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RESISTANCE ASSESSMENT OF TOMATO (*SOLANUM LYCOPERSICUM* L.) AND GBOMA (*SOLANUM MACROCARPON* L.) CULTIVARS AGAINST BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM* IN BENIN

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* is the devastating disease of Solanaceae plants including tomato and Gboma in Benin. To find out resistant cultivars against bacterial wilt, nine tomato and seven Gboma cultivars were screened in glasshouse in a completely randomized block design with 3 replications. Tomato plants were inoculated by crown region inundation with 40 ml of 10^8 CFU/ml bacterial suspension of the strain 19bLDC Phylotype 1 of *R. solanacearum* four weeks after transplanting while Gboma plants were inoculated after scarification of their roots with the strain 5aLDC Phylotype 1. Two weeks after transplanting, it was found that tomato cultivars HW7996 and PADMA were highly resistant; Platinum was resistant; Jalani, Thorgal and Mongal were moderately resistant; Nirvana was moderately susceptible (MS) and Tohouvi and TLCV15 were susceptible (S). Gboma cultivar AUB3G was appeared as resistant to *R. solanacearum*, cultivars Gboma Athiémé, Gboma PCM, CR1-01-001, CR1-13-049 and BOC15 were susceptible (S) and Gboma-teck was highly susceptible. It can be concluded that tomato cultivars PADMA and HW7996 and that of Gboma AUB3G were resistant to *R. solanacearum*. All cultivars were colonized with bacterial inoculum. Considering that resistant plants also harbor the disease, growers should practice sanitation as well for both susceptible and resistant cultivars. The resistant cultivars identified will be an important component in the management of bacterial wilt of tomatoes and Gboma and will be disseminated to farmers. They will serve as basic data for breeders and seed production firms.

Keywords: Disease of solanaceae, Management, germplasm screening.

INTRODUCTION

Vegetables production has become an activity that responds effectively to urban food demand in Benin (Simeni *et al.*, 2009). These are sociologically and economically important for the population (Assogba Komlan *et al.*, 2007). Vegetable sub-sector employs

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many people in Benin. The income generated by vegetable production allows many thousands dozen of families to survive (Sikirou *et al.*, 2001). Vegetable play also an important role in the diet of populations, contribute to the prevention of diseases due to micronutrient deficiencies (Olaniyi *et al.*, 2010) and reduce the risks of cancer and cardiovascular diseases (Bhowmik *et al.*, 2012). Many local and exotic vegetables are grown in West Africa (James *et al.*, 2010). Among these, tomato (*Solanum lycopersium*) and the African nightshade (*Solanum macrocarpum*) commonly known as "Gboma" are the main fruit and leaf vegetables

cultivated, consumed and commercialized in Benin. These are an important source of income for farmers especially during the off-season (Assogba Komlan *et al.*, 2007).

Tomato and Gboma are produced along the year (rainy season and off-season) in all agro-ecological zones of Benin (Sikirou, 2011) where several local and improved varieties are cultivated by farmers. Tomato and Gboma production fell drastically in the southern areas of Benin where tomato yield was reduced by 91.9% between 1999 and 2004 in the Ouémé valley (Sikirou *et al.*, 2017). These drops in production are due to the pressure of many fungal (Sikirou *et al.*, 2015a), bacterial (Idrissou-Touré *et al.*, 2017), virus (Hanssen *et al.*, 2010) diseases and nematodes (Pereira-Carvalho *et al.*, 2010; Affokpon, 2011). In Benin, the bacterial wilt caused by the soil-borne bacterium *R. solanacearum* is a major constraint in the production Solanaceaeous plants. Bacterial wilt has reduced the production of Solanaceae in some areas of Benin where an incidence of 72% has been recorded for tomato (Sikirou *et al.* 2017) and 75% for Gboma (Sikirou *et al.* 2017; Sikirou *et al.* 2015b). In recent years, an upsurge in bacterial wilt of Solanaceae has been observed in the main tomato production areas (Sikirou *et al.*, 2009) and Gboma (Sikirou *et al.*, 2015b) particularly in southern Benin. Yield losses caused by *R. solanacearum* vary according to country, agroecological zone and crop (Tengku Abul Malik *et al.*, 2012; Muthoni *et al.*, 2012; Habetewold *et al.*, 2015). In the United States of America, *R. solanacearum* is one of the potentially arms usable as bioterrorism (Madden and Wheelis, 2003; Hong *et al.*, 2012). This pathogen has affected the productivity of more than 80 countries

