CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Reaction of common bean lines to *Xanthomonas axonopodis* pv. *phaseoli* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

Tamires Ribeiro[1*], Cleber Vinicius Giaretta Azevedo[1], Jose Antonio de Fatima Esteves[1], Sérgio Augusto Morais Carbonell[1], Margarida Fumiko Ito[2] and Alisson Fernando Chiorato[1]

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Abstract: *The aim of this study was to evaluate the resistance of 58 common bean lines against common bacterial blight (Xanthomonas axonopodis pv. phaseoli) and bacterial wilt (Curtobacterium flaccumfaciens pv. flaccumfaciens). The experimental design consisted of completely randomized blocks, with four replications per pathogen. The results were subjected to variance analysis by the F test at 1% probability. Significant differences between the treatments indicated different resistance levels among the lines against both pathogens. According to the Scott-Knott test, six lines were resistant to Xanthomonas axonopodis pv. phaseoli, 14 moderately resistant, and 38 susceptible. To Curtobacterium flaccumfaciens pv. flaccumfaciens, 11 lines were resistant, 26 moderately resistant and 21 susceptible. Among these, the lines Pr10-3-4/1, Pr10-5-2/1 and Pr10-5-2/2 of the black bean group and C10-2-4/2 of the Carioca group were resistant to both major bacterial diseases affecting common bean in Brazil.*

Key words: *Phaseolus vulgaris L., plant breeding, common bacterial blight, bacterial wilt.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major source of vegetable protein for direct human consumption. In addition, it also contains carbohydrates, dietary fiber, B-complex vitamins, iron, calcium, and other minerals, playing an important role in the diet of the Brazilian population (Vieira et al. 2006).

According to data of CONAB (2015), the mean grain yield in Brazil is about 1.095 kg ha^{-1}, well below the productive potential of a crop which, under appropriate conditions, can yield more than 4.000 kg ha^{-1}. This low productivity can be attributed to the incidence of pests and diseases, adverse environmental conditions, low-yielding cultivars, and sowing outside the agricultural zones (Oliveira et al. 2005).

Among the main diseases affecting common bean are common bacterial blight, caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye and bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Collins & Jones. These pathogens are widespread in the producing regions, causing yield losses, especially when stimulated by favorable environmental conditions such as high temperatures (Theodoro 2004)

***Corresponding author:**
E-mail: tamires_r1@yahoo.com.br

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[1] Instituto Agronômico, Centro de Análise e Pesquisa Tecnológica do Agronegócio dos Grãos e Fibras, Avenida Barão de Itapura, 1481, 13.020-902, Botafogo, Campinas, São Paulo, Brazil
[2] Instituto Agronômico, Centro de Fitossanidade

The first pathogen symptoms of common bacterial blight appear on the shoot, consisting primarily of small water-soaked areas in the leaves, evolving to necrosis and imperfections in the seeds such as discoloration of the hilum, yellow spots, and wrinkling of the seed coat, which can reduce yields by 10 to 70% (Diaz et al. 2001, Bianchini et al. 2005). The inheritance of resistance to this pathogen is genetically complex, described by several authors as oligogenic or polygenic (Kelly et al. 2003, Santos et al. 2003, Manzanera et al. 2005). According to Zapata et al. (2010), Ferreira and Grattapaglia (2003), and Marquez et al. (2007), the number of genes, degrees and interactions involved in the expression of this trait may vary. Thus, the strong environmental influence in the evaluation period of the genotypes, can explain the low heritability observed in studies focused on the introgression of resistance into segregating common bean populations. Another factor that hampers the development of resistant genotypes for breeding programs is the genetic diversity of the pathogen (Mkandawire et al. 2004).

