International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 44 No. 1 Nov. 2024, pp. 33-42
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http://www.ijias.issr-journals.org/

Influence of pollution of the Yamoussoukro lakes watershed on the genetic diversity of *ralstonia solanacearum*: Implications for tomato productivity in Yamoussoukro

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ABSTRACT: The watershed of the central lake system of the city of Yamoussoukro in Côte d'Ivoire is subject to various forms of pollution. *Ralstonia solanacearum* (*R. solanacearum*), responsible for bacterial wilt of tomato (*Solanum lycopersicum*; 2n=2X=24Chr.), persists in market garden environments and causes tomato yield losses of up to 100%. The aim of this study was to assess the genetic diversity of *R. solanacearum,* its impact on tomato cultivation in Yamoussoukro, and to obtain genotypes of *Solanum lycopersicum* resistant to *R. solanacearum*. Samples of water, soil and wilted tomato plants was collected from June 2022 to August 2023, at six growing sites around three selected lakes. *R. solanacearum* was isolated on SMSA medium and confirmed by PCR. Genetic diversity of strains was assessed by rep-PCR. Biovar, race and pathogenicity tests were used to assess the virulence levels of strains at each site studied, and to select tomato plants potentially resistant to *R. solanacearum*. The results showed a significant variation in the incidence and severity of bacterial wilt from one site to another. The 63 *R. solanacearum* strains isolated and confirmed by PCR belonged mainly to biovar 3 and race 1. Phylogenetic analysis of *R. solanacearum* showed three distinct groups with around 70% similarity. Two groups was made up solely of strains from Lake E. A single group included all strains from all lakes. Strains from the LacE-Ch2 site showed a particularly high aggressiveness of 88%. Of the 42 tomato cultivars tested in the greenhouse, three showed no symptoms of bacterial wilt.

KEYWORDS: bacterial wilt, genetic resistance, pathogenicity, rep-PCR, taxonomy.

1 INTRODUCTION

Bacterial wilt of tomato, caused by the bacterium *R. solanacearum* (*R. solanacearum*), is a dreaded disease worldwide due to its devastating impact on crops. This disease greatly affects food security by reducing the quantity and quality of tomatoes produced [1]. *R. solanacearum* encompasses a wide range of populations that differ in host range, geographical distribution, pathogenicity and genetic characteristics. It is divided into five races based on host range [2] and six biovars based on the use of three disaccharides and three hexose alcohols [3]. Molecularly speaking, this bacterium has a circular genome of around 5.8 million base pairs, encoding numerous proteins involved in pathogenicity and virulence. Polymerase chain reaction-based DNA fingerprinting (rep-PCR) is a rapid, reliable and relatively inexpensive method for studying the genetic diversity of bacteria [4]. There are many highly conserved repetitive DNA sequences present in the genome of Gram-negative bacteria that can be used to study their genetic diversity, namely palindromic extragenic repetitive (REP) sequences [5] and enterobacterial consensus intergenic repetitive (ERIC) sequences [6].

In Yamoussoukro, the lakes watershed, where most of the region's vegetable crops are grown, presents various types of pollution conducive to bacterial propagation, according to several authors [7], [8], [9]. Morever, *R. solanacearum,* which causes bacterial wilt, can maintain itself in the rhizosphere of weed hosts, in plant debris and in healthy host plants, at a soil depth that allows it to be compete less with other microorganisms [10], thanks to environmental conditions that are favorable to its development. This pathogenic bacterium is known to colonize the vascular tissues of tomato plants, leading to rapid leaf wilting, tissue dieback and ultimately death of the infected plant [11]. Thus, *R. solanacearum* could prove detrimental to the sustainability of tomato production in Yamoussoukro [12], [13]. Knowledge of the genetic variability of *R. solanacearum* strains is important for plant breeding and, consequently, in crop improvement programs.

2 METHODOLOGY

2.1 PRESENTATION OF STUDY SITES

Three of the 10 lakes in the main lake system of the city of Yamoussoukro was selected for their anthropogenic, physicochemical and bacteriological characteristics [7]. These are Lake A, characterized by purely agricultural pollution; Lake B, characterized by pollution mainly from domestic waste (household, commercial); and Lake E, characterized by pollution from livestock waste and sewage (household and hospital). Two fields (Ch1 and Ch2) identified in the market gardening areas around each lake served as study sites, from January to August 2023. In all, the study was carried out at six sites: LacA-Ch1, LacA-Ch2 of Lake A, LacB-Ch1, LacB-Ch2 of Lake B, and LacE-Ch1, LacE-Ch2 of Lake E (Fig. 1).

