Inheritance of Anthracnose Resistance in Common Bean Accession G 2333

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ABSTRACT

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Common bean (*Phaseolus vulgaris*) germ plasm accession G 2333 (Colorado de Teopisca) from Mexico was resistant to 380 isolates of the anthracnose pathogen (*Colletotrichum lindemuthianum*) from 11 Latin American countries. Six frequently used sources of resistance were susceptible to many isolates. Inheritance of resistance was studied in a cross of resistant G 2333 × susceptible ICA Pijao. The two parents and the F_1, F_2, and backcross generations were inoculated with race 521 in both the seedling and adult-plant stages in controlled greenhouse environments at CIAT. Two independent dominant genes controlled resistance in the seedling and adult-plant stages, giving a segregation ratio of 15 resistant to 1 susceptible in the F_2, all resistant in the backcross to G 2333, and 3 resistant to 1 susceptible in the backcross to ICA Pijao.

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., is one of the most destructive diseases of common bean (*Phaseolus vulgaris* L.) in the tropics and subtropics (1,17,22). Although widely distributed, anthracnose causes particularly severe economic losses in cool and humid areas such as the highlands of Latin America and central and eastern Africa. Yield losses of 95% have been recorded in Colombia and of over 92% in Malawi (1). Farmers in Europe (especially in England, France, and the Netherlands), the United States, and Canada have managed this disease effectively for some time by using resistant cultivars and cultural practices such as disease-free seeds produced in arid climates and crop rotation (1,23). In most tropical countries, however, the ubiquity of the inoculum and high costs have made these cultural practices unfeasible for growers: almost no farmers in Latin America and Africa use commercially produced, clean, disease-free seed of common bean. Host plant resistance is a more viable option, particularly for small-scale growers with limited means. However, the effectiveness of host plant resistance is limited by the pathogen's diverse virulence in those regions, which may render a cultivar that is resistant in one location or year susceptible in another (3,9,11,12,14). Several races of *C. lindemuthianum* have been identified. This variability has been cited as the primary reason for the economic importance of anthracnose in many areas of Latin America (including south central Brazil and the highlands of Mexico, Guatemala, and Costa Rica) and central and eastern Africa (Rwanda, Kenya, Tanzania, Malawi, and Zambia) and North America (Michigan and Ontario) (6,14,17,23; J. Kelly, *personal communication*). Many national and international programs have assigned high priority to the search for new and better sources of anthracnose resistance in common bean (2,4,12,14,19).

Schwartz et al (19) evaluated several thousand germ plasm accessions of common bean under field and greenhouse conditions in Colombia with selected Latin American and European isolates of *C. lindemuthianum*. They reported that G 2333 (Colorado de Teopisca), originally collected in the state of Chiapas in southern Mexico, was among 30 accessions with resistance to all of the isolates used for screening. Subsequently, this accession, under greenhouse conditions, has been resistant to several hundred isolates of the anthracnose pathogen from around the world, and to all European races (2,3,9,17; F. Le Grand, *personal communication*). More importantly, G 2333 has shown resistance in all evaluations conducted under field conditions with severe anthracnose in Brazil, Argentina, Bolivia, Peru, Ecuador, Colombia, Central America, Mexico, and several countries of Africa (9; M. A. Pastor-Corrales, *unpublished*). Because of its resistance to anthracnose, G 2333 was released as a commercial cultivar in Rwanda, where it is known as Umubano. This cultivar is also grown in neighboring areas: Burundi, the Kivu region of Zaire, and parts of southern Uganda. Other important characteristics are its shiny red seed coat (a preferred seed color in local markets), high tolerance of low soil fertility, and stable and exceptionally high yields in diverse environments.

To facilitate additional use of G 2333 in breeding and selection studies, we investigated the inheritance of its resistance to anthracnose. We also showed how G 2333 compares with six other frequently used sources of anthracnose resistance in its reaction to several hundred isolates of *C. lindemuthianum* from 11 countries of Latin America.

MATERIALS AND METHODS

Isolates. All *C. lindemuthianum* isolates used in this study were collected during 1976 and 1993 from numerous common bean cultivars of different growth habit, seed size and color, and geographical origin. Isolates were obtained from pods, leaves, petioles, or seeds of common beans having characteristic anthracnose symptoms in naturally infected fields. In all cases, single conidial isolates were obtained from single lesions. Isolates were maintained for short periods on potato-dextrose agar (PDA) at 19–21 C. For medium-term storage, isolates were first inoculated on sterile filter papers impregnated with sterile 10% peptone and 20% sucrose. Next, the fungus-colonized filter papers were desiccated for about 8 days and stored at −20 C. To obtain abundant sporulation, cultures were grown on the PDA medium at 19–21 C in darkness for about 8 days.