worldwide leading to a loss of 90.62% under high incidence (Artal *et al.*, 2012). The complexity associated with *R. solanacearum* adaptability, viability and diversity makes its management very difficult (Subedi, 2014).

However, management approaches have been developed. They include sanitation (Aslam *et al.*, 2017), grafting (Comes *et al.*, 2009; Rivard *et al.*, 2012), the use of disease reducing plants like Alliaceae which are efficient bio-fumigant and sanitizing plants (Debert *et al.*, 2014) and crop systems by rotating or mixing none susceptible different crops (Kakuhenzire *et al.*, 2013; Getachew and Chemed, 2016). Unfortunately, pesticides targeting bacteria are rare and experimental efforts still continue to be made (Boshou *et al.*, 2005; Balestra *et al.*, 2009). Separately, neither of these methods is effective enough.

The control of this disease is mainly based on varietal resistance, which is the most effective management strategy, environment friendly and cost effective for farmers and consumers. In Benin, no information is available on the genetic assessment of tomato and Gboma cultivars to bacterial wilt. The objective of this study is to assess the resistance of tomato and Gboma cultivars against bacterial wilt.

MATERIALS AND METHODS

Germplasm collection: Tomato and Gboma germplasm were collected from different institutions, programs, farmers or purchased from a local certified seeds market (Table 1). Tomato cv. Tohounvi was the susceptible check line (Sikirou *et al.*, 2009) and HW7996 was the resistant one (Wicker *et al.*, 2009). The Gboma cv. PCM was the susceptible check line. No resistant check line was considered in Gboma.

Table 1. Crops seeds origins

Crops	Cultivars	Origins
Tomato	Platinum, Jalani, Nirvana, PADMA and TLCV15	International Fertilizer Development Center (IFDC)
	Thorgal and Mongal	Purchased from "Bénin semence"
	Tohounvi	Vegetable Program of National Agricultural Research Institute of Benin PCM/INRAB
	HW7996	Centre International de Recherche pour l'Agriculture et le Développement (CIRAD)
Gboma	AUB3G and BOC15	National Agricultural Research Center CNRA/ Ivory Coast
	CRI-13-049 and CRI-01-001	Crops Research Institute of Ghana (CRI)
	Gboma-teck and Gboma-Athiéme	Farmers
	Gboma-PCM	Vegetable Program of National Agricultural Research Institute of Benin PCM/INRAB

Experiments conditions and experimental design:
The experiments reported here were conducted twice

under controlled conditions in the Laboratory of Crop Protection (LDC) at the National Institute of Agricultural

Research of Benin (INRAB) located at Abomey-Calavi in Southern Benin (6°24'35N, 2°19'56E). The nurseries of the tomato and Gboma cultivars were raised separately in germination trays filled with previously sterilized soil for 1 hour at 80 °C. The daily temperature ranged from 26.5 °C – 28 °C for the first trial and from 29 °C - 30 °C for the second. Inoculated plants were arranged in a completely randomized block design with three (3) replications. Experiments were conducted during the rainy and the dry season to compare plants reaction under low and high temperature.

Inoculum preparation and plants inoculation: The inoculum were prepared at a concentration of 10⁸ cfu ml⁻¹ (colony-forming unit per milliliter) from the strains 19bLDC and 5aLDC of *R. solanacearum* both Phylotype 1 and of molecular characteristic ST (eg. ST43 (sequevar 31), must obtained from the Laboratory of Crop Protection (LDC) of INRAB. Four (4) week old tomato plants were transplanted after roots wounding and inoculated the same day through soil drenching with 40 ml of an inoculum per plant by the method of collar flooding. Due to the slow development of the root system, Gboma plants were inoculated two (2) weeks later (6 weeks old age) after root scarification (Vasse *et al.*, 1995; Sikirou *et al.*, 2009). After inoculation, each plant was watered daily with 100 ml of water and symptoms of bacterial wilt were observed. Control plants were applied with distilled water.