The pathogen symptoms of bacterial wilt begin with the colonization of vascular tissues, leading to the drying of apical leaflets, yellowing and gradual wilting of leaves, yellowish areas and necrosis of the parenchyma, as well as to yield drop (Maringoni 2002). According to Valentini et al. (2011), the resistance inheritance of this pathogen is polygenic and, according to Souza et al. (2006b), Wendland et al. (2008), and Torres et al. (2009b), the occurrence of genetic diversity and widespread dissemination in the producing regions of Brazil, makes the development of resistance sources even more difficult. For this reason, techniques have been developed to identify this bacterium in common bean crops (Maringoni 2002, Hsieh et al. 2005, Herbes et al. 2008), as well as to evaluate the resistance of genotypes and lines, with a view to the development of new resistant cultivars (Maringoni 2002, Souza et al. 2006a, Theodoro et al. 2007).

Both bacterial diseases are controllable by phytotechnical treatments such as crop rotation, elimination of crop residues and sowing of healthy seeds, whereas the use of resistant cultivars is the most efficient method to minimize production costs, avoiding significant yield and grain quality losses (Hsieh et al. 2005, Souza et al. 2006a, Huang et al. 2007b).

Moderate resistance to common bacterial blight or bacterial wilt was identified in the genotypes IAC Pyatã, IAC Diplomata, CNFC 10408, L 185633, IAPAR 16, UTF 6, PB 4, BRS Campeiro, IPR Chopim, XAN 159, LP 99-79, LP 93-23, L 64-5132, LP 01-51, PI 2072620, SCS 202-GUARÁ, IAPAR 81, L 264219, Iapar 80, LH 11, BRS Radiante, SM 9906, UTF 4, Iapar 20, IPR Uirapuru, and IAPAR 3 by Maringoni (2002), Rava et al. (2003), Souza et al. (2006a), Theodoro and Maringoni (2006), Costa et al. (2008), Silva et al. (2009), and Maringoni et al. (2015). These authors emphasized the importance of obtaining resistance sources to both bacterial diseases. Thus, the purpose of this study was to evaluate the resistance reaction of 58 advanced common bean lines to *X. axonopodis* pv. *phaseoli* and *C. flaccumfaciens* pv. *flaccumfaciens.*

MATERIAL AND METHODS

The experiments were conducted at the Experimental Center of Farm Santa Elisa, Instituto Agronômico-IAC, in Campinas-SP (lat 22º 54' S and long 47º 03' W, and alt 854 m asl), from January 02 to June 30, 2014. The experimental design was arranged in completely randomized blocks, with four replications for each evaluated pathogen. Each repetition consisted of one pot with two plants. Inside the greenhouse, the temperature varied from 28 °C to 32 °C during the experimental period.

Fifty-eight advanced common bean lines derived from the following crosses were evaluated: IPR Colibri x P5-4-4-1; Gen C2-1-1 x IAC Alvorada; IAC Alvorada x C6-9-10-1; Gen C4-8-2- 2 x IPR Colibri; LP02-02 x IAC Alvorada; IAC Alvorada x IAC Ybaté; Branquinho x IAC Imperador, Pr15-3-4-1 x Acesso Argentino; Pr15-5-15-1 x LP04-72; IAC Diplomata x LP04-72; IPR - Uirapuru x (IAC Una x XAN 251); IAC Una x LP04-72; P11-5-9-1 x Una IAC; IAC Diplomata x LP04-72; IAC Diplomata x (IAC Una x Acesso Argentino); (IAC Una x Acesso Argentino) x IAC Diplomata; P5-3-9-2 x IPR Colibri; IPR Colibri x IAC Imperador; LP04-72 x Pr13-3-4-1; (IAC Diplomata x LP04-72) x IAC Una; and P12-1-11-1 x LP04-72.

To evaluate the reactions of the lines to *X. axonopodis* pv. *phaseoli* (Common bacterial blight), seeds of 58 lines and the susceptible control (Rosinha G2) were disinfected with 70% ethanol and then with 1.25% sodium hypochlorite for 5 min. Subsequently, they were spread on paper sheets for germination and placed in BOD at 28 ºC for three days. After this period, the seedlings were transplanted into pots containing 500 g substrate (organic compound and soil, 1:1) and placed in the greenhouse.

