

Breeding for resistance to tomato bacterial wilt: Identification of potential sources of genetic resistance by field evaluation of 28 tomato cultivars in Daloa, Côte d'Ivoire

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Abstract. Bacterial wilt caused by *Ralstonia solanacearum* is one of the most damaging tomato diseases in the world. The best strategy for controlling this disease is to use genetically resistant tomato plants. In this study, 28 tomato cultivars were evaluated in the field for their resistance to bacterial wilt in Daloa, one of the most important agricultural regions of Côte d'Ivoire. The experiments were carried out during the dry and rainy season and the experimental design was a Randomized Complete Block Design (RCBD) with three replications. The results showed the presence of wilt disease. The streaming test and bacterial culture on growth medium proved the bacterial origin of the wilt symptoms observed. The results of the staining reactions revealed short, straight, rod and Gram negative bacteria similar to *R. solanacearum*. The severity of the disease was found to be higher in the dry season than in the rainy season. Of 28 cultivars evaluated, 7 cultivars were resistant and did not show any diseased plants during the two study periods. These cultivars constitute interesting sources of resistance for a breeding program aimed at the development of new resistant cultivars adapted to the region of Daloa.

Keywords: Bacterial wilt, *Ralstonia solanacearum*, resistance, tomato, *Solanum lycopersicum*, breeding program.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop of the Solanaceae family. It is cultivated for its edible fruits, which are not only dietary sources of vitamins, minerals and fibers, but also contain lycopene

and β -carotene which have anticancer and antioxidant properties (Tambunan *et al.*, 2018). Tomato is important in the human diet as healthy food and it represents the second most consumed vegetable in the world, after

potato (FAOSTAT, 2020). It is cultivated in all latitudes and world production greatly exceeds 100 million tonnes per year (FAOSTAT, 2020). In terms of production volume, it is the world's leading vegetable, ahead of watermelon and cabbage (FAOSTAT, 2020).

In Côte d'Ivoire, this vegetable is among the most consumed foods. Its production and sale constitute lucrative activities for many Ivorians in rural, urban and peri-urban areas. The Ivorian annual production fluctuates between 22 000 and 45 000 tonnes for an estimated need of over 100 000 tonnes (Fondio *et al.*, 2013; FAOSTAT, 2020). The deficit is filled by a significant import to satisfy the population. This import of around 70% of Ivorian consumption is mainly due to the local difficulties of growing tomatoes because of some biotic and abiotic constraints, including bacterial wilt. This disease is the cause of significant yield losses and constitutes a severe limiting factor in tomato production (Kim *et al.*, 2016a).

Ralstonia solanacearum, the causal agent of bacterial wilt, is one of the most devastating plant pathogenic bacteria with a large host range encompassing more than 200 plant species which include major agricultural crops such as tomato, potato and banana (Huet, 2014; Du *et al.*, 2019). It is a soil-borne bacterium that enters plant roots, invades xylem vessels and spreads rapidly to aerial parts of the plant through the vascular system where its high level of multiplication leads to wilting symptoms and, ultimately, plant death (Zhang *et al.*, 2017; Wang *et al.*, 2019a). The pathogen is found worldwide, primarily in tropical and subtropical regions but also in Europe and North America (Huet, 2014). To control this pathogen, several methods are used, including cultural techniques and chemical control. However bacterial wilt disease management is difficult and these methods show limits (Du *et al.*, 2019). The strains of this bacterium present an extensive genetic diversity. In addition, the ability of *R. solanacearum* to survive in soils for many years and to form latent infections within indigenous weeds contributes to the difficult eradication of the bacterium (Lebeau *et al.*, 2011; Wang *et al.*, 2019a). Hence, resistance breeding is considered the most effective and environmentally friendly strategy for the management of this devastating soil-borne disease (Kim *et al.*, 2016a; Du *et al.*, 2019).

