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Research Article

EVALUATION IN SEMI-CONTROLLED CONDITIONS OF RESISTANCE TO BACTERIAL WILT OF 19 VARIETIES OF TOMATO (*Lycopersicon esculentum* MILL.)

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A B S T R A C T

This study is part of the search for effective and efficient control methods against bacterial wilt caused by *Ralstonia solanacearum* Smith for the improvement of tomato production in Burkina Faso. A screening experiment on nineteen (19) varieties of tomato was carried out at the research station of the former plant protection laboratory, in 2016, to select resistant or tolerant varieties to bacterial wilt. The test was arranged in Fisher block with 4 repetitions. After 2-week nursery, plants from two (02) to three (03) true leaves were subcultured at a rate of one foot into 5L pots containing previously well-sterilized culture substrate. Observations and measurements focused on the susceptibility of varieties to bacterial wilt and the evaluation of phenological parameters (height, crown diameter, number of inflorescences, number of flowers and number of fruits) by variety. The results indicate that the varieties (CRA 66, BF-Okitsu and F1 Platinum) are the least susceptible to wilting compared to the resistant control (F1 Mongal). In addition, the most productive varieties are Hawai 7996, F1 Platinum, FBT3, FBT1 and TML46 oblong. It is therefore necessary to continue research in an agronomic approach in the field on heavily infested soils before any recommendation.

INTRODUCTION

Burkina Faso is a predominantly agricultural country where vegetable crops sector is very important. This sector alone contributes 8 to 9% of agricultural production and 3% of total GDP. Indeed, it is more than eighty-two (82) billion CFA that are generated annually by this sector. The major speculations that crown this are bulb onion, tomato and cabbage. The tomato is produced throughout the territory during all seasons. The production of tomato in 2014 amounted to two hundred and eighty-nine thousand five hundred and seventy-two (289,572) tons out of 11,766.4 ha of planted area or a yield of 24.61 tons / ha. The tomato alone generates more than 17.47 billion CFA francs, which represents 21% of the total turnover of the market gardening sector (MAHRA, 2011). However, the increase in production is hampered by many biotic diseases including bacterial wilt.

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The bacterial wilt caused by *Ralstonia solanacearum* Smith is one of the most harmful phytobacteriosis in the world (MANSFIELD *et al*., 2012). It exists on five continents, and is subservient to more than 250 species grouped into fifty (50) botanical families (CELLIER and PRIOR, 2010). The disease can cause 80 to 100% yield loss depending on tomato varieties (FONDIO *et al*., 2010). Some of these host plants such as potatoes, tomatoes, cabbages and peppers, which are of major economic interest in Burkina Faso, are the most infected. Indeed, the pathogen has been at the origin of the abandonment of certain speculations in market gardening perimeters in Burkina Faso (SOME, 2001). To control this bacterial disease, it is recommended to use fallow or crop rotation of the tomato with other non-host crops (corn, soy, rice, etc.). This technique seems to show limits in Burkina Faso because of the land deficit (KANDA *et al*., 2014). Producers produce the same speculations in continuous monoculture on the same portions of land (FONDIO *et al*., 2013). As a result, the search for resistant or tolerant and productive varieties seems the ultimate sustainable recourse indicated to improve the productivity of tomatoes (LEBEAU, 2010). Hence the screening of 19 varieties of tomato in the presence of *Ralstonia solanacearum* in a semi-controlled medium to determine the most resistant or tolerant to the disease. This study will evaluate the incidence of the disease on the 19 varieties of tomato in semi-controlled station. It is a question of estimating the average index of severity and the influence of the disease on the phenological caracteristics of these varieties.

MATERIAL AND METHODS

Study site

The work was carried out in the city of Bobo-Dioulasso on the semi-controlled experimental site of the former plant protection laboratory in december to january 2017. The geographical coordinates of this site are, 11,15611 ' 'North latitude and 004,28600' 'west longitude. The climate of the study area is tropical with a rainy season (May in October) and a dry season (November in April). During the experimental period, the temperature varied between 20 and 25 ° C and the relative humidity progressively evolved from 110 to 115%, the rainfall was (INERA, 2017).

