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ANSWER TO COMMON BACTERIAL BLIGHT (*Xanthomonas axonopodis p.v phaseoli*) IN THE COMMERCIAL CULTIVARS OF COMMON BEAN OF CUBA, UNDER FIELD CONDITIONS. AFFECTATION OF THE YIELDS FOR EFFECT OF THE INOCULATION

Respuesta a bacteriosis común (*Xanthomonas axonopodis p.v phaseoli*) en los cultivares comerciales de frijol común de Cuba, en condiciones de campo. Afectación de los rendimientos por efecto de la inoculación

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ABSTRACT. It is possible to find in a Cuban bean germplasm, cultivars whith some level of resistance to a pathogenic strain of *Xanthomonas axonopodis* pv. *Phaseoli* which allow to diminish the losses and increase the yield average of the crop on infected fields. The goals of this work were to evaluate differentials responses of common bean genotypes to natural infection and inoculation with the pathogenic strain of *Xap 527* and identify commercial cultivars with better behavior to damages on leaves and sheaths and there fore less losses on yield. The results allowed selecting bean genotypes, with good behavior against the studied strain *Xap*. The decreases of the yield were determined in the studied bean cultivars by its reaction of susceptibility in front of common Bacteriosis, with the inoculation of Cuban the 527*Xap* strain.

Key words: germplasm, inoculation, susceptibility, yield losses

RESUMEN. En el germoplasma de frijol común (*Phaseolus vulgaris* L.), en Cuba, es posible identificar cultivares con niveles de resistencia ante un aislamiento patogénico de *Xanthomonas axonopodis* pv. *phaseoli*), a fin de disminuir las pérdidas y aumentar el rendimiento promedio del cultivo en campos infectados con este patógeno. Los objetivos de este trabajo estuvieron dirigidos a evaluar, en genotipos de frijol común respuestas contrastantes frente a la incidencia natural e inoculación del aislamiento patogénico *Xap*527 (*Xanthomonas axonopodis*); identificar los cultivares comerciales con mejor comportamiento en follaje y vainas y con menores pérdidas de rendimiento en campos infectados por este patógeno. Los resultados permitieron seleccionar los genotipos de frijol, con buen comportamiento frente al aislamiento de *Xap* estudiado y se determinó la disminución del rendimiento en los cultivares de frijol por su reacción de susceptibilidad frente a Bacteriosis común, con la inoculación del aislamiento *Xap* 527, de Cuba.

Palabras clave: germoplasma, inoculación, susceptibilidad, pérdidas de rendimiento

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most consumed edible legumes worldwide, provides an important source of protein (22 %), vitamins and minerals (Ca, Cu, Fe, Mg, Mn, Zn) to the diet of the population in America, especially in developing countries. Annual production in developed countries exceeds 21 million metric tons, accounting for more than half of the total production of legumes for consumption in the world (1).

In Cuba, this crop production in 2009 was 110,800 metric tons, with a cultivated area of 150,584 ha (2). In the 2010-2011 campaign, 80 thousand tons of beans

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must be produced in a planting area 85 to 89000 ha with national seed (3). During the last decade, the production of beans in Cuba was in charge, the vast majority, of non-state agricultural sector, mainly consisting of small farms and plots with very different conditions and low availability of agricultural chemicals and energy inputs (4. 5). A lack of supplies joined the limited access of farmers to the new improved cultivars, or not, with adaptation to agricultural production conditions in the Cuban context. Beans produced by these farmers with limited resources, are more vulnerable to stress caused by abiotic factors such as drought and low soil fertility and biotic stress due to field and storage pests.

Among main factors which limit the production of common bean in Cuba is common Bacteriosis of bean, caused by *Xanthomonas axonopodis* pv. *Phaseoli* (Smith) Dye *(XAP)* and *Xanthomonas axonopodis* pv. *Phaseoli* (Smith) Dye *(XAP)* cv. Fuscans *(Xapf)* (6). This disease occupies the second place in the most economically important in Cuba and in the world.