The search for a source of genetic resistance to bacterial wilt has been pursued in several countries, and breeders have identified and used sources of resistance for creating bacterial wilt resistant cultivars of tomato (Upreti and Thomas, 2015; Kim *et al.*, 2016b; Aslam *et al.*, 2017; Wang *et al.*, 2019a). The tomato cultivar Hawaii7996 is the most recognized example (Upreti and Thomas, 2015; Wang *et al.*, 2019a, b). However, *R.*

solanacearum has proven to be a highly flexible bacterium capable of rapid adaptation to environmental change and able to develop the ability to counteract plant tolerance (Peeters *et al.*, 2013; Wang *et al.*, 2019a). Thus, cases of resistance breakdown under intensive culture have been observed and breeders face the challenging problem of variable efficiency of resistance expression (Lebeau *et al.*, 2011; Huet, 2014; Aslam *et al.*, 2017). Actually, available resistances to bacterial wilt generally behave differently under different environmental conditions. It thus seems like a chimera to look for universal resistances to bacterial wilt. A more realistic approach would be to seek for sources of resistance adapted only to a given ecosystem (Huet, 2014).

In Côte d'Ivoire, the presence of bacterial wilt in Daloa, one of the most important agricultural regions of the country, has been reported earlier by N'Guessan *et al.* (2012). The objectives of the present study was first to evaluate the severity of the disease on 28 cultivars of tomato and secondly to identify, in this germplasm, potential genetic resistance sources for future breeding programs.

MATERIALS AND METHODS

Experimental Site

The work was carried out in Zépréguhé, a village of Daloa, located in the west of Côte d'Ivoire, 130 km from the capital Yamoussoukro. Daloa is one of the major agricultural regions of Côte d'Ivoire (Kouamé *et al.*, 2015). The area was under humid tropical conditions with 1317 mm of rainfall per year and relative dense forest vegetation. The experiment site was located 6 ° 90 N latitude and 6 ° 37 W with an altitude of 238 m.a.s.l. (above sea level). The soil of the plot was sandy loam texture with good fertility, properly leveled and well drained. The study was carried out over two periods: a first experiment in the dry season and a second in the rainy season. The mean temperatures and relative humidities during the experimental periods varied in the dry season from 25° to 35° C and 53% to 68% respectively, and in the rainy season from 22° to 30°C and 73% to 80%, respectively.

Plant Materials

Twenty eight different cultivars of tomato (Table 1) from different origins were evaluated: 21 cultivars came from different seed companies (16 from "Technisem", 1 from "Botanic", 1 from "Tropica", 2 from "Les doigts verts", 1

Table 1. Different cultivars evaluated for the resistance to bacterial wilt in Daloa.

N°	Cultivar	Origin
1	Petomech	Technisem
2	Calinago	Technisem
3	Uc 82	Technisem
4	Savana	Technisem
5	Nadira	Technisem
6	Jaguar	Technisem
7	Kiara	Technisem
8	Mongal	Technisem
9	Cobra	Technisem
10	Sumo	Technisem
11	Lindo	Technisem
12	Copernic	Technisem
13	Carioca	Technisem
14	Rio	Technisem
15	Tropimech	Technisem
16	Thorgal	Technisem
17	Cœur de bœuf	Botanic
18	Heinz 1370	Tropica
19	Montfavet	Les doigts verts
20	St pierre	Les doigts verts
21	Caraibo	Clause vegetable seeds
22	Beams yellow pear	Gembloux Agro-Bio Tech
23	Black moor	Gembloux Agro-Bio Tech
24	Early fliston	Gembloux Agro-Bio Tech
25	California	Gembloux Agro-Bio Tech
26	Local 1	Local cultivar from Daloa
27	Local 2	Local cultivar from Daloa
28	Local 3	Local cultivar from Bouaké

from “Clause vegetable seeds”); 4 cultivars came from the collection of the Tropical Agro Ecology Laboratory of Gembloux Agro-Bio Tech (University of Liège, Belgium); 2 cultivars were local cultivars from Daloa (the region of the study); and 1 local cultivar from Bouaké (a region in the center of Côte d’Ivoire, at 245 km from Daloa).