The virulence phenotype of each isolate used in this study was characterized by inoculating a set of 12 common bean differential genotypes used internationally to identify *C. lindemuthianum* races (16). The same inoculation procedure was used to identify races of the anthracnose pathogen, to compare the reaction of G 2333 with that of other resistance sources, and to study the inheritance of resistance.

Ten seeds of each of the 12 differential cultivars were planted in vapor-sterilized soil in plastic trays and left in the greenhouse. After 7 days, the seedlings were spray-inoculated with aqueous conidial suspensions that contained 1.2×10^6 spores per milliliter of each isolate characterized. The inoculated seedlings were maintained at 20–22 C for 7 days in a 95–100% humidity chamber, which was placed in a greenhouse with a 12-hr photoperiod per day. Host responses were then scored visually to obtain the average percentage of seedling tissues covered by lesions. A 1–9 scale was used, where 1 = no visible symptoms and 9 = very severely diseased or dead (24).

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Host responses were judged susceptible or compatible (+) when most seedlings exhibited fully sporulating lesions (lesion type greater than 3). All other responses were judged resistant or incompatible (−). The cultivar La Victorie, generally compatible with all isolates, was included in each assay to check infection efficiency.

Reactions of G 2333 and other known resistance sources. The anthracnose-resistant G 2333 and six other frequently used sources of anthracnose resistance (Table 1) were inoculated separately under greenhouse conditions with 380 isolates of *C. lindemuthianum* from 11 Latin American countries. The resistance sources and their anthracnose resistance genes were Cornell 49242 (*Are*), Mexico 222 (*Mex* 1a), TO (*Mex* 2), TU (*Mex* 3), PI 207262, and AB 136; the resistance genes of the last two are undetermined (4,10,11,13,14). Of the 380 isolates, 44 were from Argentina, 26 Brazil, 47 Peru, 47 Ecuador, 107 Colombia, 69 Costa Rica, 2 Nicaragua, 5 Honduras, 3 El Salvador, 4 Guatemala, and 26 Mexico.

Inheritance of resistance in G 2333. The resistant G 2333 is a red-seeded, indeterminate cultivar with climbing growth habit type IV (20). The susceptible cultivar ICA Pijao is also indeterminate, but black seeded and with an erect growth habit type II. It was developed by the Instituto Colombiano Agropecuario (ICA). Both cultivars have small seeds (<25 g/100-seed weight) and show the typical morphological, molecular, and other characteristics of the common bean race Mesoamerica (21). Pure lines of both G 2333 and ICA Pijao were developed by repeatedly harvesting seed from individual plants separately and inoculating them in the greenhouse with monosporic cultures of the anthracnose pathogen until uniform reactions were obtained. G 2333 was resistant in both seedling and adult-plant stages (Table 2), whereas ICA Pijao was susceptible. Accession G 12488 (Ecuador 1056) was included as a check in seedling and adult-plant inoculations. This accession is resistant under field conditions at all locations where it has been tested, but it is susceptible in the seedling stage under greenhouse conditions.

G 2333 was crossed with ICA Pijao, using manual emasculation and pollination (7). Part of the F_1 seed was used to make the backcrosses onto G 2333 and ICA Pijao and also to obtain F_1-selfed F_2 seed. Several hundred flower buds were used for each cross, because relatively large quantities of seed were required. All hybridization and seed production were carried out either in the screenhouse or greenhouse to avoid contamination from foreign pollen. The two parents, F_1 and F_2 generations, and backcrosses were evaluated in the greenhouse at CIAT's headquarters near Palmira, Colombia. A randomized complete-block design was used, with three replicates each for the seedling and adult-plant screening. On the average, 10 seeds each for the parents and F_1, 60 seeds for the F_2, and 30 seeds for each backcross generation were inoculated in each replication.

Seedlings and adult plants of each treatment were inoculated simultaneously with a monosporic suspension of conidia at 1.2×10^6 ml^{-1} of the Colombian isolate CL.27.COL belonging to race 521 of *C. lindemuthianum*. Inoculated plants and seedlings were kept at 20–22 C for 7 days in a 95–100% humidity chamber, which was placed in a greenhouse with a 12-hr photoperiod per day. After 7 days, evaluations were made on individual plants and/or seedlings on a 1–9 scale, where 1 = no visible symptoms and 9 = severely diseased or dead (24). Since reactions were discrete, data were classified into class frequencies and tested for goodness-of-fit to theoretical ratios with chi-square tests.