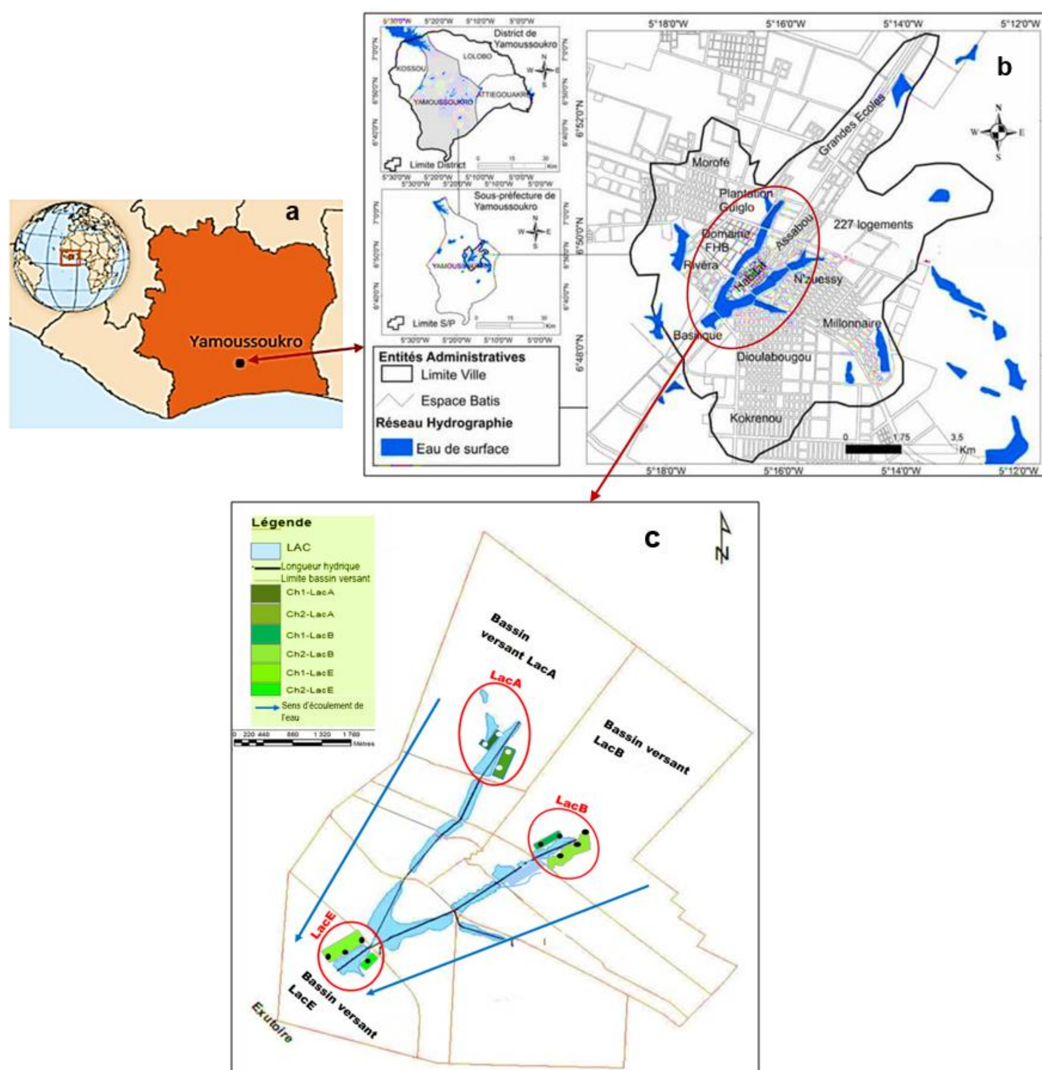

Fig. 1. Map of the town of Yamoussoukro and its hydrographic network [7]

a. Yamoussoukro in Côte d'Ivoire; b. Yamoussoukro town; c. Yamoussoukro Lakes

2.2 SAMPLING OF TOMATO PLANTS, IRRIGATION WATER, SOIL AND BACTERIAL FLUX TEST

A total of 148 lake irrigation water samples, 142 wilted tomato plants and 252 soil samples were sampled from June 2022 to August 2023. In each zone, 4 tomato fields at least 100 m apart were selected. In each field, samples of tomato plants and soil were taken from 3 locations (3 samples per field) at least 20 m apart. From each location, two concentric circles with radii of 3 m and 6 m were drawn. In the first circle, four equidistant sampling points are defined and in the second circle, 8 equidistant sampling points are defined (Fig. 2). This gives 12 sampling points for one sample. The soil was taken from the foot of the wilted tomato plants using an auger in the 0-20 cm depth stratum. The soil from each dig (550 to 600 g per dig) was collected in a bucket and mixed to form a composite sample (6.6 kg to 7.2 kg) of 12 primary samples. This mixture was placed in a polyethylene bag coded according to area, field and repeat. All the equipment is rinsed with water and then disinfected with 10% bleach at 12° before moving from one sample to another [14]. For irrigation water of lake origin, 500 ml of water was taken at each sampling point using the Canadian water sampling method, in a U-shape at a depth of 50 cm [14]. All samples were transported to the laboratory in a cooler at 4°C.