Experimental Design

The tomato seeds were first sown and nursed during 21 days in culture trays containing sterilized substrate of Jarditropic brand (Jardinova, France) in order to produce healthy and vigorous seedlings to be transplanted in field. The experimental design in field was arranged in a Randomized Complete Block Design (RCBD) with three replications separated from each other by a 2-m wide alley.

The seedlings of each cultivar were transplanted in a single-row plot with a row-to-row spacing of 100 cm and plant-to-plant spacing of 50 cm. Transplanting was done in the afternoon, when the weather was cool, in order to limit heat stress to seedlings and promote their good and fast recovery. A plant population of 5 plants per cultivar, per plot was transplanted. Thus, each cultivar was represented by 15 individual plants, *i.e.* 420 plants for the overall 28 cultivars on the experiment field.

Field Managements

Standard agronomic and management practices were adopted, but no fertilizers and insecticides were applied. Daily watering using a watering can was applied uniformly to all plots when necessary. Manual weeding by hoeing and handpicking were carried out to avoid any

competition between the crop and the weeds. Plant tutoring was also carried out.

Scoring of symptomatic plants and confirmation of the bacterial origin of the wilt

Data were collected on each plants of each cultivar in each replication. The plants were inspected daily in visual observation for the presence/absence of bacterial wilt symptoms, until the harvest. Any of the plants that showed unexplained wilted leaves were suspected to have bacterial wilt disease. Each plant was scored as asymptomatic (no wilted leaves observed) or symptomatic (wilted plant). When the wilt symptoms were observed, the next step was to confirm the presence or absence of *R. solanacearum* in the plant. For this, two confirmation techniques, *v.i.z.* bacterial streaming test and isolation of bacteria on culture media, were employed to check for the presence or absence of the pathogen in the plants displaying wilt symptoms.

Bacterial streaming test:

Five to 10 cm stem of the plant displaying wilt symptoms was cut and immersed in a glass filled with clear water. If the plant is infected by *R. solanacearum*, it is observed from the part suspended in the water, a flow of a whitish stream of exudates visible through the glass of water (Lebeau *et al.*, 2011; Ivey and Lunos, 2015; Aslam *et al.* 2017; Wang *et al.*, 2019b).

Culture on agar medium and characterization of the bacteria:

Different parts of the wilted plant, *v.i.z.* roots, stem and leaves, were sterilized, crushed and used separately for bacterial culture on Casamino acid-Peptone-Glucose (CPG) medium (1 g Casamino acid, 10 Peptone, 5g Glucose, 17g Agar and 1 L of distilled water) using the protocol of Konaté *et al.* (2015). The culture media were inspected for visible colonies after 48 hours of incubation at 28°C. A Gram stain technique was then carried out according the method of Pawaskar *et al.* (2014) to characterize the colonies obtained.

Assessment of the disease severity and identification of resistance cultivars

The disease severity (DS) in the cultivars was evaluated

using the 0-5 scale of Singh *et al.* (2019), where 0 = 0% diseased plants, 1 = less than 10% diseased plants, 2 = 10% to less than 20% diseased plants, 3 = 20% to less than 30% diseased plants, 4 = 30% to less than 40% diseased plants, 5 = more than 40% diseased plants. Any cultivar that did not show any diseased plants during the two periods of the study (dry and rainy season) was considered genetically resistant (R).

RESULTS AND DISCUSSION

Identification of symptomatic plants and confirmation of the bacterial origin of the wilt

During the inspection of the field for the identification of infected plants, the visible symptoms of the disease were seen on the foliage. First the youngest leaves at the end of the branches wilted; and a few days later (2-4 days), the whole plant wilted irreversibly, leading to its death. Several researchers (Lebeau *et al.*, 2011; Kim *et al.*, 2016a; Du *et al.*, 2019; Wang *et al.*, 2019a,b) working on bacterial wilt disease reported similar symptoms.