RESULTS

Reaction of G 2333 and other known resistance sources. The reactions of G 2333 and other resistance sources to 166 isolates (of a total of 380 used in this study) belonging to 15 selected Latin American races of the anthracnose pathogen are shown in Table 1. Most of these races of *C. lindemuthianum,* especially those that attack the well-known source of anthracnose resistance, Cornell 49242, and other sources of resistance (Mexico 222, PI 207262, TO, TU, and AB 136) occur only in Latin America. The anthracnose pathogen races alpha, beta, gamma, delta, lambda, and epsilon, reported to occur in North America, do not attack Cornell 49242 or the other five sources of resistance (22). Kelly and Tu, however, recently reported the presence of race 9 and alpha-Brazil in Michigan and Ontario, respectively (J. Kelly, *personal communication*). Both races attack the *Are* anthracnose resistance gene present in Cornell 49242. None of the 380 isolates of *C. lindemuthianum* from the 11 Latin American countries used in this study caused anthracnose symptoms on plants of G 2333. In contrast, many isolates caused severe symptoms and death on plants of the other resistance sources. Some sources, such as Cornell 49242 and PI 207262, were susceptible to a large number of isolates (Table 1). Some of these races (e.g., 9, 73, 129, and 133) have been found at many locations in several

Table 1. Reaction of six frequently used sources of resistance in common bean and of G 2333 to selected races of *Colletotrichum lindemuthianum* from Latin America

	No. isolates per country[b]						Reaction of sources of resistance[c]						
Race[a]	ARG	COL	CRA	ECU	MEX	PER	Cornell 49242	Mexico 222	TO	TU	PI 207262	AB 136	G 2333
8	1	0	0	0	0	0	S[d]	R	R	R	R	R	R
9[e]	3	28	24	0	3	2	S	R	R	R	R	R	R
65	9	0	0	1	4	0	R	S	R	R	R	R	R
73	1	1	1	0	1	0	S	S	R	R	R	R	R
129	1	6	2	12	1	2	R	R	R	R	S	R	R
133	3	6	2	14	0	2	R	R	R	R	S	R	R
136	0	0	0	0	0	1	S	R	R	R	S	R	R
385	0	6	0	0	4	0	R	R	S	R	S	R	R
448	0	0	0	0	4	0	R	S	S	R	S	R	R
521	0	11	3	0	0	0	S	R	R	S	R	R	R
901	0	1	0	0	0	0	R	R	S	S	S	R	R
905	0	0	1	0	0	0	S	R	S	S	S	R	R
1409	0	0	0	1	0	0	R	R	S	R	S	S	R
1473	0	0	0	0	3	0	R	S	S	R	S	S	R
2047	0	0	1	0	0	0	S	S	S	S	S	S	R

[a] Races determined on a set of 12 internationally accepted differential genotypes, using the binary system designation (14).
[b] ARG, Argentina; COL, Colombia; CRA, Costa Rica; ECU, Ecuador; MEX, Mexico; and PER, Peru.
[c] Known anthracnose resistance genes: Cornell 49242 (*Are*), Mexico 222 (*Mex* 1a), TO (*Mex* 2), and TU (*Mex* 3).
[d] Seedling reaction: R = resistant, incompatible reaction, with no visible anthracnose symptoms or with very small and few nonsporulating anthracnose lesions; S = susceptible, compatible reaction, with numerous medium-to-large sporulating anthracnose lesions.
[e] Race 9 also present in Brazil, Guatemala, Honduras, and El Salvador.

Table 2. Characteristics and type of reaction to *Colletotrichum lindemuthianum* under field and greenhouse conditions of seedlings and adult plants of common bean genotypes G 2333, G 12488, and ICA Pijao

Identification	Origin	Growth habit[a]	Seed		Field[b]		Greenhouse[c]	
			Color	Size	Seedling	Adult	Seedling	Adult
G 2333	Mexico	IV	Red	Small	R	R	R	R
G 12488	Ecuador	III	Purple mottled	Large	R	R	S	R
ICA Pijao	Colombia	II	Black	Small	S	S	S	S

[a]II = Indeterminate, erect bush; III = indeterminate, weak-stemmed, semiclimber; IV = indeterminate, weak-stemmed, climber (21).
[b]Reaction in Popayán, Colombia.
[c]Reaction to race 521 and several other individual races was tested.