Colonization of tomato and Gboma plants by *R. solanacearum*: Latent infection of unwilted tomato and Gboma plants was determined at 28th days after inoculation (DAI) by cutting the stem section of 2 to 3 cm per plant at 1 cm above the crown region. Each section was disinfected with 70% ethanol and then inflamed with 90% ethanol. The colonization test of tomato plant by the bacterium was performed using the imprint of the base of the section on the SMSA (Semi Selective Medium of South Africa) plate. For Gboma plants, the colonization was verified by macerating and plating on the SMSA medium according to the modified method of Elphinstone *et al.* (1996) at the rate of five imprints per plant. Plates were incubated for 72 hours at 28 °C. The appearance of *R. solanacearum* colonies on SMSA plate allowed to calculate the Bacterial Colonization Index (BCIx) according to the following formula:

BCIx = 100*(Pf + Pc)/N with
 Pf = number of wilted plants at 28 DAI; CP = number of colonized plant at 28 DAI; N = Total number of inoculated plants.

Incidence of bacterial wilt: The incidence of bacterial wilt (IBW) was calculated as the ratio of the number of wilted plants (NWP) and that of inoculated plants (NIP) by the following formula:

IBW = (NWP/NIP) x100.
 According to the classical scale of 1 = died plant, completely wilted plant or three quarters wilted plant and 0 = symptomless.

The classification level of resistance to bacterial wilt based on disease incidence according to Mew and Ho (1977) as referred to Table 2 was used.

Reaction	Disease incidence
Highly Resistant (HR)	0% wilted plant
Resistant (R)	1-10% wilted plants
Moderately Resistant (MR)	> 10-20% wilted plants
Moderately Susceptible (MS)	> 20-30% wilted plants
Susceptible (S)	> 30-70% wilted plants
Highly Susceptible (HS)	> 70% wilted plants

The area under bacterial wilt incidence progress curve: The area under bacterial wilt incidence progress curve (AUI_{bw}PC) is a function of time. AUI_{bw}PC = f(t) (Jeger and Viljanen-Rollinson, 2001). It is calculated according to the following formula:

$$AUI_{bw}PC = \sum_{i=1}^k [(IFB_i + IFB_{i+1})(t_{i+1} - t_i)] / 2$$

bw = bacterial wilt, BWI = Bacterial Wilt Incidence

STATISTICAL ANALYSIS

The one-way analysis of variance from R software, version 3.5.2 was used. It was focused on the bacterial

wilt incidence, the bacterial colonization index and AUI_{bw}PC. Means were classified into homogeneous groups using Student-Newman-Keuls test at the 5%.

RESULTS

Response of tomato cultivar to *Ralstonia solanacearum*: The incidence of bacterial wilt, the percentage of colonized plants by *R. solanacearum* and the area under bacterial wilt incidence progress curve (AUI_{bw}PC) varied among tomato cultivars (Table 2). The incidence was highly significant (P<0.001) between the

nine tomato cultivars during the two experiments. During the first one, the cv. Tohounvi was the most susceptible to bacterial wilt with 70% of wilted plants followed by the cv. TLCV15 for which 43.33% of wilted plants were recorded. Wilt was moderate for Mongal (6.66%), Thorgal (10%), Jalani (20%) and Nirvana (23.33%) cultivars, very low for Platinum (3.33%) and null (0%) for PADMA and HW7996 which did not show any wilted plants. During the 2nd experiment, the wilting trend was similar to that of the first experiment with an increasing of the incidence for more than 66% of cultivars.