It was also observed that these symptoms were able to appear at all stages of the plant's growth (from seedling to productive plant). Thus, wilted young plants were observed, as well as healthy-looking producing plants that suddenly wilted and died quickly (Figure 1). This observation showed that the infection process can last over periods ranging from several days to several weeks before the onset of symptoms, possibly depending on environmental conditions and the host plant-bacterial strain combination as reported by Lebeau *et al.* (2011). But as soon as the symptoms of leaf wilt appeared the plant dies a few days later.

To confirm the bacterial origin of the wilting, a vascular detection flow from the stem and a bacterial isolation from the main part of the wilted plants (roots, stem and leaves) on culture media were performed.

Bacterial wilt confirmation by vascular flow detection procedure

Vascular flow detection procedure also called bacterial streaming test is a common detection method for bacterial wilt disease confirmation, which can be used as a valuable diagnostic tool for quick detection of bacterial wilt in field (Tessema *et al.*, 2020).

In this study, the cross section made through the stem of the symptomatic plants, followed by its suspension in water showed after 30 seconds to 1 minute a viscous white-creamy slime streaming (Figure 2a). Tessema *et al.* (2020) reported that such streaming represents bacterial ooze exuding from the cut ends of colonized vascular bundles. Actually, once a plant is infected, bacteria multiply

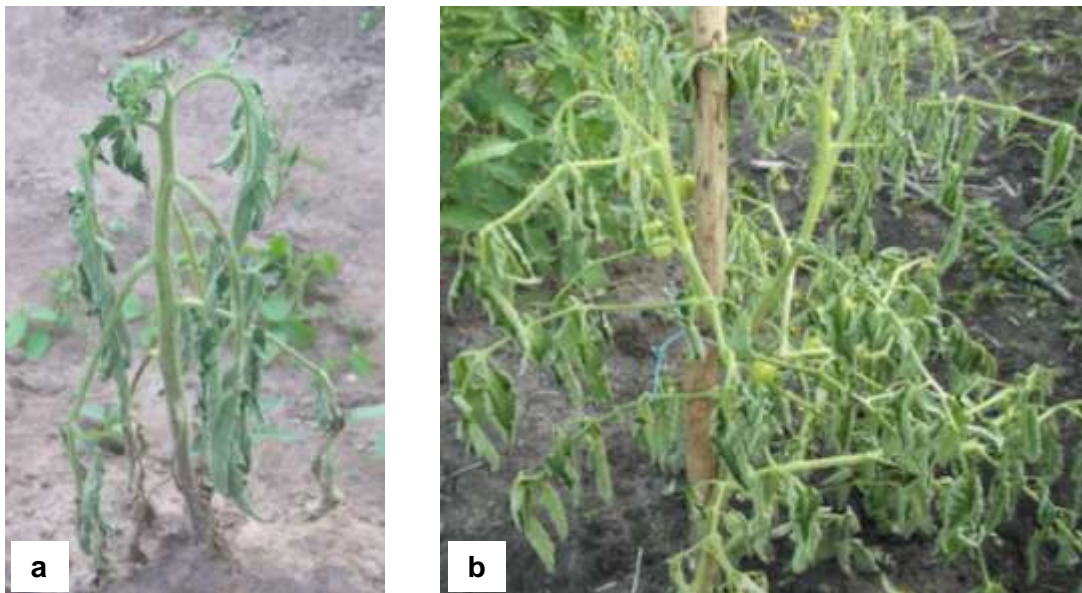


Figure 1. Tomato plants showing symptoms of bacterial wilt: a) affected young tomato plant; b) tomato plant in production affected.

quickly inside the plant tissue and the plant develops disease symptoms (Ivey and Lunos, 2015). The bacterial streaming is a phenomenon of exit of this bacterial population out of the plant tissues when the diseased area of the plant is cut and suspended in water. Hence, the white-creamy slime streaming observed in the present study suggested a bacterial origin of the wilt disease. According to several authors (Lebeau *et al.*, 2011; Ivey and Lunos, 2015; Aslam *et al.* 2017; Wang *et al.*, 2019b) the bacterium responsible for bacterial wilt in tomatoes and giving a vascular flow similar to that observed is *Ralstonia solanacearum*.