In Cuba, this disease is widely distributed; however, it is not yet known how much can affect the presence of high levels of this pathogen in the performance of Cuban commercial cultivars. Other countries have reported infection rates in pods, up to 48.5 % in susceptible cultivars; figures may become higher in the foliage, where they have found infection rates above 80 % and yield losses between 17 and 45 % (7).

In our country, it has an extensive creole collection, commercial and introduced improved ones, with multiple characters of interest for producing cultivars. However, susceptibility to common Bacterioses, can limit the grain production produced by the decrease in yields, to be presented pathogen in susceptible cultivars and with favorable environmental conditions for its development and dissemination.

The development of cultivars and resistant lines against pathogen attack or against high levels of severity of this disease should be one of the objectives of genetic improvement programs, because the country is a must to obtain high yields of beans during next years, in order to supply, with national productions, the demand of Cuban population (8).

MATERIALS AND METHODS

This study was conducted for three consecutive seasons, experiments relating to the assessment of the reaction foliage and pods (without inoculation and inoculation of 527 Cuban isolation XAP) of commercial cultivars of common bean (*Phaseolus vulgaris*) from Cuba were performed

- Commercial cultivars used against the natural incidence of the common Bacterioses

17 cultivars (Table I) were sown in plots of two rows of four meters long and 0,70 m wide for each cultivar planting at a distance of 5 cm between plants. Planting, irrigation, fertilization, cultural care and pest control were carried out following provisions for the cultivation of beans (9).

Any chemical control of diseases was made anticipating an unfavorable effect on the symptom manifestation of the common Bacteriosis.

Table I. Commercial cultivars of Cuba used in the study

Cultivars	Seed color	Progenitors	Place of origin
'CC 25-9'	Black	Selection Ticos de Costa Rica	INIFAT
'Bolita42'	Black	Selection from Holguín 3	IIG
'ICA Pijao'	Black	Porrillo Synthetic x México 11	IIG
'Güira 89'	Black	Selection Jamapa	INIFAT
'BAT 304'	Black	Porrillo Synthetic x Compound Chimaltenango	IIG
'Tazumal'	Black	(Sal 22 G4 x 11183N) x (ICA Pijao x Turrialba 1)	IIG
'Holguín 518'	Black	Black Jamapa x Turrialba 4	IIG
'BAT 832'	Black	Jutiapa 72 x Blanco 137	IIG
'CC 25-9'	Red	Selection from CC 25-9	INIFAT
'Hatuey 24'	Red	(Jamapa x Porrillo 70) x Jamapa x Prs 70)	IIG
'Velasco Largo'	Red	--------	INIFAT
'M-112'	Red	Selection from Mulangri	IIG
'Red Kloud'	Red	Red Kloud x Charlotee	IIG
'Guamá 23'	Red	(Diacol Calima x Red Kloud) x Red Kloud	IIG
'Bonita 11'	White	--------	IIG
'Chévere'	White	(Jin 108 x Ica Bunsi)x(Veranic x Jutiapa 72)	IIG
'Engañador'	Beige	(Veranic 2 x G 1320) x (Jamapa x Tara)	IIG

IIG: Instituto de Investigaciones de Granos. INIFAT: Instituto de Investigaciones Fundamentales en Agricultura Tropical Alejandro de Humboldt

The disease severity on the foliage and pods and the performance was evaluated. Reaction evaluations in foliage were performed using nine degrees scale, where 1=no visible symptoms; 9=very severe symptoms of disease (10).

The first evaluation was performed in the foliage 20 days after sowing (DAS), with an interval of seven days between them. Six evaluations were performed consecutively in total. Severity data foliage against common Bacteriosis corresponded to that taken in the last assessment (65 DAS) because this value was the maximum reaction expressed by each cultivar against this pathogen value. At this very moment the only severity assessment of the pathogen to pods was made.

♦ Commercial cultivars against the isolation *Xap527* of Cuba

Simultaneously, a trial with the same commercial cultivars mentioned above (Table I) was planted. The trial was formed following the statistical design of randomized blocks with three replications, where the experimental plot consisted of two rows of four meters long to cultivate.

