Hypersensitive reaction of tepary bean upon inoculation with the common bean blight pathogen[1]

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ABSTRACT

Tepary bean, *Phaseolus acutifolius* A. Gray, has been suggested as a source of genetic resistance to *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) for beans. However, the mechanism of resistance has not been elucidated. The tepary bean reaction to inoculation with *Xcp* suggests an induced resistance mechanism. A brown discoloration at the scratched site and surrounding tissue appeared on cotyledons after 48 h. It was more pronounced with the high virulent than low virulent strains. On detached leaves the response after 48 h was chlorosis of the inoculated tissues, but after 72 h a rapid collapse of the tissues surrounding the inoculated area was observed. The reaction resembles a hypersensitive response (HR) in attached and detached leaves and cotyledons. HR was more evident in younger leaves than in older leaves and was found associated with viable cells, but not with dead cells. This study suggests the use of the tepary bean and its HR as a key test for differentiating among virulent strains of *Xcp*.

Key words: induced resistance, *Xanthomonas campestris*

RESUMEN

Respuesta de hipersensitividad de la habichuela tepary a la inoculación del patógeno del tizón común de la habichuela

La habichuela tepary, *Phaseolus acutifolius* A. Gray, se ha usado como fuente de resistencia genética al patógeno bacteriano *Xanthomonas campestris* pv. *phaseoli* (*Xcp*). Sin embargo, al momento se desconocen los mecanismos que caracterizan esta resistencia. Durante esta investigación la reacción de la habichuela tepary al patógeno se estudió en tejidos de hojas en la planta y separadas de la planta y en cotiledones bajo condiciones de ambiente controlado. La reacción de la habichuela tepary a la inoculación de cepas del grupo *X. campestris* sugiere la existencia de un mecanismo de resistencia que se induce como respuesta a las bacterias inoculadas. Un descoloramiento en la parte inoculada y tejidos adyancentes se desarrolló a las 48 horas en cotiledones inoculados. La respuesta fue más pronunciada con cepas altamente virulentas que con mutantes de *Xcp* de baja virulencia. En hojas separadas de las plantas la respuesta a las 48 horas se caracterizó por clorosis de los tejidos inoculados, pero a las 72 horas se observó un colapso rápido de los tejidos alrededor del tejido inoculado. La reacción mos-

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tró las características de una respuesta de hipersensitividad (HR) en hojas y cotiledones. HR fue más evidente en hojas jóvenes que en hojas más viejas y se asoció con la inoculación de células viables, pero no con células muertas. Este estudio sugiere el uso de la habichuela tepary para diferenciar cepas de *Xcp* que difieren en virulencia mediante la respuesta HR.

INTRODUCTION

Common blight, caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*), has plagued the dry bean industry for many years. The extent of damage varies from year to year, depending largely on the temperature conditions. Since development of the disease is favored by a warm humid climate, losses due to bacterial blight can be very severe when beans are grown in the tropics or subtropics.

The source of primary infection could be seedborne, insect vectors, or wild or perennial plants. Sources of genetic resistance to common blight are urgently needed because the sources of resistance used up to now have been inadequate.

Very few commercial cultivars of *Phaseolus vulgaris L.* are considered tolerant to common blight, and all of them develop symptoms of the disease after artificial inoculation. Tepary bean (*P. acutifolius* A. Gray) has long been suggested as a potential source of genetic resistance for beans, because it has high tolerance to *Xcp* (Coyne et al., 1963; Honma, 1956; Sharen, 1959). Honma (1956) successfully crossed *P. vulgaris* and *P. acutifolius* and obtained segregants highly tolerant to the bacterium. Other investigators have obtained this cross as well (McElroy et al., 1985; Rabakoaribanta et al., 1979). Thus, tepary bean represents a source of resistance not commonly found in *P. vulgaris*. In theory, the exploitation of this host resistance could provide an effective and durable control of the bacterial blight disease in beans.