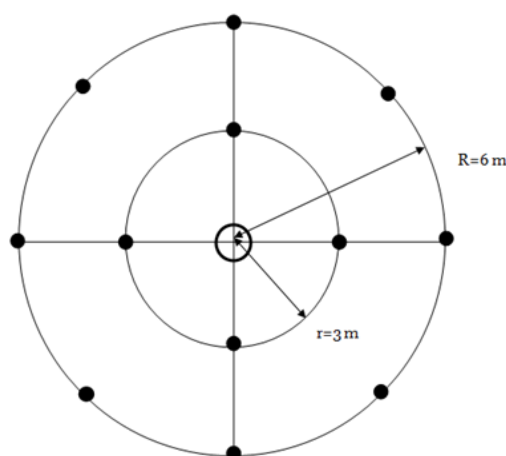

Fig. 2. Diagram of soil sampling

•basic sampling zones
○ sampling zone

2.3 ASSESSMENT OF DISEASE INCIDENCE AND SEVERITY AT CULTIVATION SITES

At the collection sites, the bacterial flux test [15] was carried out on symptomatic plants, to determine the incidence and severity of the disease in the field. After a cross-section of the stem, the tomato plants were suspended in water, and after 1 minute, those showing a viscous, creamy-white runoff were retained for the *R. solanacearum* study (Fig. 3).

The level of tomato bacterial wilt at the cultivation sites studied was assessed in terms of incidence and severity. The incidence of bacterial wilt in the field was estimated as a percentage, based on the number of plants showing bacterial flux:

$$\text{\% incidence of bacterial wilt in the field} = \frac{\text{Number of tomato plants with bacterial flux}}{\text{Number of tomato plants in the field}} \times 100$$

Bacterial wilt severity on tomato was recorded on the basis of the severity scale described by [16]: 0= no symptoms; 1= one wilted leaf; 2= two or three wilted leaves; 3= all wilted leaves; 4= bent stem or dead plant.

$$\text{Severity of bacterial wilt in the field} = \frac{\sum \text{Plant rating value}}{\sum \text{Plants}}$$

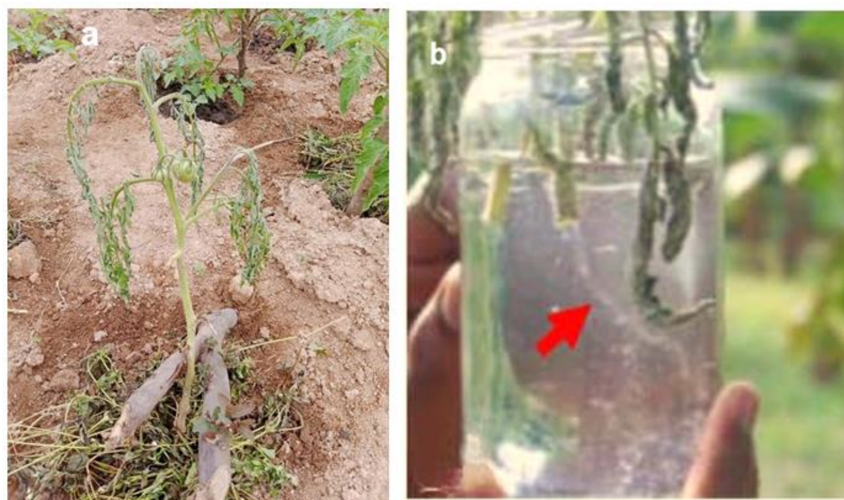

Fig. 3. Probable presence of R. solanacearum after carrying out the bacterial flow test on a wilted young tomato plant