The isolate 11.280 of *X. axonopodis* pv. *phaseoli*, from the Plant Health Center of the Instituto Agronômico-IAC, Campinas, SP, was used. The isolate was multiplied on PDA (potato, dextrose, agar) and incubated at 28°C for 48 hours. Thereafter, inoculum was prepared by addition of distilled water and sterilization in the bacterial colony, scraping with a glass slide, and concentration adjustment to 108 CFU mL^{-1}.

Plants in the V_2 stage were inoculated by the technique of multiple needles, according to Pompeu and Crowder (1973). The primary leaves were perforated with light pressure to allow the pathogen to enter the plant. Then the pots were placed in a moist chamber for 48 hours, at temperatures between 25 °C and 28 °C, and then transferred to the greenhouse.

Ten days after inoculation, the plants were evaluated on a 1 - 9 scale as follows: 1 to 2 - plants free of disease symptoms; 3 to 6 - small water-soaked areas; and 7 to 9 - plant tissue necrosis (Rava and Sartorato (1994). The resistance of genotypes was determined as follows: resistant lines had mean scores between 1 and 2; moderately resistant, between 2.1 and 5; and susceptible, between 5.1 and 9.

To evaluate reactions to *C. flaccumfaciens* pv. *flaccumfaciens,* seeds of the 58 lines and the pathogen-susceptible control (Rosinha G_2) were pre-germinated under laboratory conditions, as described above and transplanted into pots in the greenhouse.

The isolate used in this study was Feij-14627, provided by the Faculdade de Ciências Agronômicas, UNESP, in Botucatu. The isolate was multiplied in NA (Nutrient Sucrose Agar) culture medium and incubated at 28 °C for 72 hours. Inoculation was carried out in a greenhouse when the plants reached the V_3 developmental stage, by drilling two holes into the stem between the cotyledons and the primary leaves, using an entomological needle after dipping into the bacterial colony (Maringoni 2002).

Thirty days after inoculation, the plants were evaluated on a 1 - 9 scale adapted by Maringoni (2002) as follows: 0, plants without disease symptoms; 1 - mosaic symptoms on the leaves; 2 - 10% withered leaves; 5 - 25% of wilting and yellowing leaves; 7 - 50% withered leaves, yellowing and necrosis; and 9 - 75% withered leaves, yellowing and necrosis. The resistant genotypes were determined as follows: resistant lines scored between 1 and 2; moderately resistant, between 2.1 and 5; and susceptible, between 5.1 and 9.

The experiments were conducted separately. The results were subjected to analysis of variance (ANOVA) using the statistical software Genes (Cruz 2013), and differences between means were compared by the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The results of this study show the importance of knowing the resistance reaction of common bean genotypes to common bacterial blight and bacterial wilt. The evaluation of these genotypes is useful in breeding programs, to choose continuous sources of disease resistance, coupled with important agronomic traits, such as early cycle, high yield, upright growth, and resistance to grain darkening, with a view to develop superior genotypes for the productive sector.

The data of the evaluations of the 58 common bean lines regarding resistance to *Xanthomonas axonopodis* pv. *phaseoli* and *C. flaccumfaciens* pv. *flaccumfaciens* were subjected to analysis of variance by the F test at 1% probability. Table 1

Table 1. Summary of analysis of variance of 58 common bean lines inoculated with common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and bacterial wilt *(Curtobacterium flaccumfaciens* pv. *flaccumfaciens*)

		Mean square	
Sources of variation	**df**	**CBB**	**BW**
Treatments	57	236.5848**	0.9849**
Error	174	5.0785	0.02369
Total	231		
CV (%)		13.65	7.40
Mean		16.50	2.07

** Significant at 1% probability by the F test; CBB= Common Bacterial Blight and BW= Bacterial Wilt.

shows significant differences between treatments, indicating different resistance levels of the common bean lines to the two studied pathogens. The experimental precision, with coefficients of variation of 7.40% and 13.65%, indicated low environmental influence during the experiments, ensuring reliability of the results.

Differential reactions of the lines to the *X. axonopodis* pv. *phaseoli* isolate were shown by the Scott-Knott test. Of the 58 lines, 6 were resistant to the pathogen (10%), 21 moderately resistant (36.20%), and 31 were susceptible (53.44%) (Table 2).