In order to evaluate the potential of such an approach to bacterial blight disease in beans, there is need of a detailed knowledge of the nature of the resistance of this host. There is no documented evidence about the mechanisms of resistance in the tepary bean. It has been suggested that an immobilization and envelopment of the bacteria occur because of wall-like materials (Cafati et al., 1980). On the other hand, it has been observed that some tepary lines develop dark discoloration in pod tissue after inoculation with *Xcp*, thus suggesting an induced resistance mechanism (Zapata et al., 1985).

The objectives of this study were to determine the reaction of different tissues of a resistant tepary line and whether the nature of the reaction can be related to an induced resistance mechanism.

MATERIALS AND METHODS

Inoculation of attached leaves

Fifty seeds of a resistant and susceptible *P. acutifolius* were planted in 15-cm diameter clay pots containing a sterilized greenhouse soil mix. The plants were grown under environmental chamber conditions at 27°C with fluorescent lighting for 12 h daily. Eight-day-old primary leaves and the first expanded trifoliolated leaf were inoculated with four suspensions of *X. campestris* which included three isolates of *Xcp* (fuscans, 820 and a seed isolate) and one of pv. *glycines* (*Xcg*). The bacterial cell suspensions were prepared in sterile distilled water from cultures grown 48 h at 26°C. All concentrates were adjusted to 10^9 cfu/ml by means of standard turbidimetric techniques. The filtrates of each cell suspension were also inoculated in addition to a control treatment consisting of sterile distilled water.

Five plants per treatment were inoculated in three separate experiments. The method of inoculation consisted of producing 4- to 5-mm-long scratches through the epidermis of the leaves with two needles mounted 2 mm apart. A 0.05-ml drop of inoculum was deposited on the two halves of each leaf. Thus, 20 readings per treatment were recorded.

Inoculation of cotyledons

One line of *P. acutifolius* and three cultivars of *P. vulgaris* [3M-152 (susceptible to *Xcp*), Xan 161 and Tara] were included in the experiment. The seeds were covered with humid filter paper for 48 hr. After this period, seeds were peeled and the cotyledons were inoculated by scratching the surface tissue with two needles adjusted 2 mm apart. The inoculum concentration was adjusted as previously described. Readings were taken at 24, 48, and 72 h after inoculation.

Reisolation of the bacterial isolates from the inoculated areas was performed by dividing each cotyledon, previously washed in running water, into two parts. One part was treated with a low concentration of sodium hypochloride (0.2% for 2 min) and the other part without surface disinfection. Both tissues were cut in small fragments and placed on *Xanthomonas* selective medium (MXP) (Claflin et al., 1987).

Inoculation of detached leaves

Three leaves differing in position (2, 3, and 15 from the top) on 23-day-old plants of *P. acutifolius* were detached and placed in Petri dishes containing a humid paper. Six suspensions of *Xcp* were tested, four wild type *Xcp* collected in Puerto Rico (fuscans, *Xcp* 820, *Xcp* 827 and *Xcp*

