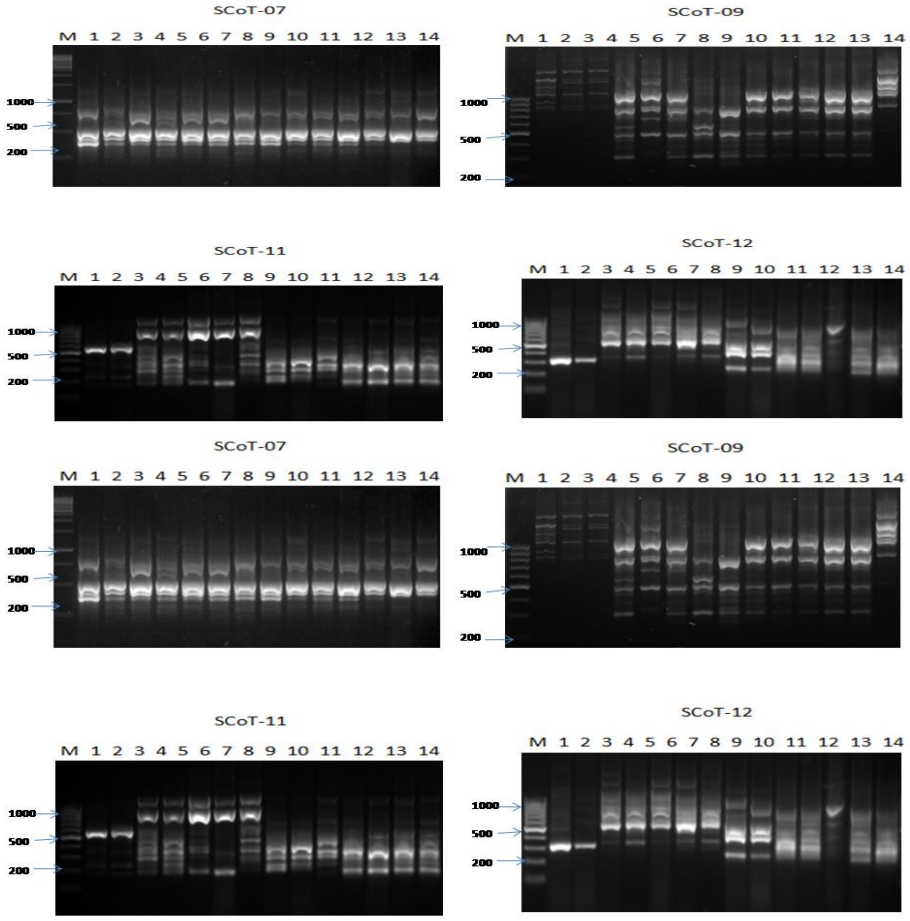

Fig. 1. SCoT amplification profiles of fourteen isolates of *R. solanacearum* using primers SCoT-1, SCoT-2, SCoT-3, SCoT-4, SCoT-5, SCoT-6, SCoT-7, SCoT-9, SCoT-11 and SCoT-12. M: 100 bp DNA ladder, Lane 1-14 isolates of *R. solanacearum*

Table 2. SCoT primers, total number of bands (TB), total number of monomorphic bands (M), total number of polymorphic bands (PB), percentage of polymorphism (%P), polymorphism information content (PIC) and size of bands observed in fourteen isolates of *R. solanacearum*.

Primer	TB	MB	PB	% P	F	PIC	Size of bands
SCoT-01	20	3	17	85	0.5	0.37	130-2100bp
SCoT-02	15	1	14	93	0.5	0.37	150-1400
SCoT-03	18	3	15	83	0.4	0.35	220-2000
SCoT-04	16	2	14	88	0.3	0.34	290-1800
SCoT-05	8	4	4	50	0.7	0.34	210-630
SCoT-06	17	5	12	71	0.5	0.37	250-1850
SCoT-07	12	5	7	58	0.7	0.37	250-1200
SCoT-09	18	4	14	78	0.4	0.37	310-1700
SCoT-11	10	2	8	80	0.6	0.37	200-1250
SCoT-12	9	2	7	78	0.5	0.37	210-1750
Total	143	31	112	-	-	-	
Average	14.3	3.1	11.2	76.4	0.5	0.36	

5. The isolates similarity indices

Isolates with the highest isolate similarity index (Si) values were isolates 6 and 5 (83%), followed by isolates detected in isolates 7 and 4 (79%), followed by isolate 12 and isolate 11 (79%). The similarity index (Si) value for isolate 13 among isolates 3 was 56% (Table 3).