All varieties were colonized with *R. solanacearum* strain 19bLDC including the resistant test line HW7996. In addition, the colonization percentage increased during the 2nd experiment for the majority of the tested cultivars. Regarding AUIbwPC, the analysis of variance

Table 3. Bacterial wilt incidence, index of colonization and area under bacterial wilt incidence progress curve of nine tomato cultivars

Tomato cultivars	Incidence (%)		Colonization (%)		AUI _{bw} PC		Reaction
	E1	E2	E1	E2	E1	E2	
Platinum	3.33 ± 5.77e	10.00 ± 0.00b	80.00	71.43	58.33 ± 101.03c	175.00 ± 00.00b	R
Jalani	20.00 ± 0.00cd	20.00 ± 10.00b	83.33	70.00	186.66 ± 106.92c	186.66 ± 132.50b	MR
Nirvana	23.33 ± 5.77cd	33.33 ± 5.77	83.33	90.00	291.66 ± 88.08c	291.66 ± 80.82b	MS
PADMA	00.00 ± 0.00e	00.00 ± 0.00c	80.00	100.00	0,00 ± 0,00d	0.00 ± 0.00c	HR
Thorgal	10.00 ± 0.00de	15.83 ± 19.43b	83.33	100.00	93.33± 72.85c	306.25 ± 388.85b	MR
Tohounvi	70.00 ± 10.00a	66.66 ± 28.86a	93.33	90.00	1166.66 ± 213.85a	1073.33 ± 470.00a	S
TLCV15	43.33 ± 11.54b	86.66 ± 23.09a	90.00	100.00	665.00 ± 185.20b	1353.33 ± 332.65a	S
Mongal	6.66 ± 5.77e	25.55 ± 6.75b	90.00	90.00	70.00 ± 92.60c	219.07 ± 113.40b	MR
HW7996	0.00 ± 0.00e	0.00 ± 0.00c	0.00	70.00	0,0 ± 0,0d	0.00 ± 0.00c	HR
P	<0.001	<0.001			<0.001	<0.001	

Means within column with the same letters are not significantly different according to Student-Newmann-Keuls test with P = 0.05. E= Experimentation

Response of Gboma cultivar to *Ralstonia solanacearum*: During the two experiments with Gboma, significant variability between cultivars was recorded for bacterial wilt incidence (P<0.05; P< 0.001), the AUIbwPC (P<0.05; P< 0.01) and percentage of colonized plants (Table 4). The highest incidence of wilted plants was recorded in cv. Gboma-teck (41.67%; 83.33%) followed by the Gboma-PCM (23.33%; 50%) in both experiments. The cv. AUB3G (8.33%; 10%) showed the lowest incidences. The disease incidence of cv. BOC15 (6.67%; 46.66%), CR1-13-049 (8.33%; 60.66%), CR1-01-001 (15.37%; 50%) and Gboma-Athiémé (15.83%; 36.66%) are in middle (Table 4). The AUIbwPC varied significantly (P <0.05; P <0.001) between cultivars of Gboma and shown similar trends to those of the incidences of bacterial wilt. The highest AUIbwPC values

showed a highly significant difference (P< 0.001) between the nine cultivars during the two experiments (Table 3). The highest AUIbwPC were noted for the cv. Tohounvi (1166.66 and 1073.33) followed by TLCV15 (665 and 1353.33) and the lowest was noted for Platinum (58.33 and 175.00). The AUIbwPC was null (0) for PADMA and HW7996 during the two experiments.

During the 2nd experiment, an increasing in the AUIbwPC was recorded for more than 50% of the tested cultivars. Based on the responses of tomato cultivars to bacterial wilt induced by the strain of *R. solanacearum* 19bLDC, the cvs. PADMA and HW7996 were highly resistant and Platinum was resistant. This resistance was moderate for cvs. Jalani, Thorgal and Mongal. In contrast, cv. Nirvana was moderately susceptible and cvs. Tohounvi and TLCV15 were susceptible.

has been recorded for cv. Gboma-teck (583.33; 1061.66) followed by cvs. CR1-13-049 (87.50; 700.00), CR1-01-001 (296.52; 641.66) and Gboma-PCM (256.66; 571.66) and the lowest value has been recorded for the cv. AUB3G (160.41; 128.33).