Material

The biological material consists of 19 tomato varieties including 11 from CORTE TAP2, 2 from East West Seed, 4 from INERA Farako-Bâ and 2 hybrids including the sensitive control Rossol and the resistant control Mongal F1 (Table 1).

Table 1: Varieties screened

Codes	Varieties	Origins
V1	F1 mongal	Technisem
V2	NC72TR4-4	INRA
V3	FBT4	INERA
V4	IRATL3	INRA
V5	FBT3	INERA
V6	TML46 oblong	INRA
V7	FBT2	INERA
V8	Hawai 7996	INRA
V9	FBT1	INERA
V10	CRA 66	INRA
V11	Okitsu Sozai N°1	INRA
V12	BF-Okitsu	INRA
V13	Rossol	Technisem
V14	L390	INRA
V15	R 3034	INRA
V16	L285	INRA
V17	CLN1463	INRA
V18	F1 Platinum	East-West Seeds
V19	F1 Padma	East-West Seeds

Two virulent bacterial strains of phylotype I (Bol1) and phylotype III (RUN 1793) were used to evaluate the resistance or tolerance of the 19 varieties of tomato.

In addition, laboratory equipment for bacterial cultures, test maintenance products, were also used: deltamethrin for foliar treatments against insect pests and mites, NPK (15-15-15). and urea 46% as fertilizer.

Methods

Experimental design

The experimental design is a randomized Fisher block composed of nineteen (19) treatments and four (04) replicates applied for each treatment compared to the two (02) strains. pots of 5L containing sterilized substrate, consisting of a mixture of potting soil (3/4) and organic manure (1/4) were used to grow the plants. Each pot contains a plant that is watered as needed and treated with Deltamethrin to reduce the pressure of insect pests.

Inoculation of plants

The technique described by N'GUESSAN *et al.* (2012) was used to infect plants. It consists first of all in scarifying the roots of the plants with a scalpel. Then a volume of 5 mL of bacterial suspension of each of the two strains calibrated at 10^8 $CFU.mL^{-1}$ is deposited at the foot of the plants and covered with soil.

Evaluation of the wilt index of the disease

Symptoms were noted daily from the third day after inoculation over four (04) weeks. The severity was noted on the plants according to the scale defined by COUPAT *et al.* (2011): 0 for no symptoms, 1 for a withered leaf, 2 for two withered leaves, 3 for at least four wilted leaves, 4 for dead plant. The evaluation of the wilting index (FI) takes into account the notes 3 and 4. Thus, IF is expressed according to the formula:

$$\text{IFB} = \frac{\text{number of fletried plants at date t k}}{\text{total number of plants}}$$

IFB is the bacterial wilt index at day X after transplant (JAR); tk is the number of JARs during the observation.

The wilting index data at different dates made it possible to evaluate the severity of the disease on these same dates and its evolution over time(AUDPC=f(t)).

$$\text{AUDPC}\ (t_k) = \sum_{i=1}^{k-1} (\text{IFB}_i + \text{IFB}_{i+1})(t_{i+1} - t_i)/2$$

AUDPC (tk) is an area under disease progressat X days after transplanting; IFBi corresponds to IFB on the previous day of observation; IFB i + 1 corresponds to IFB on the day of observation; ti + 1 is the date of the rating; ti is the date of the previous observation.

Effect of bacteria on phenological variables

The phenological variables measured were: plant height, fruit number, crown diameter, number of inflorescences and flowers. The height and diameter of the collar were measured weekly using a graduated ruler and a vernier caliper respectively. Regarding the number of inflorescences, flowers and fruits, we proceeded by weekly counting at each level of tomato.

Statistical analysis

The data obtained were analyzed with software R, version 3.4.1 (R Core Team, 2017). We made a matrix of pearson correlation of the different variables between them. The closer the correlation is to 1, the stronger the relationship. The effect of the strains on the wilting index and the production of the different varieties was evaluated by a generalized linear model and an analysis of variance. Varieties were compared in pairs by the post-hoc Tukey test (Bretz, 2010). Graphs are realised with R and Excel spreadsheet 2013. A P-value of 0.05 was used to determine significance.