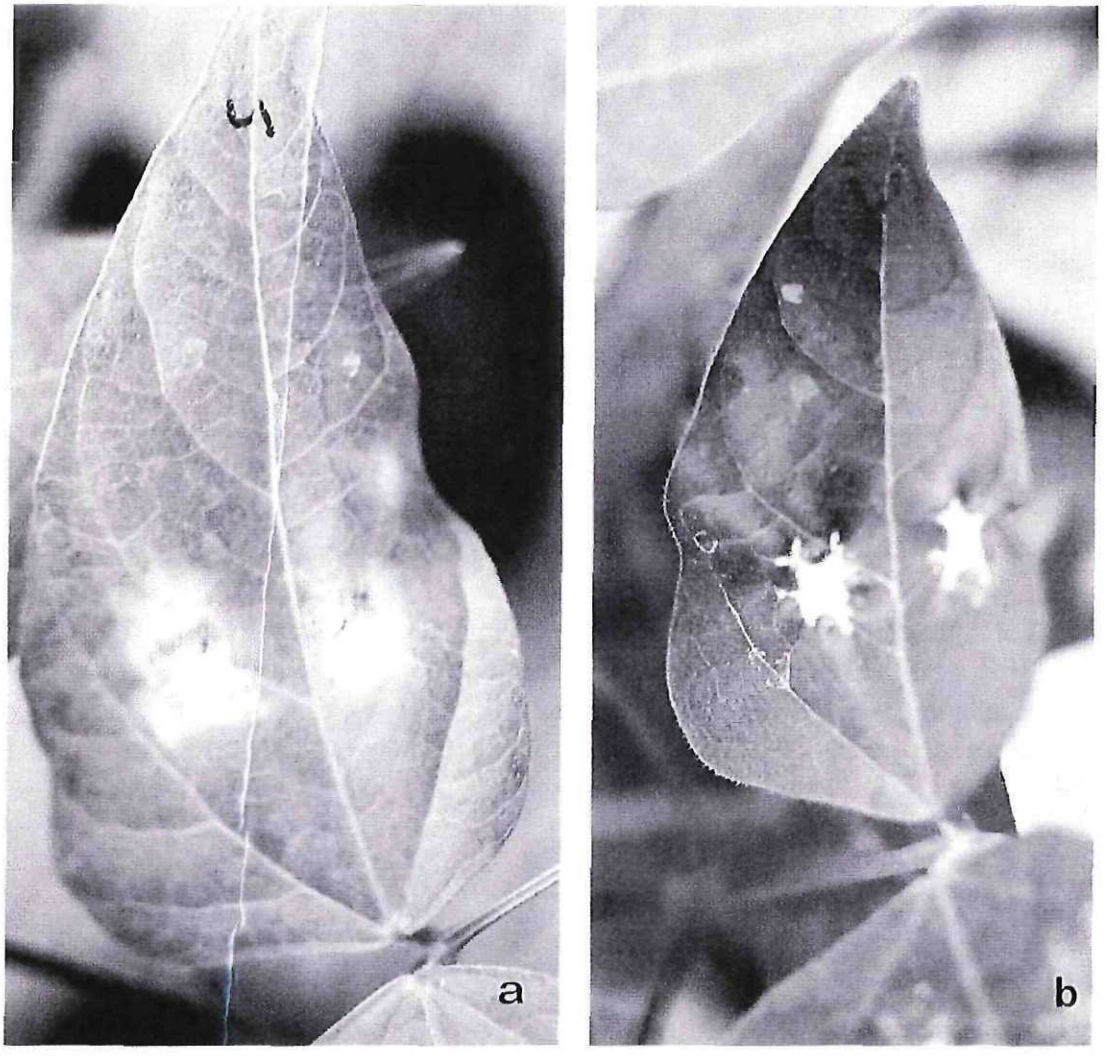

FIGURE 1. Trifoliolated leaf. (a) Susceptible tepary bean showing the translucent and progressive necrosis with irregular chlorotic borders. (b) Resistant tepary bean showing a localized dry reaction of light brown color.

seed) and two low virulent mutants (*Xcp* 121 and *Xcp* 45) (Arunakumari and Vidaver, unpubl.). A suspension consisting of heat-killed cells (100°C for 10 min treatment) and a suspension of Xcg and sterile distilled water were also included. All concentrations and inoculation techniques were adjusted and performed as previously described. The responses were recorded 24, 48, and 72 h after inoculation.

RESULTS

The response of the primary leaves (attached to the plant) to the inoculation of bacterial cell suspensions and filtrates was related to a dry, light brown discoloration induced by the cell suspensions, but not by the bacterial filtrates in the scratched area (Table 1). A similar reaction

TABLE 1.—*Response of the common blight resistant* Phaseolus acutifolius *line inoculated with cells and filtrates of* Xanthomonas campestris *pathovars on primary and trifoliolated leaves.*

Bacterial strain[1]								
Xcp (fuscans)		Xcp (820)		Xcp (seed)		Xcg		
Cell[2]	Filtrate	Cell	Filtrate	Cell	Filtrate	Cell	Filtrate	Control[3]
+[4]	-	+	-	+	-	+	-	-

[1]Bacterial strain: *Xcp* (fuscans) = *Xanthomonas campestris* pv. *phaseoli* (fuscans); *Xcp* = *Xanthomonas campestris* pv. *phaseoli* (isolate 820, isolate from seed); *Xcg* = *X. campestris* pv. *glycines*.

