



New sources of resistance to bacterial wilt identified in dry bean germplasm collection

Carlos Agustin Rava^{1*}, Joaquim Geraldo Cáprio da Costa¹, Jaime Roberto Fonseca¹ and Andréia Luiza Salgado¹.

Received 20 August 2003

Accepted 22 March 2004

ABSTRACT - Objectives of the present investigation were to obtain preliminary knowledge on the variability of *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* (Cff) and identify new sources of resistance to bacterial wilt of dry bean. Three isolates of Cff were inoculated on the cultivars Pérola, Xamego, Rosinha G-2, Diamante Negro and Valente. Since the non-environmental variation was mainly affected by isolates and cultivars, the isolate Cff CNF 4 was selected to test dry bean genotypes for disease resistance due to its high degree of pathogenicity in a set of six cultivars. Of 333 tested accessions, 12 exhibited resistance reaction, while 85 plants showed both compatible and incompatible reactions to the pathogen. The resistant plants were selected and the families derived from these plants will be further tested to confirm the resistance reaction. The resistant accessions and the selected ones that confirm the reaction class will be incorporated in the Embrapa Rice and Beans breeding program.

Key words: *Phaseolus vulgaris*, *Curtobacterium flaccumfasciens* pv. *flaccumfasciens*, disease resistance.

INTRODUCTION

Bacterial wilt was initially identified in the State of São Paulo (Maringoni and Rosa 1996, 1997), and at present found distributed in various bean-producing areas, mainly in the southeast and central-west regions of Brazil. The bacterium *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* (Hedges) Collins and Jones (Cff) is gram-positive, highly aerobic, motile with one and rarely two or three polar or sub-polar flagella, and causes hydrolysis of aesculin. The optimum growth temperature in culture medium is 37 °C, where it develops yellow or orange- coloured, and smooth, humid, and bright colonies in 48 hours (Mohan and Hagedorn 1989).

The pathogen can survive up to 24 years in infected seed and may cause seed discoloration. It does not survive in the soil but can survive in plant debris in the off-season or on weeds (Shuster and Coyne 1975). Considered a vascular pathogen, it infects plants through contaminated seed, natural openings, wounds on the aerial plant parts, wounds on roots caused by nematodes, and injuries caused by cultural practices. Mainly young plants are susceptible to systemic invasion, which occurs rapidly when the bacteria reach the vascular system of the stem or petiole (Mohan and Hagedorn 1989).

Temperatures above 32 °C under drought stress conditions favor the disease development. The initial symptom is a flaccid

¹ Embrapa Arroz e Feijão, C.P. 179, 75.375-000, Santo Antônio de Goiás, GO, Brasil. *E-mail: rava@cnpaf.embrapa.br

leaf surface during the hot periods of the day but can recover turgidity during periods of high humidity and low temperature (Zaunmeyer and Thomas 1957). Later, the leaves turn brown followed by wilt and death of the whole plant. The wilt is a consequence of the obstruction of the xylem by agglomerations of bacterial cells, gum depositions, pentosans and the presence of tyloses (Goodman et al. 1967).

Losses caused by this disease in dry beans are still undocumented, as its occurrence has only recently been reported. However, it shows great destructive potential in the fact that the pathogen can survive and be transmitted by seeds.

The most practical and economical control measure is the use of resistant cultivars, easily adopted by farmers. The first step in a breeding program is to identify the sources of resistance. Two cultivars, Ouro Branco and IPA 9, were identified as resistant in a previous investigation and three cultivars, Iapar 31, IAC Piatã and IAC Aruã, as moderately resistant (Rava and Costa 2001). The last two cultivars had been the most resistant ones among those tested by Maringoni (2000). However, in view of the possibility that the bacteria could increase its pathogenic variability, switching the reaction type of the cultivar from resistant to susceptible, the identification of new resistance sources is necessary. The original accessions of traditional cultivar collections utilized in family agriculture, where the farmers had used their own seed for several generations, are valuable sources of genetic variability for the identification of these genotypes (Fonseca 1993).

Objectives of the present investigation were to gather preliminary information on the pathogenic variability of *Cff* and to identify new resistance sources to bacterial wilt in land races, in support of Embrapa Rice and Beans' dry bean breeding program.