Isolation of the bacteria on culture medium and characterisation

The second method used to confirm the bacterial origin of the wilting was the isolation on medium culture of bacteria from symptomatic plant tissues. Extracts from different parts of the wilted plants (roots, stem and leaves) were used for bacterial culture on solid agar growth media. The result showed for all the parts of the symptomatic plants used, the apparition of visible white cream-colored bacterial colonies (Figure 2b), and the characteristic of virulent wild-type colonies of *R. solanacearum*. Indeed, according to Champoiseau and Momol (2008), virulent wild-type colonies of *R. solanacearum* are white cream while avirulent mutant

colonies are deep-red often with a bluish border. The results of the staining reactions revealed that the bacteria were short, straight rods and Gram negative in reaction (Figure 2c). Several researchers like Chaudhry and Rashid (2011) and Pawaskar *et al.* (2014) reported similar staining reaction of *R. solanacearum*. These results confirm the conclusion of the bacterial flow test and indicate that the bacterium which infected the tomato plants at the experimental site was indeed *R. solanacearum*.

The result of isolation of the bacteria on growth media also indicated the presence of the bacterium in the different part of the infected plants. This is in consonance with Kim *et al.* (2016a) who reported that once *R. solanacearum* penetrates the root epidermis via natural openings or wounds, and crosses the cortex and endodermis to finally reach the root xylem, it starts extensive colonization and spreads to the aerial parts of the infected plant along the vascular system. This explains the detection of the bacterium in the roots, the stem and the leaves in this study. According to Du *et al.* (2019), this spread of the bacterium from the roots to the shoots is accompanied by further growth and the secretion of large quantities of extracellular polysaccharides. It is eventually, the density of the population and the quantity of the polysaccharides present in the xylem that attain high levels, which block the water flux from the roots to the shoots and lead to the death of the plant by wilting.

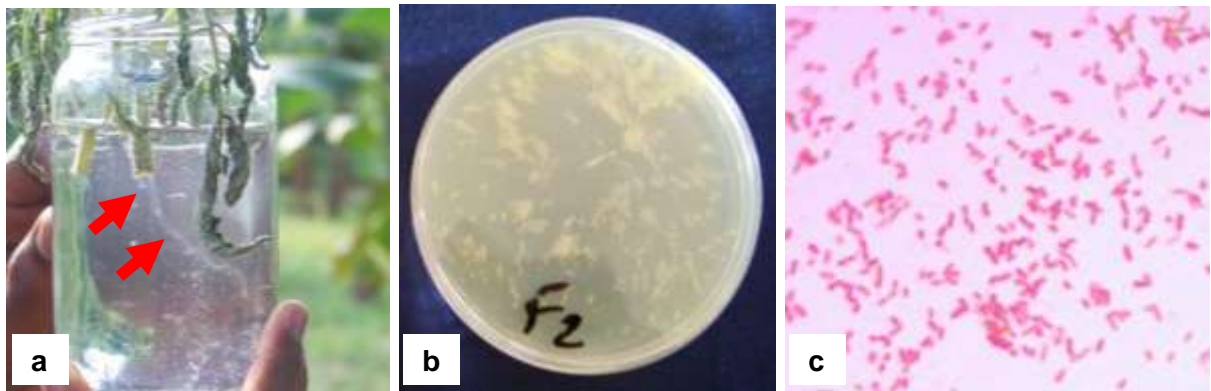


Figure 2. Confirmation test of the bacterial origin of the wilt observed in the tomato plants: a) Bacterial exudation (arrow) from the cut stem of an infected plant suspended in water, b) Bacterial colonies isolated on a culture medium from an infected plant tissues, c) Gram negative bacteria observed after Gram staining.