a. Tomato plant affected by bacterial wilt; b. Bacterial flux flow

2.4 Isolation Of R. Solanacearum Bacterial Strains From Soil, Irrigation Water And Tomato Plants

To isolate *R. solanacearum* from the soil, 100 ml of sterile distilled water was added to 25 g of soil for each sample. The mixture was stirred at 250 rpm using a rotary shaker for 1 hour. The resulting solution was filtered through a 1 mm diameter sieve to remove debris and then stored in a sterile 100 ml tube for isolation of *R. solanacearum*. To isolate *R. solanacearum* from plants, 100 g of tomato plants from each sample were surface disinfected with 70% ethanol for 30 seconds and macerated in 50 ml of 10 mM phosphate buffer using a stomacher. The resulting solution was stored in a sterile 100ml tube. The irrigation water samples (500 ml) were filtered through a 0.45 µm nitrocellulose membrane, followed by washing of the filter in 50 ml of 10 mM phosphate buffer, and then stored. 1 ml of the solutions obtained was dissolved in 2 first 9 ml tubes of 10 mM phosphate buffer, then homogenised by vortexing. 1 ml of the resulting dilution (1/10) was also transferred to 2 other 9 ml tubes of 10 mM phosphate buffer (1/100), then 1 ml of the resulting dilution was transferred to 2 other new 9 ml tubes of 10 mM phosphate buffer (1/1000). Next, 100 µl of each resulting solution and the stock solution were spread directly onto semi selective medium from South Africa (SMSA) developed by [17] and further modified by [18]. Petri dishes were incubated at room temperature (28 ± 5°C) for 48 hours [17], in an inverted position as water condensation causes colonies to flow into each other, limiting separation.

2.5 DNA Extraction And Confirmation Of R. Solanacearum Bacterial Strains By PCR

The bacterial isolates obtained after culture on SMSA medium, were transferred to SMSA broth and cultured overnight at 28°C on a rotary shaker (150 rpm). Two ml of culture were used for genomic DNA extraction. Ultrapure sterile water was used as negative control. DNA was extracted by the CTAB (Cetyltrimethylammonium bromide) method. The DNA content and purity of the extracts were determined by measuring the absorbance at 260 and 280 nm [18].

PCR was performed in a GeneAmp System 9700 thermal cycler (Applied Biosystem, Foster City, USA) with the primer pair OLI-1 and Y-2 according to the protocol of [19]. The reaction mixture of 25 µl in total, contained 1.25 µl of each primer OLI-1 (20 µm) (5'-GGGGGTAGCTTGCTACCTGCC-3') and Y-2 (20 µm) (5'-CCCACTGCTGCCTCCCGTAGGAGT-3'); 0.25 µl of each dNTP (20 µm); 2.5 µl of 10X PCR-Buffer (MgCl2 15Mm); 0.1 µl of Taq polymerase (5U/ µl), 17.65 µl of ultrapure water and finally 2 µl of template DNA. PCR conditions were as follows, an initial denaturation step at 96°C for 2 min, followed by 35 cycles at 94°C for 20 s, 68°C for 20 s and 72°C for 30s. Final extension at 72°C was carried out for 10 min. The presence of the specific 288 bp PCR product was determined by 1% agarose gel electrophoresis (FMC BioProducts, USA) in 0.5 X TBE buffer. A 100 bp DNA marker (Gibco, BRL) was used as a size marker. DNA was detected by ethidium bromide staining after staining the gel in 0.5 g/ml ethidium bromide. The gels were photographed under UV light.

2.6 Genetic Typing By Rep-PCR

The genetic diversity of sixty-three *R. solanacearum* isolates was determined by a repetitive PCR assay (rep-PCR), using primer pairs BOXAIR, ERIC, and REP [20], [21]. Twenty-five µl of PCR reaction mixture containing 5.0 µl of 5X Taq PCR buffer, 2.0 µl of 25 mM MgCl2, 0.2 µl of BSA, 2.5 µl of DMSO, 1.25 µl of 25 mMdNTP, 1.0 µl of primer, 1 unit of Taq polymerase (Promega), and 1 µl of 100 ng of DNA templates was used. The rep-PCR conditions were carried out in a thermocycler (BIORAD, Thermocycler C100TM) according to the

following program: an initial denaturation step at 95°C for 7 min, followed by 30 cycles at 94°C for 1 min, 53°C for 1 min (BOX) or 46°C for 1 min (ERIC) or 48°C for 1 min (REP) and 65°C for 8 min with a final extension of 65°C for 15 min, followed by a hold time at 4°C until samples were collected. Fingerprinting of PCR products was performed by gel electrophoresis in 1.2% ethidium bromide-stained agarose and photographed using the gel documentation system (BIO-RAD, GEL).

2.7 Phylogenetic Relationships Between Strains Based On Rep-Pcr Banding Patterns

Rep-PCR fingerprint patterns were used to measure genetic similarity among strains. Each band with different electrophoretic mobility was assigned a position number and scored as 1 or 0 depending on the presence or absence of the band, respectively, for that position. Variations in band intensity among isolates were not considered as differences. Similarity coefficients for all possible pairs of strains based on fingerprint groups were estimated by the Dice method [22]. The dendrogram was generated from the similarity coefficient data by the unweighted pair group method with arithmetic mean clustering (UPGMA) [23].