They were used as control crops 'XAN 112' and 'Engañador' and as susceptible 'Velasco Largo' and 'ICA Pijao' cultivars. Materials were set as follows: control resistant and one susceptible followed of five cultivars to study, subsequently controls were placed again, but alternately, the first susceptible followed resistant, placing five cultivars and so on, to be made throughout the trial to assess reaction to common Bacteriosis.

Assessments of disease severity on foliage and pods were performed as described in the previous section "Commercial cultivars against natural incidence of common Bacteriosis".

♦ Inoculation of *Xap527* isolation of Cuba in field trials

The inoculum was prepared from pure isolation 527, to increase, the pure isolation was seeded in a YCDA medium in 10 Petri plates were grown for 48 hours and washed with sterile distilled water. The suspension obtained was diluted in 12 L of distilled water, (necessary for motor backpack, of conical nozzle, with engine) until a bacterial concentration of 5×10^7cel mL; this concentration was measured at 650 nm optical density, in a Milton Roy spectrophotometer (Spectronic 20) (11, 12).

The inoculation is made under field conditions in each test, by the method of pressure spraying with motor backpack with engine, to ensure a uniform infection of plants in experimental plots. The first inoculation was performed at 20 days DAS; the following were made at 27 and 34, subsequent to the last 40DAS, at which susceptible controls ("Velasco Long 'and' ICA Pijao ') presented severe symptoms of the disease. Inoculations were performed in the tests after 4:00 pm to avoid solar radiation affect the viability of the inoculated bacteria.

During the execution of studies some rules were established in order to avoid possible risks to the environment with inoculation and spread of *Xap* other adjacent areas to these experiments:

- Seeds were planted in areas far from the center of the farm, biological barriers were established planting two rows of corn *(Zea mays)* all along the experimental area as this crop is not host of this pathogen
- After making leaf inoculations, frequent checks of the surrounding area were made to identify possible sprouts of the disease in other host crops.
- When the study completed, we proceeded to incorporate crop residues more than 20 cm deep, then rotating the area with crops such as maize *(Zea mays)*, sorghum (*Sorghum bicolor* L.) or other non-host of *Xap,* avoiding that the pathogen remains viable crop residues from a campaign to another.

Data taken in the tests

♦ Reaction of disease symptoms in leaves and pods

The classification of cultivars was conducted according to the reaction in the foliage, taking into account the average value of the latest assessment reply to cultivate. In assessing the reaction in pods, the value is taken only once, in the formation stage and grain filling (R^8), an assessment was performed on each cultivar or line in each of the replicates. For both classifications the overall scale was used to assess the reaction of bean to bacterial and fungal pathogens (10).

♦ Grain yield per plot

Once harvested the experiment, the performance data of each genotype in grams per plot were taken and converted to kg ha^{-1}.

♦ Depressive index of yield due to inoculation effect

With yield values obtained by commercial cultivars inoculated and not inoculated with 527 *Xap* Cuban isolation, depressive index was estimated following the formula:

$$\text{Depressive index} = \frac{\text{Yield var. No Inoculated - yield var. Inoculated}}{\text{Yield var. No Inoculated}}$$

Statistical Analysis

The values obtained were determined the average (x). For performance, it is applied a test T between inoculated and non-inoculated plants p≤0,05.

All statistical analysis was performed with the use of Infostat/Professional program. Version-2011 Windows (13).

RESULTS AND DISCUSSION

Commercial cultivars against natural incidence and inoculation of isolation 527 *Xap*

Table II presents evaluations foliage reaction under natural incidence of Salmonella, highlighting 'Tazumal' and 'Hatuey 24 cultivars ',as the less affected ones in the pathogen presence, with reaction values of 3 (resistant), according scale used (10), similar to the value obtained by the 'Engañador' witness (3), followed by' CC25-9 (N) '' Holguin 518 ',' 832 BAT, 'CC25-9(R)', 'M-112' and 'Bonita 11', with intermediate values of 4, against this pathogen.