[2]Cell = refers to bacterial cell suspensions in water; filtrate refers to the solution obtained after filtration of the bacterial cell suspension through a bacterial filter (0.2μ).

[3]Control = sterilized distilled water.

[4]Response reaction: + = brown pigmented area; - = no reaction when compared with the control plant tissue. The response represents the summary of 10 leaf readings per treatment 72 hours after inoculation in three experiments.

was developed in the trifoliolated leaves of the resistant tepary line. The susceptible line developed chlorosis and progressive necrosis (a yellow irregular margin around translucent necrotic tissue) (Figure 1).

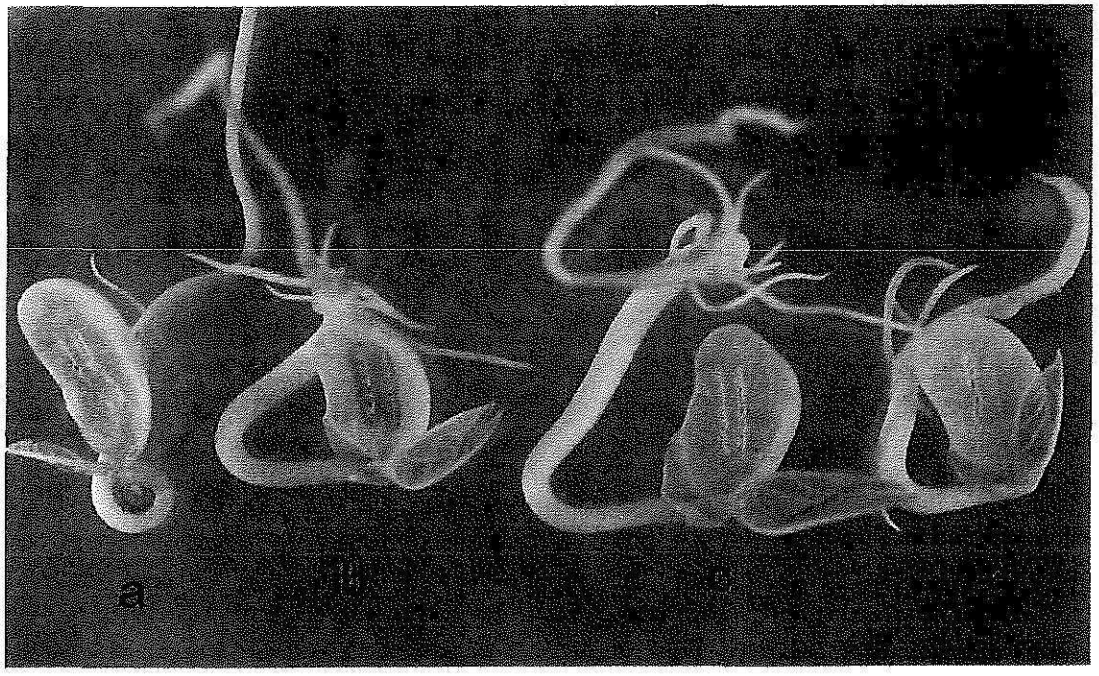

FIGURE 2. Response of the tepary bean cotyledons to the inoculation of *Xanthomonas campestris*: (a) pv. *phaseoli (fuscans)*; (b) pv. *phaseoli* (isolated from seed); (c) pv. *glycines*; (d) control.

The less intense discoloration was induced by the *X. campestris* pv. *glycines* (*Xcg*) cell suspensions. No visible difference was observed between the strains *Xcp* fuscans, *Xcp* 820, and *Xcp* seed. The scratched area treated with water showed a clear white color.