RÉSULTS

Correlation between phenological variables

The largest correlations were observed for factors that measure flowering and fruiting: number of flowers, number of inflorescence and number of fruit. This correlation of the order of 0.79; 0.81 and 0.96 for respectively number of inflorescences and fruits; number of flowers and fruit and finally number of inflorescences and flowers. The collar diameter and height factors determining the vigor of the plant are also well correlated with each other, this correlation is 0.65 (Table 2). As a result, the wilting index and fruit number were retained for further analysis. Indeed, the performance of a tomato plant necessarily takes into account its ability to produce fruits regardless of biotic and abiotic constraints. Thus, the number of fruits being generally well correlated with other phenological variables, the results obtained for the number of fruits are generalizable to others.

Table 2 Correlation between phenological variables observed

Phenological variables	Height	Fruit Number	Collar diameter	Inflorescence number	Flower number
Height	1	0,4463437	0,6565619	0,6095046	0,5956732
Fruit Number	0,4463437	1	0,4272097	0,7903065	0,814467
Collar diameter	0,6565619	0,4272097	1	0,5934451	0,5876671
Inflorescence number	0,6095046	0,7903065	0,5934451	1	0,9645913
Flower number	0,5956732	0,814467	0,5876671	0,9645913	1

Wilt index of 19 varieties against strains of bacteria

The analysis of the variances reveals a significant difference (p<0,05) between the wilt indices observed whatever the bacterial strain. In fact, compared to the reference strain (RUN 1793, phylotype III), the F1 Platinum variety does not wither and the most sensitive varieties are FBT2, FBT4, L285, FBT3 and Okitsu Sozai N ° 1 with wilting indices similar to that of the susceptible control, while the varieties: L390, Padma F1, CRA66, CLN1463, BF-Okitsu recorded wilting indices statistically equal to that of the Mongal F1 resistant control. However, the varieties: Okitsu Sozai N ° 1, CLN1463, F1 Platinum, R3034, FBT3, FBT4, IRATL3, L390 and the TML46-Oblong give wilting indices close to that of the sensitive control Rossol.

Moreover, the analyzes carried out on the IFs of the local strain BOL1 showed that the varieties BF-Okitsu and CRA66 do not wilt and that the most sensitive varieties are the NC72TR4-4, the Hawai7996 and the F1 Padma, while the FBT1, FBT2 and L285 have a wilting index statistically equal to that of the Mongal F1 resistant control. On the other hand, the varieties: Okitsu Sozai N ° 1, CLN1463, Platinum F1, R3034, FBT3, FBT4, IRATL3, L390 and the TML46-Oblong give wilting indices close to that of the sensitive control Rossol. As a result of this analysis, regardless of the bacterial strain used, BF-Okitsu F1 Platinum varieties are resistant to the disease. Varieties L390 and CLN1463 have a wilting index relatively close to that of the sensitive control (F1 Mongal) (Table 3).

Table 3 Wilt index of 19 varieties against bacterial strains

Varieties	Average wilting indices	
	Local strain Bol1	Reference strain RUN 1793
BF okitsu	0,000 (0,000) a	0,125 (0,342)b
CLN1463	0,375 (0,500)bc	0,187 (0,403)b
CRA66	0,000 (0,000)a	0,250 (0,447)b
F1 Mongal	0,250 (0,440)b	0,125 (0,341)b
F1 Padma	0,500 (0,516)c	0,250 (0,447)b
F1 Platinum	0,375 (0,500)bc	0,000 (0,0)a
FBT1	0,125 (0,341)b	0,437 (0,512)bc
FBT2	0,250 (0,447)b	0,750 (0,447)c
FBT3	0,437 (0,512)bc	0,500 (0,516)c
FBT4	0,437 (0,512)bc	0,687 (0,478)c
Hawai 7996	0,562 (0,512)c	0,437 (0,512)bc
IRATL3	0,437 (0,512)bc	0,373 (0,500)bc
L285	0,250 (0,447) b	0,562 (0,512)c
L390	0,437 (0,512)bc	0,250 (0,447)b
NC72TR4-4	0,750 (0,447)c	0,312 (0,478)bc
Okitsu Sozai N°1	0,312 (0,478)bc	0,500 (0,516)c
R3034	0,375 (0,500)bc	0,375 (0,500)bc
Rossol	0,437 (0,512)bc	0,500 (0,516)c
TML46 Oblong	0,437 (0,512)bc	0,312 (0,478)bc

Mean wilting index values followed by the same letters did not differ significantly (P <0.05).