The rest of the crops had reactions between 5,0 and 6,0, which allowed classifying them as intermediate reaction genotypes. These results are consistent with previously reported assessments made in commercial bean cultivars and lines under conditions of natural incidence of this pathogen (14).

After the inoculation with the isolation of Cap 527, more severe reactions were observed in the foliage for these same cultivars; however, 'BAT 304', 'Hatuey 24', 'M-112', 'Guamá 24' and 'Chévere' were the less affected by the presence of this isolation, reaction with intermediate values between 5,3 and 6, 6; the 'Engañador' witness remained in this range of reaction with a value of 5,6. Remaining evaluated cultivars had reactions of susceptibility to this insulation values ranging between 7,0 and 8,3. They are highlighted by the presence of severe symptoms of the disease and higher susceptibility values are presented in foliage 'Furball 42' and 'Güira 89' and 'Velasco Largo' and 'ICA Pijao' susceptible witnesses with reactions between 8,0 and 8,3.

In the evaluation to pods under natural incidence (Table II), it observed that in most commercial cultivars no symptoms of the disease were presented and they maintained within the range of resistance reaction, according to the scale used (10), with values between 2 and 3. 'Engañador' witness, within the same group, but with resistance reaction level 1. A compound group by 'CC 25-9 (N)', 'BAT 304' and 'ICA Pijao' cultivars, showed slight symptoms of the disease, which classifies them according to their reactions, as intermediate values of 4,0.

After the inoculation with the aforementioned isolation was observed in all cultivars increased values of reaction to this disease in pods, including the 'Engañador' witness; these values ranged from 4,0 to 6,6 (intermediate). 'Hatuey 24', 'M-112', 'Chévere' highlighted with the lowest reaction intermediate values, 4,0 to 4,6. Only 'Velasco Largo' cultivar presented severe symptoms of the disease, allowing its classification as susceptible, with a value of 7,3.

The results presented so far show in most cultivars, a different behavior under the natural incidence and against challenge with the pathogen of *Xap*527 isolation (Table II), where the effect of this increased favoured reactions resistant cultivars intermediates in some cases as 'Hatuey 24' and 'Engañador' witness and in others, intermediate to susceptible like: 'CC 25-9 (N)' and (R), 'Furball 42', 'ICA Pijao ',' Güira 89 ',' BAT 832 'for foliage and the ' Velasco Long ' cultivar for both reaction the foliage and pods.

Under natural field conditions, without direct inoculation of the pathogen, symptoms of common Bacterioses are presented in commercial cultivars that are used in production in Cuba. However, once inoculated, the reaction against this disease varies and it is expressed in greater or lesser degree depending on the resistance level of evaluated cultivars and inoculum pressure. So, for a more reliable evaluation and selection of cultivars to this disease, it becomes necessary inoculations with isolates pathogenic *Xap* evaluating cultivar reactions against high pressure of this pathogen. Otherwise, it runs the risk of selecting cultivars that do not express their true potential under these conditions.

They also observed an increase in reaction ranges foliage, ranging from intermediate and susceptible (6,0 and 8,3) greatly exceeding the values observed reaction in pods, ranging from intermediate ranges (4,6 to 6,6).

Se observó, además, un aumento en los rangos de reacción en el follaje, oscilando entre intermedios y susceptibles (6,0 y 8,3) superando en gran medida a los valores de reacción observados en la vainas, que oscilaron entre los rangos intermedios (4,6 a 6,6).

This may be because of reactions to the pathogen observed in the foliage and pods were determined by the effect of genes, which for some reason are in leaves and pods, or vice versa.

Statements that are consistent with these results were exposed by those who showed that PI207262 bean line presented different reactions in leaves and pods to *Xap* isolation, and this was determined by the effect of different genes (15).