Table 3. Segregation for resistance to race 521 of *Colletotrichum lindemuthianum* in a cross of common bean genotypes ICA Pijao × G 2333

Pedigree	Generation	Seedling					Adult				
		No. plants		Expected ratio	χ^2	*P*	No. plants		Expected ratio	χ^2	*P*
		Resistant	Susceptible				Resistant	Susceptible			
ICA Pijao	P_1	0	30	...	...	...	0	30	...	...	...
G 2333	P_2	30	0	...	...	...	30	0	...	...	...
ICA Pijao × G 2333	F_1	30	0	...	...	...	30	0	...	...	...
ICA Pijao × G 2333	F_2	169	10	15:1	0.13	0.71	167	10	15:1	0.10	0.74
ICA Pijao × F_1	BC_s	66	23	3:1	0.03	0.85	84	6	3:1	16.13	0.00
G 2333 × F_1	BC_r	90	0	1:0	0.00	1.00	90	0	1:0	0.00	1.00

Latin American countries, while many others (such as 8, 136, 448, 901, 905, 1409, 1473, and 2047) have been found at only one location.

Inheritance of resistance in G 2333. G 2333 was resistant to race 521 of *C. lindemuthianum* in the seedling and adult-plant stages, under both field and greenhouse conditions (Table 2). In contrast, ICA Pijao was susceptible in both plant stages and under both environmental conditions. The check, G 12488 (Ecuador 1056), was resistant under field conditions but susceptible in the seedling stage in the greenhouse.

Table 3 gives the observed and expected frequencies for resistant and susceptible reactions of the parents G 2333 and ICA Pijao, the F_1 and F_2, the F_1 backcrossed to ICA Pijao, and F_1 backcrossed to G 2333 for seedling and adult plants. The chi-square values revealed that a good fit was obtained for the segregation ratio of 15 resistant to 1 susceptible in the F_2, for 3 resistant to 1 susceptible when the F_1 was backcrossed to ICA Pijao, and for all resistant when the F_1 was backcrossed to G 2333. Results were similar in both the seedling and adult-plant stages, although there was a deficiency of susceptible plants for the adult-plant reaction in the backcross of the F_1 into ICA Pijao.

DISCUSSION

Successful development of common bean cultivars with durable anthracnose resistance depends partly on the sources of resistance gene(s) used in hybridization and on the number and types of pathogen isolates used to screen populations and advance generation lines. It is particularly important to select parents with durable resistance to a variable pathogen. Only G 2333 was resistant to all 380 isolates of *C. lindemuthianum* used in this study. This accession was also resistant to all Brazilian isolates and to all European and North American races of *C. lindemuthianum* (2,3,9,17). The other six sources were susceptible to a large or small number of isolates belonging to several races of the anthracnose pathogen (Table 1). Thus, G 2333, which has wide adaptation, high seed yield in many environments, and tolerance of low soil fertility, is a valuable parent for breeding programs, particularly where *C. lindemuthianum* is highly variable.

The segregation ratio of 15 resistant to 1 susceptible in the F_2 and the good fit to the expected ratios in the backcross generations (Table 3) indicate that the resistance of G 2333 to race 521 is controlled by two independent dominant genes with equal effects. Because the reactions in seedling and adult-plant stages were similar and had a similar mode of inheritance, the same genes probably control resistance in both plant stages. Cardenas et al (8), Muhalet et al (15), and Peloso et al (18) also reported that duplicate dominant genes were responsible for anthracnose resistance in some bean crosses. But in other crosses, these same authors, as well as other researchers, including Bannerot et al (5) and Mastenbrock (13), reported monogenic dominance and other types of genetic control for anthracnose resistance in common bean.

The results of studies on the nature of inheritance depend, among other factors, on the tester genotype used as the susceptible parent, the isolate or race of the pathogen used for inoculation, and the stage at which the host plant is inoculated. Because these factors vary greatly among studies, and seed from original stocks is often not available, we made no attempt to test for allelism with known sources of resistance. Nor did we assign gene symbols to the two resistant genes found in G 2333. But because G 2333 is resistant to a much broader range of pathogen populations, we infer that its two resistance genes are different from those found in the other six sources of anthracnose resistance. Because two independent genes with equal effects control the resistance, it is likely to be more durable than the resistance controlled by the single gene found in Cornell 49242, Mexico 222, TO, and TU.

The dominant nature of inheritance should make transferring anthracnose resistance from G 2333 to susceptible cultivars relatively easy with any selection method. However, care should be taken to ensure that both dominant alleles are transferred into the desired cultivar. Also, when anthracnose resistance from G 2333 is combined with resistance to other diseases and desirable traits, selection for anthracnose resistance can be delayed until later generations if necessary, giving preference to selection for other traits in the early generations.

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