Stump effect and variety on the number of fruits

In view of the lack of fruit before the fourth week, the analyzes focused on week 4. Indeed, the best model is a linear model that explains the number of fruit by variety and strain. The effect of these two variables is significant at the 5% level (p <0.05). In sum, the plants infected with the reference strain Run1793 give more fruits than those infected with the local strain Bol1. Indeed, the analysis of the prediction diagram (Figure 1) shows that whatever the bacterial strain used, the most productive variety is Hawai7996 and the least productive variety is Okitsu Sozai N ° 1. There are four (04) varieties (F1 Platinum, FBT3, FBT1 and TML46 Oblong) that yield more fruit compared to F1Mongal resistant, while R3034, FBT4 and FBT2 have a similar production to Mongal F1. . However, the varieties: BF-Okitsu, L390 and NC72TR4-4 have a similar production to the sensitive control Rossol. The other five (05) varieties (CLN1463, L285, IRATL3, CRA66 and F1 Padma) have intermediate production between the two (02) controls used.

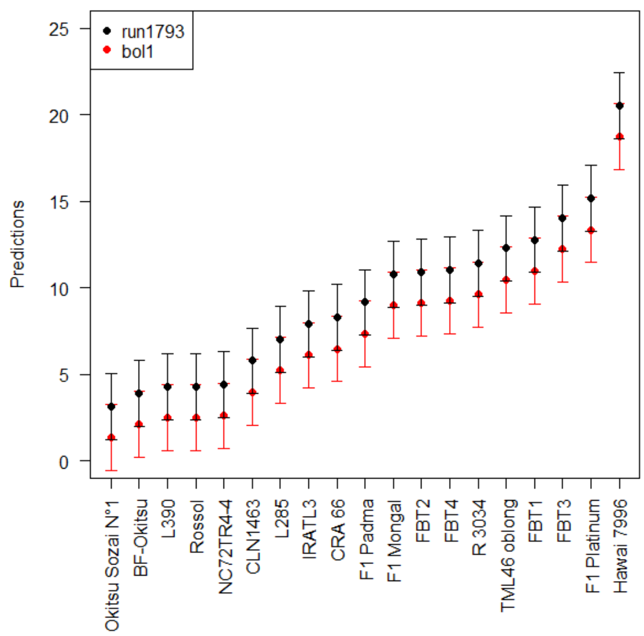
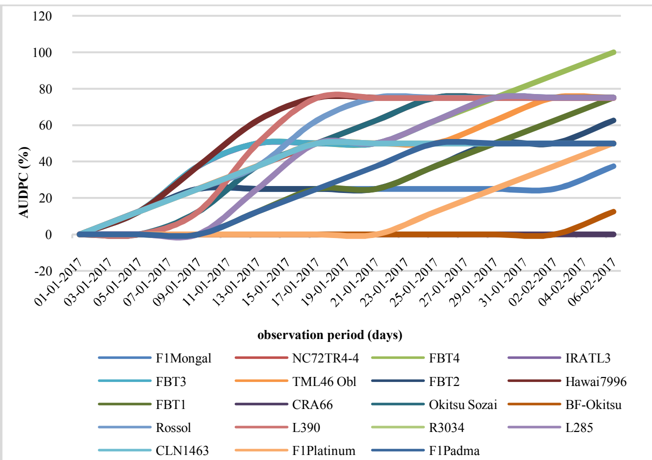
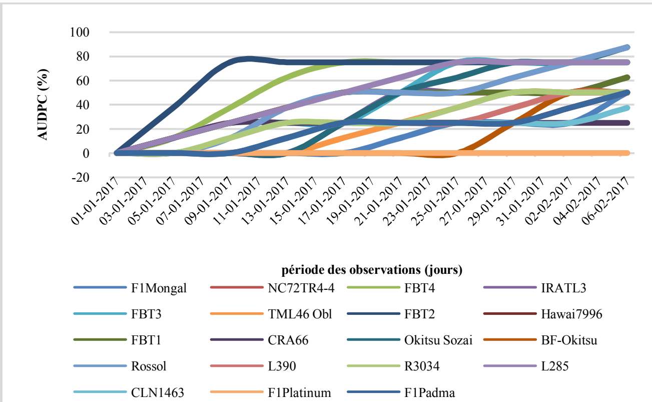


