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Evaluation of dry beans for resistance to ashy stem blight[1]

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ABSTRACT

Experiments were conducted to determine the effectiveness of two inoculation methods in the field evaluation of beans for resistance to ashy stem blight. Seed inoculation resulted in lower germination and greater infection by *Macrophomina phaseolina* than that in nontreated plots. Stem inoculation did not significantly differ from the control in percentage disease. Least significant differences among genotypes were smaller toward the end of the growing season. Therefore, bean genotypes should be evaluated for ashy stem blight infection near senescence. Spearman rank correlations between percentage germination and percentage infection near senescence were significant in 2 years. Seed inoculation combined with germination tests may be useful in the preliminary screening of bean genotypes for resistance to ashy stem blight.

RESUMEN

Resistencia de la habichuela a la pudrición carbonosa del tallo

Se realizaron experimentos de campo para determinar la efectividad de dos métodos de inoculación para evaluar la resistencia de la habichuela a la pudrición carbonosa del tallo. Al comparar las parcelas tratados con las sin tratar, el método de inocular la semilla resultó en una germinación más baja y una infección de *Macrophomina phaseolina* más alta. No hubo diferencias significativas para el porcentaje de infección al inocular el tallo en parcelas tratados en relación con las sin tratar. La diferencia mínima significativa entre los genotipos de habichuela podrían evaluarse para la pudrición carbonosa del tallo cerca de la senescencia de las plantas. Las correlaciones de rango de Spearman entre el porcentaje de germinación y el porcentaje de infección cerca de la senescencia fueron significativas en ambos años. La inoculación de la semilla combinada con las pruebas de germinación debe ser útil en selecciones preliminares de genotipos de habichuela resistentes a la pudrición carbonosa del tallo.

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INTRODUCTION

Ashy stem blight caused by *Macrophomina phaseolina* (Tassi) Goid has been reported on beans in the United States (7) and in Latin America (6). When beans are planted in the summer in Puerto Rico and the Dominican Republic, this disease is favored by the warm dry growing conditions and as a consequence ashy stem blight infection can be severe (2).

Although techniques for screening beans for ashy stem blight resistance in a greenhouse have recently been reported (1,3), reliable field evaluations for ashy stem blight are difficult to obtain. The distribution of natural infection of *M. phaseolina* in the field is usually not uniform. Moreover, evaluation based on natural infection requires almost the entire growing season since the severity of the disease is more intense toward senescence. Another factor which might reduce the reliability of ashy stem blight readings is the existence of isolates which vary in virulence (1). Resistance to ashy stem blight has been reported for beans (3,5). However, the durability and the nature of inheritance of these sources of resistance have not been determined. The principal objective of this research was to measure the effectiveness of seed and stem inoculation in field evaluation of bean genotypes for resistance to ashy stem blight.

MATERIALS AND METHODS

Two field experiments were conducted at the Arroyo Loro Experiment Station in the San Juan Valley of the Dominican Republic. The experiments were planted 3 October 1984 and 3 October 1985. The experimental design for both experiments was a split-plot arrangement of a randomized complete block with three replications. The whole plots consisted of two inoculation methods and a control. The sub-plot treatments consisted of 15 bean genotypes for the 1984 experiment and 18 genotypes for the 1985 planting. Experimental units consisted of one row 2.5 m long with 0.5 m between rows. Thirty seed were planted in each row.

The test isolate originated from microsclerotia of *M. phaseolina* from infected bean stems obtained from the Arroyo Loro Experiment Station. The fungus was isolated and cultured for 2 weeks on potato-dextrose-agar (PDA) at 28° C.

Seed inoculation consisted of soaking bean seed for 1 minute in a suspension containing a concentration of approximately 1000 microsclerotia per ml. After the soaking, the seed were allowed to dry at 25° C for a 12-hour period. Dried seed was stored in a refrigerator at approximately 10° C until the time of planting.

The other treatment consisted of stem inoculations performed 15 days after emergence (R1) (4). We inoculated plants by rubbing the base of

TABLE 1.—*Mean percentage of bean plants with ashy stem blight in 1984*

Genotype	Percent infection 35 days after planting			Percent infection 42 days after planting			Percent infection 49 days after planting			Percent infection 67 days after planting		
	Control	Seed inoc.	Stem inoc.	Control	Seed inoc.	Stem inoc.	Control	Seed inoc.	Stem inoc.	Control	Seed inoc.	Stem inoc.
Pompadour checa	4	10	11	5	11	10	13	9	14	12	8	12
José Beta	18	14	12	10	10	1	11	16	17	17	8	12
Constanza	8	20	9	1	12	2	12	24	11	9	13	12
A 475	15	18	8	10	12	5	14	33	14	33	42	14
PAI 43	24	49	17	20	32	6	31	30	20	24	47	24
PAI 92	5	8	6	5	12	6	9	24	7	7	17	12
B-190	8	8	7	5	8	6	6	16	12	13	16	20
La Vega	5	10	7	4	4	2	7	21	9	9	17	10
2B-5-1	9	8	17	4	9	12	7	9	15	13	26	13
Venezuela 44	6	1	4	7	4	5	10	6	6	8	11	11
XAN149	1	13	3	1	9	5	5	9	5	9	8	10
XAN154	7	5	3	4	3	1	11	5	4	19	14	10
BAT 240	10	9	13	4	10	6	10	18	13	15	14	7
CC4462-3-1-6	6	4	3	7	5	4	8	9	5	12	9	3
CC4462-12	10	15	7	4	9	9	19	11	21	28	14	35
Treatment mean	9	12	8	6	10	5	11	16	12	15	18	14
Overall mean		10			7			13			16	
L.S.D. (0.05)[1]		3			3			N.S.			N.S.	
L.S.D. (0.05)[2]		13			9			10			9	

[1]Least significant difference (0.05) to compare treatment means.