2.8 Characterization Of R. Solanacearum Biovars

R. solanacearum isolates were differentiated into biovars based on their ability to utilize disaccharides (sucrose, lactose, maltose) and sugar alcohols (manitol, sorbitol, and dulcitol) using the KB009 HiCarbohydrateTM kit (HiMedia Laboratories Pvt. Limited), which contains the above-mentioned disaccharide and sugar alcohols as previously described by [3], [24]. 50 µl of bacterial suspension prepared from a 48-h-old culture of *R. solanacearum* strains (0.1 OD at 600 nm) was inoculated into each well by the surface inoculation method and incubated at 35 ± 1 °C. Color change observations were recorded after 18 hours of culture inoculation up to 21 days.

2.9 Characterisation Of R. Solanacearum Races

To determine the races of *R. solanacearum* isolated, the method of [2] based on the differential host of solanaceous crops was used. The solanaceous crops used in our study were potatoes (*Solanum tuberosum*), aubergines (*Solanum melongena*), chillies (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*). The semis were surface sterilised with 12% bleach, rinsed with sterile water and sown in a separate tray. They were grown in a greenhouse on a sterile loam-vermiculite substrate (80/20) in a randomised block design with 2 replicates. Seedlings bearing 4 to 5 leaves were used to perform pathogenicity tests for race assignment. Approximately 10 ml of bacterial inoculum of each *R. solanacearum* isolate (10^6ufc/mL), obtained from a 24-48 h culture, and were used to incubate the seedlings. Two seedlings of each strain were inoculated by soaking the injured roots for 30 minutes for up to two weeks. The inoculated plants were regularly watered and maintained at temperatures between 25°C and 28°C and 90% relative humidity. They were then kept under observation for 28 days.

2.10 Evaluation Of The Pathogenicity Level Of Isolated R. Solanacearum Strains

The sterilization of tomato seeds for their greenhouse planting, then their infection with bacterial suspensions, was carried out as previously described in the characterization of *R. solanacearum* races. The tomato variety *Rossoi* was used. The symptoms were evaluated every two days according to the scale of Horita and Tsuchiya [16] (Table 1).

Table 1. Disease symptom rating scale

Pathogenicity of the strain	Condition of the plant
0	No symptoms
1	One wilted leaf
2	Two or three wilted leaves
3	All leaves wilted
4	Bent stem or dead plant

At the end of the trial, isolation on tomato stems was performed on asymptomatic plants to verify the presence or absence of *R. solanacearum*. Sections of 2 to 3 cm of the stem of each plant were made at the collar, followed by disinfection with alcohol. These sections were transferred to 5 mL of distilled water and left for 2 hours at room temperature to promote the release of bacterial colonies in the distilled water. A volume of 50 µL of each extract was inoculated on SMSA medium. The Petri dishes were then incubated at 28°C for 3 to 4 days. Asymptomatic plants were observed to be positive for latent infection when colonies characteristic of *R. solanacearum* were observed. The data obtained on latent infections were used to calculate the colonization index (CI) [15].

$$CI = WI + (NS \times RS)$$

With **NS**: Percentage of asymptomatic plants; **RS**: Percentage of asymptomatic plants with latent infection; and **WI**: Percentage of wilt index.

3 Results

3.1 Assessment Of The Incidence And Severity Of Bacterial Wilt At Cultivation Sites

The results showed that in the six selected tomato growing sites, namely LacA-Ch1, LacA-Ch2, LacB-Ch1, LacB-Ch2, LacE-Ch1 and LacE-Ch2 spread across three lakes Lake A, Lake B and Lake E, significant variation was observed in terms of incidence and severity of bacterial wilt (Table 2). The highest incidence of bacterial wilt (22.65%) was recorded at site LacE-Ch2, which corresponds to field 2 of the most downstream lake in the lake system, Lake E. The lowest incidence (14.36%) was recorded at site LacA-Ch2, which corresponds to field 2 of the most upstream lake in the lake system, Lake A. Regarding the severity of bacterial wilt, the highest severity (3.80) was recorded at site LacE-Ch2 as well, i.e. field 2 of Lake E, and the lowest severity (2.93) was recorded at site LacA-Ch1, which corresponds to field 1 of Lake A, the most upstream lake in the Yamoussoukro lake system.