MATERIAL AND METHODS

The following four *Cff* isolates are found in the Embrapa Rice and Beans pathogen collection: isolate *Cff* CNF 1 (propriety of Professor Antônio C. Maringoni, of the Faculdade de Ciências Agrônômicas da UNESP, Campus de Botucatu, originally denominated Feij 2502); isolates *Cff* CNF 2, *Cff* CNF 3 and *Cff* CNF 4, which were obtained from infected material collected in Silvânia, State of Goiás, Taquarituba, São Paulo and Luziânia, Goiás, respectively. The preliminary test demonstrated avirulence of isolate *Cff* CNF 3, which was consequently excluded from further testing. The three *Cff* isolates were tested for pathogenicity by inoculation on dry bean cultivars of Pérola, Xamego, Rosinha G-2, Diamante Negro and Valente.

Nine days after sowing, the plants were inoculated at two punctures on the stem, between the cotyledonary and primary leaves, made by a needle previously moistened in the bacterial

colonies developed in Petri plates containing PDA, during 72 hours at 28 °C. The experiment was a factorial 3 x 6 (18 treatments), using a randomized complete block design with three replications. Each plot was represented by one pot with three plants.

The symptoms were evaluated 12 days after inoculation on a nine-grade scale, originally developed for Fusarium wilt (Rava et al. 1996). Three hundred and thirty-three dry bean accessions collected in the States of Rio Grande do Sul, Santa Catarina, Paraná, Espírito Santo, Bahia and Minas Gerais were tested utilizing the isolate *Cff* CNF 4 and the inoculation and evaluation method described earlier. The randomized complete block design with five replications was used, but only four repetitions were inoculated, keeping one set as control. Each plot consisted of one pot with three plants. The plants that attained a disease score equal to or less than 3 were considered resistant.

RESULTS AND DISCUSSION

In the first experiment, the non-environmental variation was primarily due to main effects of isolates and cultivars. Even though the interaction isolate x cultivar was significant, it was of little magnitude, representing 20.2% of genetic variation of treatments, due, basically, to the performance of isolates in the cultivars Diamante Negro and Valente (Figure 1). Isolates *Cff* CNF 1 and *Cff* CNF 2 induced low intensity of symptoms in these cultivars, whereas isolate *Cff* CNF 4 was highly pathogenic. Isolate *Cff* CNF 4 was therefore selected to test dry bean genotypes for resistance to bacterial wilt, since it also presented the highest pathogenicity in the whole set of six cultivars.

Twelve accessions showed resistance reaction, i.e., the average symptom intensity of these plants was inferior to score 3 on the disease scale (Table 1), representing only 3.6% of the tested accessions. The identification of resistant accessions

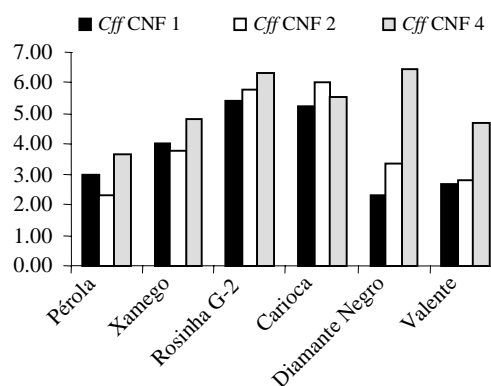


Figure 1. Reaction of six dry bean cultivars inoculated with three isolates of *Curtobacterium flaccumfasciens* pv. *Flaccumfasciens* (*Cff*).

is of great value to genetic breeding work. Additional studies are required to study the inheritance of resistance of different genotypes as well as the identification of molecular markers for their use in marker-assisted selection (MAS) of these traits.

Eighty-five accessions showed plants with compatible and incompatible reactions to the pathogen. This was to be expected because, according to Walder (1976), Duarte (1977) and Vieira et al. (1983) the cultivars used by farmers in the Zona da Mata region, State of Minas Gerais, consist, in most cases, of genotype mixtures. Plants with resistance reaction were transplanted to pots in order to obtain seeds. Families derived from these plants will have to be tested again to confirm the resistance reaction. The original resistant accessions as well as those selected based on the confirmation of resistance reaction will be incorporated in the dry bean disease resistance breeding program of Embrapa Rice and Beans.

Table 1. Dry bean accessions that showed resistance reaction in inoculation tests with isolate Cff CNF 04 of *Curtobacterium flaccumfasciens* pv. *Flaccumfasciens* (Cff)

Accessions	Dry bean cultivars	Mean score ¹
CF 800314	Mulatinho	2.67
CF 800316	Feijão Branco	1.83
CF 800393	Desconhecido	1.42
CF 220222	Amarelinho	2.25
CF 220224	Enxofre/Amarelo	2.75
CF 220225	Coquinho Enxofre	1.33
CF 810284	Jalinho	2.92
CF 810457	Amarelo Arroxeado	2.25
CF 220225	Rapezão	2.08
CF 220234	Feijão Mourinho	2.92
CF 220240	Feijão Baetão	1.92
CF 220277	Vermelho 1 Epamig	1.25

¹ Scale for determining disease reaction (Rava et al. 1996), where: 0 = absence of symptoms and 9 = dead plants or close to collapse. Resistant plants: score ≤ 3 (mean of four repetitions).