The resistant tepary cotyledons' response to the inoculation of the strains of *X. campestris* showed a discoloration produced in the scratched area and also in the areas near the scratch. This response was observed after 24 h and was more pronounced at 48 h. The response was more pronounced after the inoculation of the virulent strains *Xcp* fuscans, *Xcp* 820, *Xcp* seed and very slightly when inoculated with *Xcg*. No symptoms were observed in the control (Figure 2).

The cotyledons of the *P. vulgaris* cultivars differed in their reaction. The susceptible host, 3M-152, showed a yellow exudate in the inoculated area without discoloration of the scratched area. The reaction of Xan 161 showed discoloration of scratched area. The reaction for Xan 161 showed discoloration of the inoculated tissue for the *Xcp* 820, *Xcp* seed and *Xcg* (Table 2).

The reisolation of the *Xanthomonas* strains from the dark pigmented areas in *P. acutifolius* cotyledons and from the susceptible 3M-152 cultivar showed that the bacterium was viable in both species (Table 3).

The detached tepary bean leaves showed a chlorotic response in the inoculated area after 48 h on the younger leaves (2 and 3 position) but not on the older leaf (15 position). At 72 h the reaction resembled a hypersensitive response in which the whole leaf (2 and 3 position) showed chlorosis and exudated drops with a coffee color pigmentation. No changes were observed on the older leaf (15 position). No response was observed to the control and heat-killed cell treatments (Table 4).

DISCUSSION

There are no reports in the literature on *Xcp* which indicate that high inoculum concentration results in a hypersensitive reaction in common or tepary bean such as that reported with *Erwinia* and *Pseudomonas*. The brown color reaction to the inoculation of the different strains of *Xanthomonas* on the tepary bean leaves and cotyledons suggests an induced resistance mechanism in which cells near the inoculated area are also involved in the interaction with the bacterium. This response suggests the accumulation of inhibitory resistance substances in the inoculated tissues toward the different strains, especially the virulent ones. In contrast, the mechanisms that occur in Xan 161 and Tara (both of these have *P. acutifolius* germoplasm) have some similarities in their response to some strains, but not with all the

TABLE 2.—*Response of the common blight resistant* Phaseolus acutifolius *and* P. vulgaris *cotyledons to the inoculation of* Xanthomonas campestris *pathovar suspensions using the scratch technique.*[1]

	Bacterial strain[2]				
Phaseolus species	*Xcp* (fuscans)	*Xcp* (820)	*Xcp* (seed)	*Xcg*	Control[3]
acutifolius	brown	brown	brown	li-brown	wh
vulgaris					
3M-152	wh, exud	wh, exud	wh, exud	wh, exud	wh
Xan 161	brown	brown	wh	wh	wh
Tara	brown	wh	wh	wh	wh

[1]Symptom response was observed as discoloration on the inoculated (scratched) area: brown; li-brown = light brown; wh = white at 72 hours after inoculation; exud = bacterial exudates were observed at 7 days after inoculation.

[2]Bacterial strain: *Xcp* (fuscans) = *Xanthomonas campestris* pv. *phaseoli* (fuscans); *Xcp* (820) = *X. campestris* pv. *phaseoli* isolate 820; *Xcp* (seed) = *X. campestris* pv. *phaseoli* isolate from seed; *Xcg* = *X. campestris* pv. *glycines*.

[3]Control = sterilized distilled water.

virulent strains. Possible explanation of these phenomena may be related to the presence of different inhibitory compounds, differences in accumulation rates, different mechanisms of resistance, or different physiological *Xcp* races.

The response to inoculation of *Xcp* on cotyledons and young primary and trifoliolate leaves of the tepary bean suggests an induced resistance mechanism that occurs in a period of 48 to 72 h. The rapid collapse of cells in the leaves indicates a hypersensitive reaction (HR). Besides having altered physiology, detached leaves reacted differently according to leaf position. Leaves at positions 2 and 3 were similar in reaction, but different in reaction from that of position 15. Leaves at positions 2 and 3 showed chlorosis and fast yellowing versus the immune response of leaf at position 15. The differences in responses may be explained by leaf age. Old leaves may have a different mechanism of defense against the bacterium from that of the young leaves. HR may be regarded as a defense mechanism in which an increased permeability, drying, and death of host cells occur in the neighborhood of the infected tissues.