The low percentage of resistant genotypes to common bacterial blight can be explained by the occurrence of additive and non-additive effects, resulting in complex inheritance, as reported by Marquez et al. (2007). Six QTLs in F_3 plants resulting from the BAC-6 and HAB-52 cross were identified by Santos et al. (2003). Five of these QTLs were associated with resistance of leaves and one of pods, with a phenotypic variation from 12.7 to 68.7% for leaf and 12.9% for pod resistance. These results highlight the complexity of the trait, where the genes that control leaf resistance are not the same as those that control pod resistance, indicating the occurrence of oligo- or polygenic interaction, reinforcing the complex nature of resistance to the pathogen.

Among 56 evaluated cultivars, Silva (2009) identified 21 as resistant to common bacterial blight and among 61

Table 2. Resistance of 58 common bean lines to common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and bacterial wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*)

Line	Mean		Line	Mean	
	Common Bacterial Blight	Bacterial Wilt		Common Bacterial Blight	Bacterial Wilt
1. Pr10-4-4/11	1.00 a**R**	3.50 bMR	30. C10-2-4/36	7.00 fS	5.00 dMR
2. Pr10-5-2/1	1.00 a**R**	2.00 a**R**	31. C10-2-5/8	7.00 fS	5.00 dMR
3. Pr10-5-2/2	1.00 a**R**	1.50 a**R**	32. P10-1-2/13	7.00 fS	3.25 bMR
4. C10-2-4/2	2.00 b**R**	1.00 a**R**	33. Pr10-4-3/13	7.00 fS	4.50 cMR
5. Pr10-3-4/1	2.00 b**R**	1.75 a**R**	34. Pr10-4-4/14	7.00 fS	7.50 fS
6. Pr10-3-5/10	2.00 b**R**	7.50 fS	35. C10-2-16/1	7.50 fS	7.00 fS
7. C10-2-4/57	3.75 cMR	2.00 a**R**	36. P10-1-3/1	7.50 fS	6.00 eS
8. P10-1-3/16	3.75 cMR	5.50 eS	37. P10-1-4/2	7.50 fS	7.00 fS
9. P10-1-1/12	4.50 dMR	2.00 a**R**	38. Pr10-5-1/14	7.50 fS	3.75 cMR
10. P10-1-9/38	4.50 dMR	2.00 a**R**	39. Pr10-5-1/2	7.50 fS	3.00 bMR
11. Pr10-8-3/2	4.50 dMR	7.50 fS	40. Pr10-5-2/4	7.50 fS	7.00 fS
12. Pr10-4-4/4	4.5 dMR	6.00 eS	41. Pr10-7-1/3	7.50 fS	4.00 cMR
13. Pr10-4-4/5	4.75 dMR	4.00 cMR	42. C10-2-16/5	8.00 gS	3.00 bMR
14. C10-2-17/1	5.00 dMR	3.00 bMR	43. C10-2-16/9	8.00 gS	3.00 bMR
15. C10-2-4/35	5.00 dMR	2.00 a**R**	44. P10-1-1/19	8.00 gS	4.50 cMR
16. C10-2-4/41	5.00 dMR	6.50 eS	45. Pr10-3-4/2	8.00 gS	6.00 eS
17. P10-1-4/23	5.00 dMR	5.00 dMR	46. Pr10-4-4/27	8.00 gS	3.00 bMR
18. Pr10-4-2/10	5.00 dMR	3.50 bMR	47. C10-2-17/4	8.50 gS	5.50 eS
19. Pr10-5-2/3	5.00 dMR	2.00 a**R**	48. Pr10-3-2/35	8.50 gS	8.50 gS
20. Pr10-7-1/6	5.00 dMR	8.50 gS	49. Pr10-4-3/14	8.50 gS	5.00 dMR
21. Pr10-3-3/8	5.25 eS	3.25 bMR	50. Pr10-7-1/16	8.50 gS	5.50 eS
22. C10-2-16/8	6.00 eS	3.25 bMR	51. C10-2-16/7	9.00 hS	6.50 eS
23. C10-2-17/3	6.00 eS	2.00 a**R**	52. P10-1-3/17	9.00 hS	4.00 cMR
24. C10-2-4/12	6.00 eS	5.50 eS	53. Pr10-3-3/9	9.00 hS	4.00 cMR
25. C10-6-2/11	6.00 eS	4.00 cMR	54. Pr10-3-3/10	9.00 hS	8.00 gS
26. P10-1-1/8	6.00 eS	4.50 cMR	55. Pr10-4-4/19	9.00 hS	3.25 bMR
27. Pr10-3-5/35	6.00 eS	3.75 cMR	56. Pr10-4-4/39	9.00 hS	8.00 gS
28. P10-1-9/39	6.75 fS	2.00 a**R**	57. Pr10-5-1/15	9.00 hS	3.25 bMR
29. C10-2-17/2	7.00 fS	7.50 fS	58. Pr10-8-3/1	9.00 hS	8.00 gS