Novas fontes de resistência à murcha-de-*Curtobacterium* identificadas em coletas de feijoeiro comum

RESUMO - Os objetivos do presente trabalho foram a obtenção de informação preliminar sobre a variabilidade de *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* (Cff) e a identificação de novas fontes de resistência à murcha-de-*Curtobacterium* no feijoeiro comum. Três isolados de Cff foram inoculados nas cultivares Pérola, Xamego, Rosinha G-2, Diamante Negro e Valente. Como a variação não ambiental foi devida principalmente aos isolados e às cultivares, o isolado Cff CNF 4 foi selecionado para o teste de genótipos de feijoeiro comum para resistência à doença devido à sua maior patogenicidade no conjunto das seis cultivares. Dos 333 acessos testados, 12 apresentaram reação de resistência, enquanto 85 deles apresentaram plantas com reações compatíveis e incompatíveis com o patógeno. As plantas resistentes foram selecionadas e as famílias provenientes delas serão testadas para confirmação de resistência. Os acessos resistentes, e os selecionados que confirmarem sua classe de reação serão incorporados no programa de melhoramento da Embrapa Arroz e Feijão.

Palavras-chave: *Phaseolus vulgaris*, *Curtobacterium flaccumfasciens* pv. *flaccumfasciens*, coletas de feijão.

REFERENCES

- Duarte AO (1977) **Situação da cultura do feijão em nove municípios da Zona da Mata de Minas Gerais.** MSc. Thesis, Universidade Federal de Viçosa, Viçosa, 33p.
- Fonseca JR (1993) **Emprego da análise multivariada na caracterização de germoplasma de feijão (*Phaseolus vulgaris* L.).** PhD Thesis, Escola Superior de Agricultura de Lavras, Lavras, 123p.
- Goodman R, Király Z and Zaitlin M (1967) **The biochemistry and physiology of infectious plant diseases.** Van Nostrand, Princeton, 353p.
- Maringoni AC (2000) **Caracterização de isolados de *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* e avaliação da resistência de cultivares de feijoeiro comum à murcha-de-*Curtobacterium*.** PhD Thesis, Faculdade de Ciências Agronômicas da Unesp, Botucatu, 73p.
- Maringoni AC and Rosa EF (1996) Ocorrência de *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* em feijoeiro no Estado de São Paulo. **Fitopatologia Brasileira** 21:336.
- Maringoni AC and Rosa EF (1997) Ocorrência de *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* em feijoeiro no Estado de São Paulo. **Summa Phytopathologica** 23:160-162.
- Mohan SK and Hagedorn DJ (1989) Additional bacterial diseases: bacterial wilt. In: Schwartz HF and Pastor-Corrales MA (eds.) **Bean production problems in the tropics.** CIAT, Cali, p.303-306.
- Rava CA and Costa JGC (2001) Reação de cultivares de feijoeiro comum à murcha-de *Curtobacterium*. In: **Anais do I Congresso de Melhoramento de Plantas.** Sociedade Brasileira de Melhoramento de Plantas, Embrapa Arroz e Feijão, Goiânia (CD-ROM).
- Rava CA, Sartorato A and Costa JGC (1996) Reação de genótipos de feijoeiro comum ao *Fusarium oxysporum* f. sp. *phaseoli* em casa de vegetação. **Fitopatologia Brasileira** 21:296-300.

Schuster ML and Coyne DP (1975) **Survival factors of plant pathogenic bacteria**. Agricultural Experiment Station, University of Nebraska, Lincoln, 53p. (Research Bulletin 268).

Vieira RF, Vieira C, Euclides RF and Silva CC (1983) Avaliação preliminar do germoplasma de *Phaseolus vulgaris* L. da Microrregião Homogênea 192 (Zona da Mata de Minas Gerais). **Revista Ceres** 30:419-450.

Walder VLMS (1976) **Qualidade das sementes de feijão (*Phaseolus vulgaris* L.) utilizadas pelos agricultores em 28 municípios da Zona da Mata de Minas Gerais**. MSc. Thesis, Universidade Federal de Viçosa, Viçosa, 64p.

Zaumeyer WJ and Thomas HR (1957) **A monographic study of bean diseases and methods for their control**. USDA, Washington, 255p. (USDA Technical Bulletin 868).