Table 2. Incidence and severity of bacterial wilt on tomato growing sites and number of R. solanacearum bacterial isolates found per growing site

Lakes	Sites	Incidence (%)	Severity (scale 1 to 5)	Quantity of isolates
Lake A	LacA-Ch1	20.00b	2.93	4
	LacA-Ch2	14.36c	3.00	3
Lake B	LacB-Ch1	20.56b	3.00	8
	LacB-Ch2	19.98ab	3.13	9
Lake E	LacE-Ch1	22.52a	3.26	20
	LacE-Ch2	22.65a	3.80	19

3.2 Isolation And Confirmation Of Bacterial Strains By PCR

Sixty-three (63) strains of *R. solanacearum* were isolated from all samples after bacterial culture on SMSA medium. At the LacA-Ch1 site, 4 strains of *R. solanacearum* were isolated from irrigation water and crop soils. No strains were isolated from sampled plants. At the LacA-Ch2 site, 3 strains of *R. solanacearum* were isolated, including 2 from crop soils and 1 strain from sampled plants. At the LacB-Ch1, LacB-Ch2, LacE-Ch1 and LacE-Ch2 sites, respectively, 8; 9; 20 and 19 strains of *R. solanacearum* were isolated from irrigation water, crop soils, and sampled plants. All collected *R. solanacearum* colonies exhibited a fluid, irregular, creamy-white morphology with a pink center. These isolates were characterized using biochemical and pathogenicity tests. All strains were confirmed as *R. solanacearum* by PCR, yielding an expected 288-bp fragment after amplification with *R. solanacearum*-specific primers OLI 1-Y2.

3.3 Differentiation Of R. Solanacearum Isolates Into Biovars And Races

The results of biovar tests showed that all 63 *R. solanacearum* isolates oxidized disaccharides (sucrose, lactose, maltose) within 3–5 days, but not sugar alcohols (manitol, sorbitol, and dulcitol). The oxidation reaction was indicated by a color change from blue to yellow, indicating oxidation of sugars by bacterial isolates. According to these results, all groups of *R. solanacearum* isolates belong to biovar 2.

The racial identification of the 63 strains of *R. solanacearum* obtained from market gardening areas around the Yamoussoukro lakes was carried out using differential hosts such as inoculated aubergine, tomato, potato and chilli plants. All 63 isolates of *R. solanacearum* were able to cause wilting symptoms in these plants and potatoes. This indicates that they could belong to race 3.

3.4 Genetic Diversity Of R. Solanacearum Strains

Rep-PCR generated by the BOX, ERIC and REP primer sets combined, produced for the 63 *R. solanacearum* isolates, 97.06% polymorphic bands and 2.94% monomorphic bands. These results are illustrated in Figure 3 for the ERIC primer. The REP primer generated 3–13 bands per isolate, with the maximum number of 13 amplicons found in isolate LacE-Ch2-17 collected from Lake E. The ERIC primer produced 4–10 bands per isolate, with the maximum number of 10 amplicons found in isolate LacE-Ch1-1 collected from Lake E. The BOX primer produced 4–9 bands per isolate, with the maximum number of 9 amplicons found in isolate LacB-Ch2-10 collected

from Lake B. All these primers produced a highly polymorphic banding pattern, facilitating differentiation into groups and confirming the extreme genetic heterogeneity within *R. solanacearum*.

Phylogenetic analysis of 63 *R. solanacearum* strains, based on fingerprint data generated by rep-PCR, formed 3 distinct groups with a similarity coefficient of 70% (Fig. 3). Group 1 consisted of 8 strains, belonging only to the LacE-Ch1 site. Group 2 consisted of 10 strains belonging to the LacE-Ch1 site and 16 strains belonging to the LacE-Ch2 site. Group 3 grouped the strains belonging to all the sites studied. These are 2 strains from the LacE-Ch1 site, 3 strains from the LacE-Ch2 site, 8 strains from the LacB-Ch1 site, 9 strains from the LacB-Ch2 site, 4 strains from the LacA-Ch1 site and 4 strains from the LacA-Ch2 site. In summary, groups 1 and 2 included only strains from Lake E, while group 3 included all strains from all lakes (Fig. 4).