Furthermore, all cultivars of Gboma were colonized by *R. solanacearum* strain 5aLDC with an increasing of colonized plants during the second experiment for all cultivars except AUB3G.

Considering the reactions of the cultivars of Gboma to the strain 5aLDC of *R. solanacearum* from Gboma, it follows that only the cv. AUB3G was resistant. The cvs. CR1-13-049, CR1-01-001, BOC15, Gboma-PCM and Gboma-Athiémé were susceptible and the cv. Gboma-teck was highly susceptible.

Table 4. Bacterial wilt incidence, index of colonization and area under bacterial wilt incidence progress curve of seven Gboma cultivars

Gboma cultivars	Incidence (%)		Colonization (%)		AUI _{bw} PC		Reactions
	E1	E2	E1	E2	E1	E2	
Gboma-teck	41.67 ± 14.43a	83.33 ± 15.27a	45.83	100.00	583.33 ± 91.07a	1061.66 ± 297.67a	HS
Gboma-Athiémé	15.83 ± 19.41b	36.66 ± 5.77b	16.66	43.33	224.58 ± 30.64b	478.33 ± 199.01b	S
AUB3G	8.33 ± 14.43b	10.00 ± 10.00c	15	20.00	160.41 ± 77.84b	128.33 ± 141.45b	R
CR1-01-001	15.37 ± 8.35b	50.00 ± 10.00b	18.70	53.33	296.52 ± 162.61b	641.66 ± 222.279ab	S
BOC15	6.67 ± 11.54b	46.66 ± 5.77b	6.67	60.00	140.00 ± 42.48b	396.66 ± 20.20b	S
CR1-13-049	8.33 ± 14.43b	60.00 ± 17.32b	8.33	76.66	87.50 ± 51.55b	700.00 ± 311.08ab	S
Gboma-PCM	23.33 ± 15.27b	50.00 ± 10.00b	60.00	80.00	256.66 ± 248.31b	571.66 ± 165.40ab	S
P	<0.05	<0.001			<0.05	<0.01	

Means within column with the same letters are not significantly different according to Student-Newmann-Keuls test with P = 0.05. E= Experimentation

DISCUSSION

One of the most effective crop disease control approaches is the use of resistant materials. In this study, the reaction of some tomato and Gboma cultivars was assessed. For the two crops, these reactions were variable against *R. solanacearum*. The results showed that the local cv. Tohounvi showed more wilted plants of tomato with an incidence varying between 66.66% and 70% during the dry and rainy periods respectively. The tomato cv. Tohounvi has previously been identified as susceptible to bacterial wilt after disease incidence records in the field varying between 72% and 100% (Sikirou *et al.*, 2009). It is the most cultivated tomato cultivar for years by farmers because it is easier for farmer for self- seeds production. It wilts in all agroecological zones where *R. solanacearum* occurs in Benin. The current results demonstrate the high susceptibility of tomato cv. Tohounvi to the local strain and confirm its use as a susceptible test cultivar to *R. solanacearum* (Sikirou *et al.*, 2017).

The tested cultivars pointed out some susceptible, moderately resistant and resistant tomato cultivars to *R. solanacearum*. These results corroborate those of N'guessan *et al.* (2012) who reported a variation in tomatoes susceptibility against bacterial wilt. It ranged from highly susceptible to more resistant varieties. According to Grimault *et al.* (1995) and Monma *et al.* (1997), resistance to bacterial wilt in tomato is due to dominant single or recessive genes. Moreover, Oliveira *et al.* (1999) reported additive effects of genes for bacterial wilt resistance. During the two stages of experimentation, no wilted plants of the cultivar HW7996 was observed. The reaction of this cultivar to the bacterium is related to the resistance genes that it holds. The variety HW7996 is reported as resistant to *R. solanacearum* by many authors (Grimault *et al.*, 1995; Wang *et al.*, 2000; Carmeille *et al.*, 2006; Wicker *et al.*, 2009). These previous results with