Disease severity and identification of cultivars with genetic resistance

The result of the assessment of disease severity and plant mortality rates for the tomato cultivars evaluated are presented in Table 2. The disease severity varied from 0 to 5 for each of the two seasons of study on a 0-5 scale disease severity score. This result indicates that the bacterial wilt disease is active in the experimental site, confirming the findings of N'Guessan *et al.* (2012) who reported the presence of virulent strains of *R. solanacearum* in the region of Daloa. The mean severity score was higher for the dry season (2.39) than for the rainy season (1.82). In the same trend, the mean mortality rate of plants by bacterial wilt was higher (23.14%) in the dry season than in the rainy season (15.24%). These results showed that the disease was more severe during the dry season than during the rainy season. This is in accordance with Champoiseau and Momol (2008) who stated that the expression of symptoms of bacterial wilt is favored by high temperatures.

When considering both the two seasons (Table 2), the disease severity score was 0 for seven cultivars, 1 for four cultivars, 2 for four cultivars, 3 for five cultivars, 4 for six cultivars, and 5 for two cultivars. Thus, out of 28 tomato cultivars evaluated over the two seasons, 21 cultivars showed a severity score greater than 0. Plant mortality rates recorded for these 21 cultivars ranged from 4.17% to 58.33% with a mean of 18.75% (Table 2). The highest mortality rates were recorded for cultivar Local#3 (58.33%) followed by Montfavet (52.17%); while the lowest rates were shown by Sumo (4.17%) followed by Carioca (5.88), Copernic (9.09) and Thorgal (9.09). These 21 cultivars cannot be recommended to farmers in

the Daloa region as they present a risk of yield loss and therefore may not be economically profitable. In addition, their culture can contribute to the spread of the bacteria. Therefore, none of these cultivars should be selected as resistance donor parents in future breeding programs for the development of new tomato cultivars resistant to bacterial wilt in Daloa. In contrast, the remaining seven cultivars (Cobra, Lindo, Caraibo, Beams yellow pear, Early fliston, Local#1 and Local#2) that showed no wilted plants during the two seasons of the study could be considered resistant to local strains of *R. solanacearum*.

The two local cultivars (Local#1 and Local#2) from Daloa, unlike Local#3 from Bouké, were resistant. This result shows that these two local Daloa cultivars were well adapted to their region (Daloa) and could therefore be good sources of resistance for tomato improvement, even if they are not well appreciated by the population because of their strong acidity. The other five resistant cultivars identified in this study are also interesting sources of resistance for breeding.

The resistance process to bacterial wilt in tomato is less well understood. But it is clear that the resistant plants to *R. solanacearum* may tolerate a limited growth of the bacterial pathogen within their vascular systems and they may have the ability to contain the bacteria in the lower parts of the plants (Wang *et al.*, 2000). Lebeau *et al.* (2011) and Kim *et al.* (2016a) reported two possible types of resistance mechanisms in the stem that both block bacterial movement in resistant plants, but in different ways. In the first type, the resistant plants confined bacteria to the primary xylem, possibly through thickenings of pit membranes in stem xylem tissue. In the susceptible plant, bacteria were found in secondary xylem tissues of the stem or moved into the pith (Nakaho *et al.*, 2004). The second means of preventing bacterial

Table 2. Disease severity score and mortality rates in tomato cultivars evaluated during the dry and rainy season