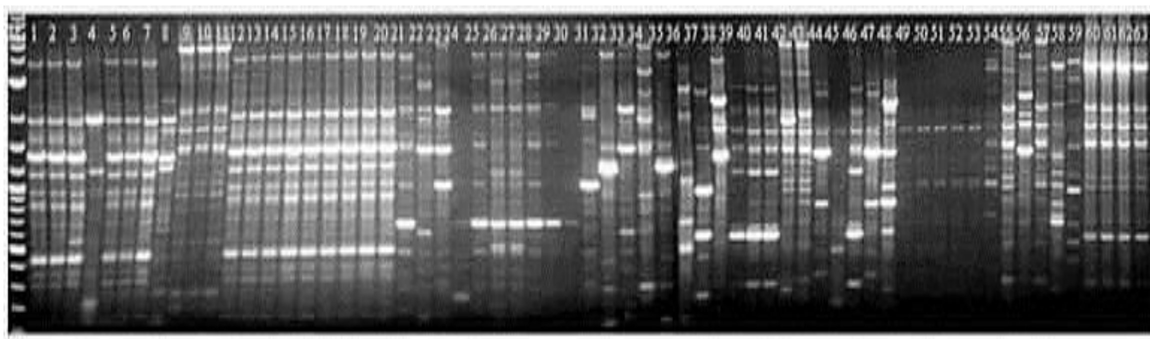

Fig. 4. ***Repetitive sequence polymerase chain reaction (Rep-PCR): Case of ERIC primer showing the genetic profiles of the 63 strains of R. solanacearum collected from market garden sites in the main lake system of the town of Yamoussoukro. MM of 10 kb DNA***

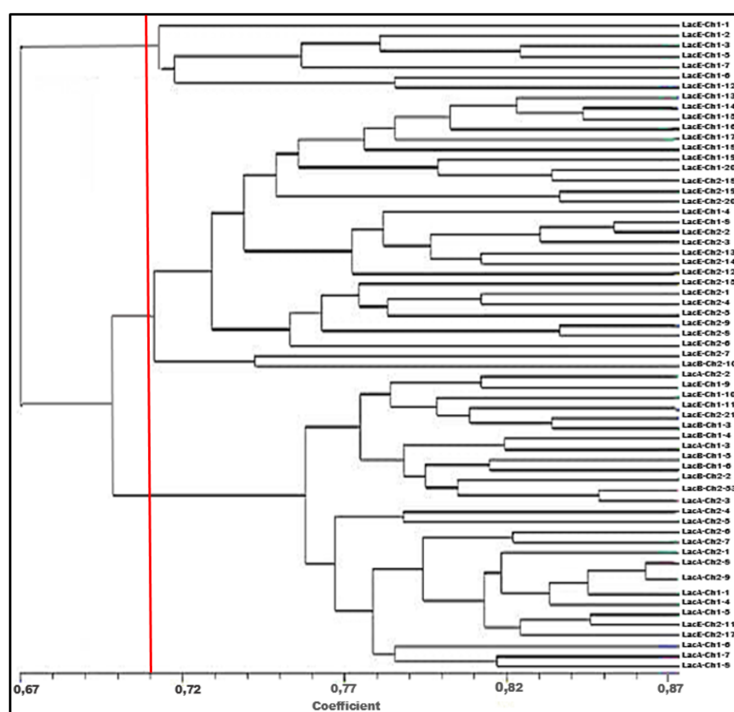

Fig. 5. ***Dendrogram obtained by the UPGMA method based on the pattern of bands obtained by Rep-PCR analysis for sixty-three isolates according to Jaccard's index***

3.5 Results Of The Pathogenicity Tests Of Strains

The results of the pathogenicity tests revealed that the 63 isolates of *R. solanacearum,* confirmed by PCR, were able to cause wilting symptoms in greenhouse tomatoes. However, these strains showed three levels of aggressiveness that were significantly different between them. The most aggressive groups of strains were found in the LacE-Ch1 and LacE-Ch2 sites, with aggressiveness levels higher than 55% and up to 77%.

The strains encountered on the LacB-Ch2 and LacB-Ch1 sites showed slight aggressiveness between 40% and 60%, with mainly strains isolated from crop soils and tomato plants. The strains encountered on the LacA-Ch1 and LacA-Ch2 sites showed low aggressiveness between 7% and 22%. This is the most heterogeneous group with as many virulent strains from crop soils, tomato plants and irrigation water of lake origin. (Figure 5). Nevertheless, out of the 42 cultivars evaluated, three did not show any diseased plants. These cultivars would appear resistant to the *R. solanacearum* strains present in the experimental site.

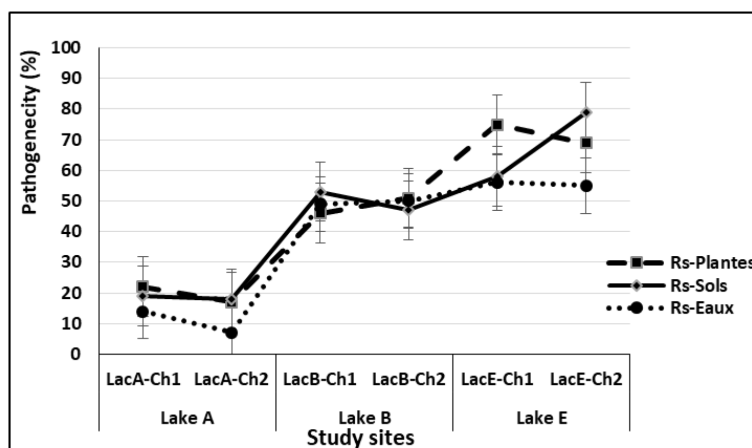

Fig. 6. ***Level of pathogenicity of groups of R. solanacearum isolates at the different study sites***