6. Genetic relationships between *R. solanacearum* isolates

using Nei's genetic distance and UPGMA, dendrograms revealed that the *R. solanacearum* isolates segregated into two main clusters (Figure 2). Isolates 1, 11, 12, 13, and 14 were grouped into cluster 1, and cluster 2 was divided into two major subclusters. Subcluster 1 contains isolates 5 and 6 in one subcluster and isolates 2 and 3 in a second subcluster. The second subcluster is divided into two subclusters and one isolate (isolate 10). The first subcluster contained isolates 4 and 7 and the second subcluster contained isolates 8 and 9. The greatest genetic distance (0.70) was detected between isolates 1 and 14.

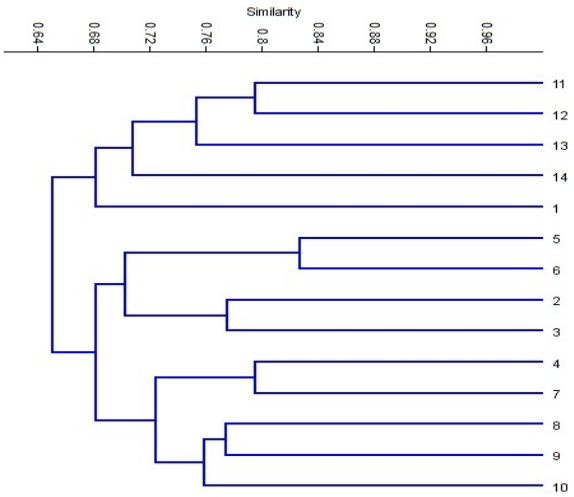

Fig. 2. Tree SCoT marker generated by unweighted pair-group method using arithmetic mean (UPGMA) show dissimilarity between 14 isolates of *R. solanacearum*.

Table 3. Nei's (1972) genetic distance values between the fourteen isolates of *Ralstonia solanacearum*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.0													
2	0.73	1.0												
3	0.62	0.77	1.0											
4	0.62	0.63	0.73	1.0										
5	0.67	0.72	0.75	0.70	1.0									
6	0.62	0.64	0.70	0.77	0.83	1.0								
7	0.61	0.62	0.69	0.79	0.66	0.75	1.0							
8	0.69	0.68	0.60	0.67	0..63	0.69	0.68	1.0						
9	0.68	0.67	0.65	0.78	0.67	0.75	0.70	0.77	1.0					
10	0.68	0.67	0.65	0.75	0.68	0.72	0.76	0.76	0.76	1.0				
11	0.70	0.62	0.62	0.66	0.65	0.73	0.67	0.68	0.66	0.74	1.0			
12	0.69	0.61	0.59	0.68	0.69	0.72	0.66	0.68	0.65	0.68	0.79	1.0		
13	0.67	0.58	0.57	0.66	0.63	0.66	0.65	0.71	0.64	0.67	0.72	0.78	1.0	
14	0.66	0.62	0.60	0.61	0.59	0.59	0.60	0.67	0.62	0.69	0.76	0.70	0.66	1.0

Discussion

In this study, there was significant heterogeneity in the frequency and bacterial wilt severity via different potato-growing regions, suggesting variability in *R. solanacearum* populations. This is most likely a result of altered host responses and genetic diversity of the populations of the pathogen. Variations were documented for the frequency and severity of wilt disease as a result of pathogen host plant type, the bacterial wilt pathogen phenotype and genotype, its wide range geographic appearance, and different conditions of environment that suite the bacterial wilt disease. The SCoT study found that all *R. solanacerum* can be divided into two groups. Horita and Tsuchiya (2000) found low average similarity among Biovar III strains, which are divided into five groups. Isolation of 14 *R. solanacearum* isolates was demonstrated by genetic distance-based dendrogram by Nei (1972) using UPGMA, *R. solanacearum* is divided into his two groups, Cluster one and Cluster two. RsB-1, RsB-2, RsB-3, and RsP-1 are assigned to the main cluster, and clusters RsP-2, RsP-3, and RsP-4 are assigned. Although it is common for isolates from the same host to be distributed in the same clusters, The one isolates group isolated only from potato, RsP-1, is associated with the three isolates (RsB-1, RsB-2, and RsB-3). These results revealed that this group of isolates is a clone of field isolates that were first introduced to the region. Clustery Y has divided into two groups, RsP-2 belongs to one and RsP-3 and RsP-4 belong to the other. According to this study, RsB-1 and RsB-2 were more closely related in cluster 1, with the smallest genetic distance (0.0357), while RsB-2 and RsP-4 had the largest genetic distance (0.4293). This was on remotely connected cluster 2. In this study, all primers produced different banding patterns, with 96.4% of polymorphic bands from Solanacearum isolate. All these primers exhibited highly polymorphic banding patterns, indicating considerable diversity within the field population of this soil-borne bacterium. It is unknown when this fungus was introduced to the country, but the types found are astonishing. Genetic diversity was very high, with yields ranging from 57% to 83% similarity between isolates. Indeed, fragment size frequencies reflect regions that differ significantly from one isolation to another. Low-band frequency variability means that these specific sections of the *R. solanacearum* genome play sensitive roles in survival and are thus tolerant to change.