Means values followed by different lowercase letters are significantly different between lines by the Scott-Knott test, at 5% probability and different uppercase letters indicate reactions of the lines to the common bacterial blight and bacterial wilt isolates (R= resistant: scores between 1.00 and 2.00; MR= moderately resistant: scores between 2.10 and 6,00 S= susceptible: scores between 6.10 and 9.00).

genotypes, Costa et al. (2008) identified the cultivars Magnifico, Radiante and BRS Pontal as resistant. In our study, six lines were selected, one of which belongs to the Carioca group (C10-2-4/2) and five to the black bean group (Pr10-4-4/11, Pr10-5-2/1, Pr10-5-2/2, Pr10-3-4/1 and Pr10-3-5/10). These six resulted from the respective crosses: IAC Alvorada x C6-9-10-1; IAC Una x LP04-72; IPR-Uirapuru x (IAC Una x XAN 251); IPR-Uirapuru x (IAC Una x XAN 251); (IAC Diplomata x LP04 -72) x IAC Una and (IAC Una x Acesso Argentino) x IAC Diplomata (Table 3).

The resistant lines of the black bean group (Pr10-4-4/11, Pr10-5-2/1, Pr10-5-2/2, Pr10-3-4/1 and Pr10-3-5/10), were derived from the parents IPR-Uirapuru, IAC Una or IAC Diplomata. The former two were classified, respectively, as resistant and moderately resistant by Silva et al. (2009), while IAC Diplomata was classified as susceptible by Azevedo et al. (2015). The resistant line of the Carioca group C10-2-4/2 was derived from the parent IAC Alvorada, classified as susceptible to common bacterial blight by Azevedo et al. (2015).

The difficulty in the development of resistant genotypes motivated several authors to approach this problem by identifying bacteria in seeds. However, Denardin and Agostini (2013) and Silva et al. (2013) described the complexity of pathogen identification on seeds and highlighted the importance of finding resistance sources, due to the wide dissemination of the pathogen in the producing areas of common bean.

The Scott-Knott test showed a differential reactions among the lines to *C. flaccumfaciens* pv. *flaccumfaciens*. Among the 58 lines, 11 were resistant to the pathogen (18.96%), 26 moderately resistant (44.82%) and 21 were susceptible (36.20%) (Table 2).

Of 333 tested genotypes, Souza et al. (2006a) found 18% to be resistant, which is consistent with our results. The low percentage of genotypes resistant to bacterial wilt was mentioned by Theodoro and Maringoni (2006). These authors evaluated 73 lines and found only two resistant cultivars (Mouro Piratuba and Vagem Amarela). According to Valentini et al. (2011), the low rate of resistant genotypes can be explained by the occurrence of additive and non-additive effects in the inheritance of bacterial wilt resistance. These authors identified more than three resistance genes in two populations resulting from the crosses IAC Carioca Aruã x SCS Guará and IAC Carioca Pyatã x Perola.