Table II. Foliage and pod reaction induced by common Bacterioses and reduction of yields in commercial crops in Cuba

Cultivars	Severity against Xap 527				R (Kg P^{-1})	P R (%)
	F	Cat	V	Cat		
CC 25-9 (N)						
NI	4,0	I	4,0	I	1424	44,38
I	7,3	S	6,6	I	792	
*P**					0,0225*	
Bolita 42						
NI	5,0	I	3,0	R	1188	55,6
I	8,0	S	5,0	I	527	
*P**					0,0015**	
ICA Pijao						
NI	5,0	I	4,0	I	1504	48,93
I	8,0	S	6,0	I	768	
*P**					0,0001***	
Güira 89						
NI	6,0	I	3,0	R	1244	30,78
I	8,0	S	5,0	I	861	
*P**					0,007**	
BAT 304						
NI	5,0	I	4,0	I	1078	31,35
I	6,3	I	5,3	I	740	
*P**					0,019*	
Tazumal						
NI	3,0	R	2,0	R	1579	40,21
I	7,3	S	5,0	I	944	
*P**					0,0048**	
Holguín 518						
NI	4,0	I	3,0	R	1474	46,47
I	7,3	S	5,3	I	789	
*P**					0,0085**	
BAT 832						
NI	4,0	I	2,0	R	1454	42,50
I	7,3	S	6,0	I	836	
*P**					0,0005***	
CC 25-9 (R)						
NI	4,0	I	2,0	R	1165	29,78
I	7,0	S	5,0	I	818	
*P**					0,020*	
Hatuey 24						
NI	3,0	R	1,0	R	1634	48,77
I	6,0	I	4,6	I	837	
*P**					0,007**	
Velasco Largo						
NI	6,0	I	5,0	I	932	52,47
I	8,3	S	7,3	S	443	
*P**					0,003**	
M – 112						
NI	4,0	I	2,0	R	848	41,62
I	6,6	I	4,6	I	495	
*P**					0,020*	
Red Kloud						
NI	6,0	I	3,0	R	523	0,76
I	7,3	S	7,0	I	519	
*P**					0,968 N.S	
'Guamá 23'						
NI	5,0	I	2,0	R	955	80,73
I	7,3	S	6,6	I	184	
*P**					0,0012**	
Bonita 11						
NI	4,0	I	3,0	R	1325	29,81
I	7,6	S	4,6	I	930	
*P**					0,008**	
Chévere						
NI	5,0	I	3,0	R	1016	28,93
I	6,6	I	4,0	I	722	
*P**					0,003**	
Engañador (T)						
NI	3,0	R	1,0	R	1265	3,90
I	5,6	I	4,6	I	1215	
*P**					0,771 N.S	

* * p≤0,05 ** p≤0,01 Significant for test of T between inoculated and uninoculated plants with p<0,001 . XAP: pathogenic isolation of Cuba. F (foliage); Cat (Category per scale of 9 degrees.); P (pods); Y (Yield); kg/P-1 (kilograms per parcel); PY (percent yield), NI (non-inoculated) and I (Inoculated)

Something very important and should be taken into account is symptom presence in pods, as this involvement may decrease the quality thereof and producing affectation in seeds. A damaged or stained seed as a result of this bacterium can be a source of primary inoculum, safe, for future campaigns, and a way of disease effective dissemination, if proper management and a selection of healthy seeds with quality are not done before sowing.

Many of these crops are being used in the beans production in Cuba. The distribution of commercial cultivars and not implementing appropriate disease management strategies can raise pathogen levels in fields by the infected seed spreading, thus facilitating the rapid development of the disease and resulting yield losses

For the above reasons should be taken into account that the use of 'Hatuey 24' and 'Engañador' cultivars, to present lower values of reaction foliage and pods before (resistant) and after (intermediate) inoculation 527 *Xap* insulation in areas where the pathogen is present the common Bacteriosis. By contrast, the 'Tazumal' cultivar showed a more severe disease in leaves and pods varying its reaction from resistant before inoculation to susceptible (foliage) to intermediate (pods) against high pressure of 527 *Xap* isolation.