Figure 1 Stump effect and variety on the number offruits

Cinetics of disease progression caused by Bol1 and RUN1793 strains on 19 varieties

Figures 2 and 3 provide information on the evolution of bacterial wilt during the observation period for all the varieties studied. Indeed, the observations of the local strain Bol1 show that the disease is induced by it on most varieties one week after inoculation. However, the disease settled towards the end of the trial on F1 Platinum and BF-Okitsu varieties. No symptoms were noticed on the CRA66 variety. The most sensitive varieties compared to the Rossol susceptible control with an average disease progression of 37.75% are L390, FBT4 and Hawai7996, each with a respective increase of 51.25%; 52.5% and 56.25%. On the other hand, the varieties least susceptible to the disease with regard to the resistant F1Mongal control with an average progression of 18.75% are the varieties BF-Okitsu and CRA66 with each an increase of respectively 6.25% and 0% (Figure 2). Regarding the reference strain RUN1793, it is found that the disease is induced in the majority of varieties 2 weeks after inoculation. However, after the induction of the disease one week after inoculation in the variety CRA66, the evolution remained constant until the end of the collections. In addition, no signs of disease were observed with the F1 Platinum variety during the observation period. The most sensitive varieties compared to the Rossol susceptible control with an average progression of the disease of 43.75% are the varieties FBT2, FBT4 and L285 which each record an average progression of 63.75%; 56.25% and 48.75%. On the other hand, the varieties least susceptible to the disease with regard to the resistant F1Mongal control with an average progression of 25% are the varieties CLN1463 CRA66 and F1 Platinum with each an increase of 18.75% respectively;12.25% and 0% (Figure 3).





DISCUSSION

Positive correlations between the different phenological variables (crown height and diameter) that provide information on plant vigor and variables such as inflorescences, flowers and fruits that provide information on plant productivity indicate that they are all intrinsically linked. The genetic performances of each variety. In fact, the more vigorous a variety is, the more its resilient capacity increases, all which contributes to its greater productivity. Since the vigor of the plant evolves over time, then the mechanism of resistance or defense would not be effective enough to inhibit the pathogenicity of the pathogen from the first moments. Therefore, the less vigorous varieties would have a younger tissue structure and therefore less able to control the disease (SORO *et al.,* 2008). Moreover, the strong correlations between the productivity variables are due to the fact that the productivity of the plant depends on its ability to give many more inflorescences and therefore flowers.