regard to the behavior of tomato cv. HW7996 justify its choice as a reference resistant cultivar in this study. Our results confirm those of Hanson *et al.* (1998) who stated that the varieties HW7996, HW7997, CRA66 and TML114 are the main sources of resistance in tomato to bacterial wilt. They also claimed that among these varieties, HW7996 is resistant to *R. solanacearum* phylotype I and II. Among all tested tomato cultivars, only PADMA came as the closest to HW7996 in terms of resistance to wilt and was identified as a resistant cultivar to strain 19bLDC. The Acc 99, Acc 151, Hy 5 and Sweet 72 genotypes have been reported resistant to *R. solanacearum* by Tewari (1986). Similar results were obtained for tomato cultivars Sonali (Patil *et al.*, 1990), BWR-1 and BWR-5 (Bora *et al.*, 1993) and BT-18 (Mishra *et al.*, 1995). Sharma *et al.* (2006) identified resistance genes to bacterial wilt in cultivars Swarna Naveen, Swarna Lalima and B-17. Scott *et al.* (2009) also demonstrated a high level of resistance to *R. solanacearum* in large fruit breeding tomato lines from eight crosses of the F5 generation.

All tested tomato cultivars have been colonized by the bacteria. The cv. PADMA which appeared as resistant with no wilted plants was colonized at 80% by the bacteria during the second experimentation. This can be explained by the latency of *R. solanacearum* in resistant tomato cultivars vessels as reported by Prior *et al.* (1989). The fact that several resistant species and genotypes can harbor the pathogen without showing symptoms have already been reported (Jyothi *et al.*, 2012). Unlike PADMA, the cv. HW7996 was not colonized by the used isolate during the first experiment. According to Prior *et al.* (1996), resistance to bacterial wilt results from the non-penetration of the bacteria into the plant or from its less propagation in vascular tissues.

For Gboma, this study reports for the first time its reaction to bacterial wilt during screening test. The

cultivars Gboma-Athiémé, CR1-01-001, BOC15, CR1-13-049 and Gboma-PCM were susceptible with an incidence varying between 36.66% and 60%. The Gboma cultivar AUB3G was resistant to the disease. The susceptibility of cv.Gboma-PCM (the most cultivated cultivar) to the bacterial wilt caused by *R. solanacearum* was reported by Sikirou *et al.* (2015b). Moreover, all tested Gboma cultivars were colonized by *R. solanacearum*. This result assumes that these Gboma cultivars tested in this study are penetrated by the bacterium *R. solanacearum*. This suggests that farmers have to avoid burying plants residues after harvesting tomato and Gboma as green manure in soils to avoid increasing soil inoculum for subsequent crops susceptible to *R. solanacearum*.

For tomato or Gboma cultivars, incidences as well as percentages of colonized plants by *R. solanacearum* were low during the first experiment installed in fresh period (27.65 °C) and high during the second experiment installed in a dry period (29.50 °C). This finding confirms many previous results which demonstrated that temperature and humidity are the main development and multiplication factors of phytopathogenic bacteria. The general increasing of bacterial wilt incidence and colonized plant percentage may be due to the upward temperature variation which favored the multiplication of bacteria in plant stems. Also, in the dry season, with the low available water at soil level, plants absorb more individuals of *R. solanacearum* through the roots by drawing up the soil water. The same phenomenon had been reported by Techawongstien *et al.* (2009) who noted that bacterial wilt of tomatoes is more severe in the dry season than in the rainy season for tomato plants installed on infected soils.

CONCLUSION

Tomato and Gboma cultivars show variations in their resistance response to *R. solanacearum*. Two tomato cultivars HW7996 and PADMA were found highly resistant, one Platinum was resistant, and three Jalani, Thorgal and Mongal were found as moderately resistant. One Gboma cultivar AUB3G appeared resistant to *R. solanacearum*. The moderately resistant cultivars are recommended for cultivation under integrated production systems. New resistant tomato and Gboma cultivars are to be developed to increase the number of available resistant cultivars in Benin.

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