- Inoculation effect on yield, in commercial cultures of Cuba

In evaluating the performance under natural incidence of the pathogen (Table II), a group of cultivars with highest values were noted, in kg ha^{-1}, they are: 'Hatuey 24' (1634), 'Tazumal' (1579), 'ICA Pijao' (1504), 'Holguin 518' (1474), BAT 832 '(1454), and' Bonita 11 '(1325), superior to that obtained by the ' Engañador' witness in 1265. The other cultivars had lower yields than these, ranging between 523 and 1244 kg ha^{-1}. Similar results were reported previously to evaluate the behavior of some of these commercial cultivars in Havana province, Mayabeque at present, under the same conditions of natural incidence of the pathogen (16).

After the inoculation a significant decrease in yields of these same cultivars 'Hatuey 24' (837 kg ha^{-1}), 'Tazumal' (944 kg ha^{-1}), 'ICA Pijao' (768 kg ha^{-1} was observed), 'Holguin 518' (789 kg ha^{-1}), 'BAT 832' (836 kg ha^{-1}) and 'Nice 11' (930 kg ha^{-1}). The highest value was obtained by the commercial witness 'Deceiver' with 1215 kg ha^{-1}, with a minimum impact on its performance, by this pathogen.

When analyzing performance values achieved by commercial crops, before and after inoculation performed, using T test, a significant decrease in yield was observed in most of them, which shows the effectiveness of inoculation made to a better assessment of yield behavior against this bacterial pathogen (Table II).

'Red Kloud' and 'Engañador' cultivars (Witness), no significant losses were presented on yields due to the inoculation, obtained values were 0,76 and 3,90 respectively. Projecting with lowest values of losses yields against challenge insulation 527 of *Xap* cultivars as 'Bonita 11' 29 with 81; 'Chevere' with 28,93; 'CC 25-9 (R)' with 29,78; 'Güira 89' with 30,78 and 'BAT 304' 31 with 35. All of them can be classified as tolerant cultivars to common Bacteriosis, always remember that tolerance is an intermediate quality between susceptibility and resistance (17) and it is satisfied when the presence of a pathogen on a plant tolerant lead to less damage (expressed as less reduction in yields), or the presence of symptoms in them, are lighter than in a sensitive plant (18).

Similar results of yield losses due to the inoculation with *Xap* isolates have been obtained in previous studies in Mexico (19), Spain and Puerto Rico (20, 21).

The highest yield loss value was obtained in 'Guamá 23' cultivar with a reduction from 955-184 kg ha^{-1} for an 80, 73 % accompanied by susceptibility reactions being inoculated with insulation 527 of this pathogen. Susceptibility is expressed as severe or very severe symptoms of the disease that can cause significant yield losses, or death of the plant (11).

This analysis allowed knowing the behavior of commercial cultivar yields when inoculated with the pathogenic isolation of Xap 527 and determining the reduction on yields under field conditions. Tolerant cultivars include: 'Tazumal', 'Nice 11' and 'Hatuey 24', followed by 'CC 25-9 (R)', 'BAT 304', and 'Güira 89' because despite having presented different levels to react to this isolation, their yields were not affected by the inoculation effect.

Incorporating them management strategies is suggested to reduce damages caused by the common Bacteriosis and avoid the decline and losses of crop yield in production.

Also, incorporate 'Hatuey 24', 'M112', 'Chevere' and 'Engañador' cultivars as potential progenitors in the National Program for Improving the disease resistance, led by the Grain Research Institute and the National Institute of Agricultural Sciences (INCA) for their reactions in leaves and pods under the natural incidence and inoculation of the pathogen of the common Bacteriosis, under field conditions.

Obtaining commercial cultivars tolerant to this bacterial disease, it is an additional protection within a system of integrated pest management for this crop, which helps to reduce losses caused by this disease under production conditions.

CONCLUSIONS

- There is variability in the response of bean genotypes against the Cuban isolation of the common Bacteriosis (*Xanthomonas axonopodispv. Phaseoli* (XAP)), which allowed the selection of 'Hatuey 24', 'M-112', 'Chevere' and 'Engañador' commercial cultivars as the most tolerant to this pathogen attack.
- Damage caused by common Bacteriosis on foliage and pods, product inoculation with Xap527 isolation, determined yield losses in cultivars and bean lines studied in field.

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