There are no standard criteria for assessing plant resistance to disease. Indeed, several authors who have worked on the incidence of R. solanacearum for various speculations do not agree on the rate of tolerance or resistance to the disease. Thus, ADAMOU (2011) considers that a variety of potato with a wilting rate of 9% is tolerant, it would be sensitive to 19.05% and very sensitive from 38%. Moreover, WANG *et al.* (1997) estimate that a variety with a wilting index of 11% is resistant, it would be intermediate resistance from 57%, and very sensitive from 90%. N'GUESSAN *et al.* (2012) showed that there are different levels of susceptibility to bacterial wilt ranging from highly susceptible to most resistant varieties in Ivory Coast. Thus, the 19 varieties screened behaved differently with respect to both strains. These differences in varietal behavior vis-à-vis strains are related to the intrinsic characteristics of each variety (N'GUESSAN *et al*., 2012). The most resistant varieties are CRA 66, BF-Okitsu and F1 Platinum, the first two are long used as rootstocks because of their resistance to the disease (BLANCARD and PRIOR, 2013). . All varieties behaving like Mongal F1, a control resistant to bacterial wilt and its adaptation to hot and humid climate (VAN DER VOSSEN *et al*., 2004), by analogy are considered tolerant to the disease.However, this tolerance of Mongal F1 remains unstable in that it has been circumvented at times. These results are consistent with those of CHESNEAU and ROUX-CUVELIER, (2012) where 90% of Mongal F1 plants were found to be susceptible to bacteriosis. Also the work of BORO (2014) showed that the bacterium is not without incidence on the variety Mongal F1. On the other hand, those with the highest rate of wilting similar to Rossol are the most sensitive. The absence of symptoms on some tomato varieties (BF-Okitsu, CRA66 and F1 Platinum) can be explained by a late manifestation of the pathogen, in that no variety has recorded a zero colonization index. Indeed, the values of 20 to 25 ° C average temperature and average humidity of 110 observed during the test do not promote the manifestation of the disease. These same climatic conditions would have an inhibitory effect on the genes responsible for the resistance of the tomato varieties. Modern marker-assisted genetic methods reveal that resistance to bacterial wilt in L285 and Hawaii is polygenic and is carried by 2 or more chromosomes. Both QTLs showed stronger effects in the warm season than in the cold season. However, Bwr-4 and Bwr-8 QTLs were only detected in the warm season, demonstrating that environmental factors may influence the expression of resistance against the strain (LEBEAU *et al*., 2011).

Moreover, since the resistance of any plant to a disease is a function of its ability to recognize the signal or signals produced by the pathogen at the first moments of infection, the kinetics of detection pathogen remains important in plant resistance, as too slow detection is often the cause of host susceptibility (LEBEAU, 2010). This resistance seems to be related to the genetic characteristics of these varieties. Indeed, varieties such as Hawaii 7996, BF-Okitsu, TML 46 oblong were tested resistant by WANG *et al*. (2000) in 12 localities in different agro-ecological zones. The analysis of the progression of the disease shows that it is very early with the local strain (Bol1) than the reference strain (RUN 1793). Also, the number of fruits per variety is higher with the reference strain than with the local strain. This finding could be explained by the difference in aggressiveness between the two strains. Indeed, the OUEDRAOGO (2016) study has shown that the local Bol1 strain originating from a market garden site in Orodara in Kénédougou is more aggressive (IF = 95%) than the reference strain RUN 1793 (IF = 89%)).

The high fruit numbers of the Hawai7996 and F1 Platinum varieties compared to the other two resistant varieties such as the CRA 66 and the BF Okitsu would be linked to the only ability of the varieties to be produced under the same conditions. This result is in agreement with that of DJIDJI *et al*. (2010) for which, the difference in yield observed during a season is due to the characteristics and genetic performances of the variety.

CONCLUSION

This study shows that the measured phenological parameters are strongly correlated with each other. Indeed, the number of fruits being very correlated with the other phenological parameters. There is a stump effect on the different varieties. Thus, regardless of the bacterial strain, of the 19 varieties of screened tomato, BF-okitsu, Platinum F1, L390 and CLN1463 have a wilting index close to that of the F1 Mongal resistant control. The disease remains much earlier with the local strain than the reference strain. Taking into account the two strains, we find that the disease is more aggressive on the L390, FBT4, Hawai7996, FBT2 and L285 varieties, while the BF-okitsu, CRA66, F1 Platinum and CLN1463 varieties are resistant to disease. The strongest and weakest productions were found respectively with the varieties Hawai 7996 and Okitsu SozaiN ° 1. Our study reveals that the most productive variety remains sensitive to the disease and that the less sensitive remains unproductive. Indeed, it is desirable in complementarity with a team of breeders to cross the said varieties in order to combine the two performances. Furthermore, it is desirable to continue the research with the varieties (FBT2, FBT4, R3034, Oblong TML46, FBT1, FBT3, F1 Platinum and Hawai7996) with agronomic characteristics quite similar to the field-resistant control on soils with high bacterial inoculum. . Anything that will make it possible to appreciate their behavior with regard to bacterial wilt on the one hand, but also their net yields in fruts.

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