The results showed that *R. solanacearum* exhibits very high degrees of genetic variations among isolates derived from different loci clustered at low levels of similarity index, regardless of host. indicates that This explains the high degree of variability observed in this R group. Solanacerum is also related to soil-dwelling plants. Pathogens have to cope with the changing soil environment while existing saprophytically. Soil traits can play an important role in genetic divergence, as shown for other soil-borne bacterial species by Nguyen and Ranamukhaarachchi (2010). Ecologists and population geneticists have long believed that environmental structure is related to the maintenance of microbial diversity, Kassen (2002). This bacterium is known for its ability to survive in a variety of ecological environments and to interact with a variety of biotic and abiotic stimuli. This may explain the increased variability in bacterial evolution and maintenance.

To our knowledge, this publication is considered as one of view publications on the genetic diversity of *R. solanacearum* field isolates which were isolated from different locations in Egypt. However, more isolates from different host plants are required to obtain stronger correlations between isolates and host sources. Despite these facts, the current study suggests that SCoT markers are one of the more sensitive, simple, and efficient techniques for analysis of the genetic diversity within *R. solanacearum*, suggesting their genetic relationships and tracking well. However, using more bacterial isolates and primers for SCoT and other molecular markers to assess the genetic diversity of *R. solanacearum* show the complex population structure of this soil-borne bacterium in Egypt. The results revealed that host resistance is the most effective way to control bacterial wilt. The first step in developing a resistance breeding strategy is to collect information and data about the presence of variability in local pathogen populations and determine host range. Finally, the information presented in this study on *R. solanacearum* strains will help initiating and optimizing pathogen population assessment and breeding efforts in Egypt.

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استخدام المعلم الجزيئ SCoT لتحديد التنوع الوراثى للمسبب المرضى للذبول البكتيرى *Ralstonia solanacearum*

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الملخص

مرض الذبول البكتيرى ، الذي تسببه *Ralstonia solanacearum* هو مرض شائع وخطير يؤثر على العديد من أنواع المحاصيل المهمة اقتصاديًا. يُظهر *R. solanacearum* نطاقًا واسعًا من التواجد والشدة في البطاطس ومحاصيل العائلة الصليبية الأخرى مثل الباذنجان والطماطم والفلفل الأخضر. تهدف هذه الدراسة إلى تقييم التنوع الوراثى بين 14 عزلة من *R. solanacearum* من مناطق جغرافية بيئية مختلفة في مصر من خلال تطبيق تقنية بدء الكودون المستهدف (SCOT). تم استخدام عشرة بادئات SCOT لتضخيم علامات SCOT ، مع الأخذ في الاعتبار التنوع الوراثي والمسافة التطورية بين العزلات المستهدفة. أظهرت النتائج أن هناك 143 حزمة بأحجام تتراوح من 130 إلى 2100 pb ، منها 112 حزمة (76.40٪) متعددة الأشكال و 31 حزمة (23.60٪) أحادية الشكل. المسافة الوراثية لـ Nei وطريقة (UPGMA) قسمت الشجرة الوراثية للعزلات الأربعة عشر إلى مجموعتين رئيسيتين. كانت العزلات 1 و 11 و 12 و 13 و 14 معًا في المجموعة الأولى ، بينما تم تقسيم المجموعة الثانية إلى مجموعتين فرعيتين رئيسيتين ، حيث تم تجميع العزلات 5 و 6 في المجموعة الفرعية الأولى وتم جمع العزلات 2 و 3 في المجموعة الفرعية الثانية. تم تقسيم المجموعة الثالثة إلى ثلاث مجموعات فرعية حيث اشتملت المجموعة الفرعية الأولى على العزلة رقم 4 والعزلة رقم 7. كما احتوت المجموعة الفرعية الثانية على العزلة رقم 8 والعزلة رقم 9. ومن المثير للاهتمام أن العزلة 10 كانت بمفردها في المجموعة الفرعية الثالثة ، مما أظهر مظهرًا فريدًا للتنوع وعلاقة أقل بالعزلات الأخرى. يمكن أن تكشف هذه العزلة عن خصائص مختلفة فيما يتعلق بخطورة المرض ، والتي سيتم النظر فيها في المستقبل.

الكلمات الدالة : التنوع الوراثى- الذبول البكتيرى-البرايمرات البادئة