N°	Cultivar	Dry season		Rainy season		Combined dry season + rainy season	
		Severity score	Mortality rate (%)	Severity score	Mortality rate (%)	Severity score	Mortality rate (%)
1	Petomech	4	30.00	4	33.33	4	32.00
2	Calinago	2	12.50	2	13.33	2	13.04
3	Uc 82	5	40.00	0	0.00	2	16.67
4	Savana	4	33.33	4	30.77	4	31.82
5	Nadira	3	20.00	4	36.36	3	28.57
6	Jaguar	5	44.44	0	0.00	2	17.39
7	Kiara	3	20.00	5	41.67	4	31.82
8	Mongal	0	0.00	4	30.77	3	19.05
9	Cobra	0	0.00	0	0.00	0	0.00
10	Sumo	2	11.11	0	0.00	1	4.17
11	Lindo	0	0.00	0	0.00	0	0.00
12	Copernic	2	10.00	1	8.33	1	9.09
13	Carioca	2	11.11	0	0.00	1	5.88
14	Rio	5	42.86	4	30.77	4	35.00
15	Tropimech	5	87.50	0	0.00	4	35.00
16	Thorgal	2	10.00	1	8.33	1	9.09
17	Cœur de bœuf	0	0.00	3	26.67	3	21.05
18	Heinz 1370	3	25.00	3	27.27	3	26.32
19	Montfavet	5	50.00	5	53.33	5	52.17
20	St pierre	5	60.00	3	21.43	4	37.50
21	Caraibo	0	0.00	0	0.00	0	0.00
22	Beams yellow pear	0	0.00	0	0.00	0	0.00
23	Black moor	0	0.00	3	21.43	2	13.64
24	Early fliston	0	0.00	0	0.00	0	0.00
25	California	5	60.00	0	0.00	3	27.27
26	Local 1	0	0.00	0	0.00	0	0.00
27	local 2	0	0.00	0	0.00	0	0.00
28	Local 3	5	80.00	5	42.86	5	58.33
Average		2.39	23.14	1.82	15.24	2.18	18.75

movement may be through the accumulation of tyloses in stem xylem vessels. Tyloses are outgrowths of xylem parenchyma cells that balloon into the lumen of adjacent xylem vessels, creating a physical barrier that may prevent bacterial movement (Kim *et al.*, 2016a).

At the genetic level, the mechanism of resistance to

bacterial wilt in tomato has been described in different studies as quantitative (polygenic) with the involvement of several QTL (quantitative trait loci) controlling this trait (Wang *et al.*, 2000; Huet, 2014; Kim *et al.*, 2016a; Aslam *et al.*, 2017). However, despite its polygenic nature, this resistance is most often strain specific and can become

ineffective in a geographical different region due to the extensive worldwide genetic diversity exhibited by the strains of *R. solanacearum* (Huet, 2014). Globally four phylotypes corresponding roughly to the strains' geographic origin are recognized (phylotype I in Asia, II in the Americas, III in Africa, and IV in the Australia-Indonesia region), but each phylotype is extremely diverse (Lebeau *et al.*, 2011; Wang *et al.*, 2019a; Wang *et al.*, 2019a). According to Huet (2014), the polygenic resistance to bacterial wilt in the tomato cultivar Hawaii7996, one of the most recognized resistance against *R. solanacearum* was strain specific, and QTLs (Quantitative Trait Loci) in Hawaii7996 may deploy a phylotype-specific resistance. This example illustrates the difficulty of obtaining a worldwide resistance to *R. solanacearum* (Huet, 2014). The genetic and phenotypic plasticity of *R. solanacearum* strains strongly hinders the use of a specific varietal resistance as a universal control strategy (Aslam *et al.*, 2017). A better strategy would be to identify sources of resistance adapted only to a given ecosystem and use them to develop new suitable cultivars resistant to the disease. From this point of view, the sources of resistance to bacterial wilt found in the present study are a good start in the development of resistant cultivars for the Daloa region. These cultivars could be used as breeding resources in the improvement of tomato for bacterial wilt resistance in Daloa.

CONCLUSION

In conclusion, this study revealed that bacterial wilt, a damaging disease of tomatoes caused by *R. solanacearum*, is indeed present in Daloa (Côte d'Ivoire). The results highlighted that the severity of the disease is more important in dry season than in rainy season. Of the 28 cultivars evaluated, seven showed no diseased plants during the two study periods (dry season and rainy season). These cultivars appear resistant to the strains of *R. solanacearum* present in the experimental site and therefore constitute interesting sources of resistance for the development of resistant cultivars adapted to this important agricultural region of Côte d'Ivoire. However, the results obtained need to be validated by multi-site tests in the Daloa region before being widely used in future breeding programs.

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