In plant pathogenic bacteria, the HR in tobacco is considered a key test of the LOPAT groupings for fluorescent pseudomonads (Shaad, 1988) and a general screening procedure for most potential pathogens although some pathogens do not give positive reactions. *Erwinia amylovora* and most pathogenic *Pseudomonas* will induce a positive HR

TABLE 3.—*Reisolation of* Xanthomonas *strains from brown pigmented and non-pigmented areas on cotyledons of the resistant* Phaseolus acutifolius *and* P. vulgaris.

	Xanthomonas campestris pathovars[1]									
	Xcp (fuscans)		*Xcp* (820)		*Xcp* (seed)		*Xcg*		Control[3]	
Phaseolus[2] species	Ster[4]	Non-ster	Ster	Non-ster	Ster	Non-ster	Ster	Non-ster	Ster	Non-ster
acutifolius	6/6[5]	8/8	6/6	6/6	6/6	6/6	8/8	9/9	6/0	6/0
vulgaris	6/6	6/6	8/8	8/8	6/6	8/8	8/8	6/6	6/0	6/0

[1]Bacterial strains: *Xcp* (fuscans) = *Xanthomonas campestris* pv. *phaseoli* (fuscans); *Xcp* = *X. campestris* pv. *phaseoli*: (isolate 820 and isolate from seed); *Xcg* = *X. campestris* pv. *glycines*.

[2]*Phaseolus* species: *acutifolius* response represents the summary of the susceptible and resistant lines and *vulgaris* response represents the summary of the lines 3M-152, Xan 161 and Tara.

[3]Control = sterilized distilled water.

[4]Each cotyledon was washed with running water and divided into two parts; one part was surface sterilized with a 0.2% sodium hypochloride solution (ster) and the other was not surface sterilized (non-ster).

[5]Represents the number of fragments planted on the semi-selective medium MXP versus the number in which bacterial growth was detected.

TABLE 4.—*Response of* P. acutifolius *detached leaves to the inoculation of* Xanthomonas campestris *suspensions at 24, 48, and 72 h after inoculation.*

	Xcp fuscans[2]			*Xcp* 820			*Xcp* seed			*Xcp* 827			*Xcp* 121			*Xcp* 45			*Xcg*			Control		
Leaf[1]	24[3]	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
2	-	chl	chl'	-	chl	chl'	-	chl	chl'	-	chl	chl	-	chl	chl'	-	chl	chl'	-	chl	chl'	-	-	-
3	-	chl	chl'	-	chl	chl'	-	chl	chl'	-	chl	chl	-	chl	chl'	-	chl	chl'	-	chl	chl'	-	-	-
15	-	sd	sd	-	sd	sd	-	sd	sd	-	sd	sd	-	sd	sd	-	sd	sd	-	sd	sd	-	-	-

[1]Leaf = refers to position of the leaves from top. Plant age was 23 days.

[2]*X. campestris* pv. *phaseoli* (*Xcp*) includes four virulent isolates (fuscans, 820, seed, 827); two low virulent mutants (121, 45), and one heat-killed cell suspension prepared from *Xcp* (fuscans) data not shown because reaction was the same as that of the control; *Xcg* = *Xanthomonas campestris* pv. *glycines*; Control = sterilized distilled water.

[3]Responses were recorded at 24, 48, and 72 h after inoculation; - = no response; chl = chlorotic tissue at the inoculated area; chl' = chlorosis is present in the inoculated area plus the adjacent tissue, and sd = scar discoloration.

over a relatively wide range of conditions, but xanthomonads and agrobacteria produce reactions only under a restricted range of temperature and light conditions. One application of the information obtained by this study is to use the tepary bean as a key host for testing strains of the *Xcp* group. In the near future other experiments will be conducted to determine the antibacterial inhibitory substances that occur in the tepary bean and the genetic role in the resistance to *Xcp*. Attempts will be made to determine whether the reaction is restricted to the *Xanthomonas campestris* group or whether it could be induced by another genus.

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