3.6 DISCUSSION

In this research carried out in Yamoussoukro, on market gardening areas near lakes, the results showed a high severity of bacterial wilt, regardless of the site studied. This increase in severity could be explained by the virulence and highly pathogenic nature of *R. solanacearum* strains on solanaceous plants [25]. However, the results of the incidence of bacterial wilt in the fields revealed significant variation from one site to another. Tahir *et al.* [26] stated that the variability in bacterial wilt action could be due to the competitive environment and genetic diversity in tomato (Solanum lycopersicon.L). According to Santiago *et al.* [27], the pathogen must cope with the variable soil environment during its saprophytic survival. Zheng *et al.* [28] added that this variation in bacterial incidence could indicate variation in the *R. solanacearum* population, due to the diversity of crops grown. Indeed, sixty-three isolates of *R. solanacearum* were isolated from tomato plants, crop soils and irrigation water collected from the sites identified for the study, and these strains were confirmed as *R. solanacearum* by PCR, and mostly belonged to Race 3 and Biovar 2. The predominance of Race 3 and biovar 2 could be explained by better adaptation to the environment. This result has also been demonstrated among strains collected in Côte d'Ivoire [12] and Burkina Faso [29]. In addition, Race 3 and biovar 2 have been reported to be more competitive than other race and biovar, in fairly tropical environments [30]. To assess genetic diversity and predict the geographical origin of *R. solanacearum,* rep-PCR, generated by the BOX, ERIC and REP primer sets, produced a pattern of highly polymorphic bands, facilitating differentiation into groups and confirming the extreme genetic heterogeneity within *R. solanacearum*. The results showed 97.06% polymorphic bands. This bacterium is well known to survive in diverse ecological conditions, interacting with a milieu of biotic and abiotic factors [29]. This could be the reason for the evolution and maintenance of greater variability in this bacterium [3]. This extreme diversity reflects the presence of several distinct genetic groups [31]. Nevertheless, the phylogenetic analysis carried out on the basis of rep-PCR fingerprint data, with a similarity coefficient of 70%, enabled these strains to be grouped into 3 main groups. Groups 1 and 2, made up solely of strains from Lake E, and group 3, made up of strains from all the sites studied. The bacterial strains isolated from Lake E showed the highest levels of aggressiveness, above 55%. Several previous studies have shown increased pollution in the catchment area of the lakes [7], [8], particularly in Lake E, which is subject to pollution such as agricultural, livestock, hospital and household waste, while the other two lakes (Lake A and Lake B) only have a specific type of pollution [7]. Thus, soil and water properties may play an important role in the genetic differentiation of *R. solanacearum,* as well as other bacterial species [32]. Ecologists and population geneticists have long suspected that the structure of the environment is linked to the maintenance of micro-organism diversity [32], [33]. This could explain the great variability observed in this population of *R. solanacearum* in Yamoussoukro, linked to its terricultural nature. For this reason, genetic variation within *R. solanacearum* was determined in order to design effective control strategies for the pathogen. Furthermore, of the 42 tomato cultivars assessed in the greenhouse, three showed no diseased plants. These cultivars could be resistant to the strains of *R. solanacearum* present at the experimental site, and therefore constitute interesting sources for the development of resistant cultivars adapted to the Yamoussoukro region in Côte d'Ivoire.

4 Conclusion

The aim of this study was to characterise the diversity and pathogenicity of *R. solanacearum* strains isolated from market garden areas located in the catchment area of the main lake system in the town of Yamoussoukro in Côte d'Ivoire. Water, wilted tomato plants and crop soil from two fields per lake were sampled from three lakes in order to provide an accurate representation of the distribution of strain diversity. The presence of *R. solanacearum* isolates in almost all the sites sampled demonstrated the challenge faced by farmers in Yamoussoukro, as well as the need for locally adapted strategies to combat this disease.

Acknowledgment

We would like to thank LANADA-CI for the microbiological analyses and the CEA-VALOPRO (**C**entre d'**E**xcellence **A**fricain pour la **Valo**risation des déchets en **Pro**duits à haute valeur ajoutée = African Centre of Excellence for the Valorisation of Waste into High Added Value Products) for the financial assistance provided to carry out molecular analyses.

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