In this study, 11 lines were classified as bacterial wilt resistant, 7 of which belong to the Carioca group (C10-2-4/2, C10-2-4/57, P10-1-1/12, P10-1-9/38, C10-2-4/35, C10-2-17/3, and P10-1-9/39) and 4 to the black bean group (Pr10-5-2/1,

Table 3. Common bean lines and their respective original crosses resistant to common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and bacterial wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*)

Common bacterial blight		
Line	**Cross**	**Market class**
1. C10-2-4/2	IAC Alvorada x C6-9-10-1	Carioca
2. Pr10-3-5/10	(IAC Una x Acesso Argentino) x IAC Diplomata	Black
3. Pr10-3-4/1	(IAC Diplomata x LP04-72) x IAC Una	Black
4. Pr10-5-2/1	IPR - Uirapuru x (IAC Una x XAN 251)	Black
5. Pr10-5-2/2	IPR - Uirapuru x (IAC Una x XAN 251)	Black
6. Pr10-4-4/11	IAC Una x LP04-72	Black
Bacterial Wilt		
Line	**Cross**	**Market class**
1. C10-2-4/2	IAC Alvorada x C6-9-10-1	Carioca
2. C10-2-4/57	IAC Alvorada x C6-9-10-1	Carioca
3. P10-1-1/12	IPR Colibri x P5-4-4-1	Carioca
4. P10-1-9/38	P5-3-9-2 x IPR Colibri	Carioca
5. C10-2-4/35	IAC Alvorada x C6-9-10-1	Carioca
6. C10-2-17/3	Gen C2-1-1 x IAC Alvorada	Carioca
7. P10-1-9/39	P5-3-9-2 x IPR Colibri	Carioca
8. Pr10-5-2/1	IPR - Uirapuru x (IAC Una x XAN 251)	Black
9. Pr10-5-2/2	IPR - Uirapuru x (IAC Una x XAN 251)	Black
10. Pr10-3-4/1	(IAC Diplomata x LP04-72) x IAC Una	Black
11. Pr10-5-2/3	(IAC Diplomata x LP04-72) x IAC Una	Black

Pr10-5-2/2, Pr10-3-4/1, and Pr10-5-2/3), resulting from the respective crosses IAC Alvorada x C6-9-10-1, IAC Alvorada x C6-9-10-1, IPR Colibri x P5-4-4-1, P5-3-9-2 x IPR Colibri, IAC Alvorada x C6-9-10-1, Gen C2-1-1 x IAC Alvorada, P5-3-9-2 x IPR Colibri, IPR-Uirapuru x (IAC Una x XAN 251), IPR-Uirapuru x (IAC Una x XAN 251), (IAC Diplomata x LP04-72) x IAC Una, and (IAC Diplomata x LP04-72) x IAC Una (Table 3).

Of these 11 lines, C10-2-4/57, Pr10-3-4/1, Pr10-5-2/1, Pr10-5-2/2, C10-2-4/2, C10 2-17/3, and C10-2-4/35 were originated from the parents IAC Diplomata, IAC Alvorada, IAC Una, or IPR-Uirapuru. IAC Diplomata and IAC Alvorada were classified as resistant, while IAC Una was classified as susceptible by Maringoni et al. (2015). Parent IPR-Uirapuru was classified as susceptible to *C. flaccumfaciens* pv. *flaccumfaciens* by Theodoro et al. (2007) and Maringoni et al. (2015).

According to Souza and Maringoni (2008), resistant genotypes involve the pathogen by protoplasmic projections, preventing its installation in the xylem vessels, while in susceptible genotypes the water transport is obstructed by the presence of bacterial cells. These results are related to the disease symptoms, e.g., plant wilting, yellowing, underdevelopment, and death, observed at different levels of aggressiveness in the 58 lines evaluated in this study.

The lines C10-2-4/2, Pr10-3-4/1, Pr10-5-2/1, and Pr10-5-2/2 (Table 3) were resistant to common bacterial blight and bacterial wilt, the two major bacterial diseases affecting common bean in Brazil. The development of these cultivars is extremely important to maintain the yield and grain quality of common bean, given the lack of resistant cultivars in the productive sector, the physiological variability and wide dissemination of the pathogens in crop areas.

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