[2]Least significant difference (0.05) to compare genotype means within a treatment.

the stems with small pieces of cheesecloth which has been previously soaked in a suspension containing approximately 1,000 microsclerotia per ml. and 10 g of carborundum per L of supension.

The number of plants per row was counted at emergence. Percentage germination was calculated as follows: (number of plants per row at emergence/number of seed planted per row) × 100. The first evaluation for ashy stem blight was conducted 2 weeks after emergence, and additional readings were conducted at weekly intervals until senescence. During the evaluations, the plants showing infection were counted in each row. A plant was considered infected if it had symptoms characteristic of ashy stem blight such as a stem rot with tissue covered with microsclerotia. The plants identified as infected were marked so that the progress of the infection could be observed. Percentage infection was calculated as follows: (number of plants infected per row/number of plants at emergence) × 100. At maturity (R9) seed was harvested from each row.

TABLE 2.—*Mean percentage of bean plants with ashy stem blight in 1985*

	Percent infection 57 days after planting			Percent infection 80 days after planting		
Genotype	Control	Seed inoc.	Stem inoc.	Control	Seed inoc.	Stem inoc.
XAN 149	18	20	14	22	21	19
Venezuela 44	17	31	20	23	33	27
A 475	27	63	30	36	84	48
Pompadour checa	33	34	34	35	37	43
Constanza	19	41	24	27	55	29
PC 50	29	24	31	34	31	34
PC 157	40	17	46	48	25	60
PC 23	34	37	29	41	44	37
RIZ 30	35	35	23	39	59	35
PAT 12	34	17	11	38	37	35
PAT 9	23	16	19	30	30	32
PAT 11	24	17	16	32	34	26
PAT 6	21	19	13	24	39	23
BAT 240	22	25	27	29	45	37
H270	24	19	11	31	46	47
Talamanca	21	15	29	23	21	40
ICA Pijao	12	18	23	17	27	32
XAN 93	21	20	19	25	36	30
Treatment mean	25	26	25	31	39	35
Overall mean		25			35	
L.S.D. (0.05)[1]		N.S.			5	
L.S.D. (0.05)[2]		18			10	

[1]Least significant difference (0.05) to compare treatment means.

[2]Least significant difference (0.05) to compare genotype means within a treatment.

Genotype and stem inoculation treatment means were compared by least significant differences at the 0.05% probability level. Spearman rank correlations were used to measure association of percentage germination with percentage infection.

RESULTS AND DISCUSSION

During both years there was an increased incidence of ashy stem blight as the plants approached senescence (tables 1 and 2). Disease frequency was greater in 1985 than in 1984. This difference might be due to an increase in the initial inoculum since the trials were conducted on the same site both years of the experiment. The seed inoculation treatment significantly reduced percentage germination during both years (tables 3 and 4). The magnitude of the reduction, however, varied among genotypes. Experimental units which had received the seed inoculation treatment also had a greater percentage infection than the control for the 7 and 14 October 1984 readings (3 and 4 weeks, respectively, after inoculation) and the 29 November 1985 reading (6 weeks after inoculation). On the other hand, the percentage infection of the experimental

TABLE 3.—*Mean percentage germination and seed yield per plot of beans in 1984*

Genotype	Percent germination			Seed yield per plot		
	Control	Seed inoc.	Stem inoc.	Control	Seed inoc.	Stem inoc.
					g	
Pompadour checa	89	93	84	123	145	109
José Beta	89	86	95	63	122	112
Constanza	91	83	82	137	94	341
A 475	93	69	93	80	66	90
PAI 43	89	43	89	172	98	140
PAI 92	96	79	91	197	192	202
B-190	84	44	89	179	183	165
La Vega	88	84	85	232	163	186
2B-5-1	93	89	88	154	98	175
Venezuela 44	93	86	93	237	195	212
XAN149	91	83	83	215	148	242
XAN154	89	92	93	264	173	224
BAT 240	94	73	90	161	240	153
CC4462-3-16	86	86	86	174	173	218
CC4462-12	74	73	69	84	81	49
Treatment mean	89	77	87	164	144	174
Overall mean		85			161	
L.S.D. (0.05)[1]		5			N.S.	
L.S.D. (0.05)[2]		14			124	

[1]Least significant difference (0.05) to compare treatment means.

[2]Least significant difference (0.05) to compare genotype means within a treatment.

TABLE 4.—*Mean percentage germination and seed yield per plot in 1985*

Genotype	Percent germination			Seed yield per plot		
	Control	Seed inoc.	Stem inoc.	Control	Seed inoc.	Stem inoc.
					g	
XAN 149	87	73	84	126	163	143
Venezuela 44	94	81	90	76	74	47
A 475	81	27	87	15	37	19
Pompadour checa	89	84	92	51	59	61
Constanza	96	51	93	71	47	48
PC 50	84	69	82	49	64	60
PC 157	90	91	96	63	85	61
PC 23	91	88	93	66	148	12
RIZ 30	83	64	88	24	24	66
PAT 12	86	46	91	32	32	46
PAT 9	93	71	94	38	83	82
PAT 11	85	79	88	43	49	66
PAT 6	91	70	95	32	58	70
BAT 240	88	79	90	64	100	50
H 270	83	69	87	127	86	89
Talamanca	91	83	91	153	112	91
ICA Pijao	76	76	86	72	31	41
XAN 93	93	70	88	88	69	86
Treatment means	88	71	90	66	73	63
Overall mean		83			68	
L.S.D. (0.05)[1]		8			N.S.	
L.S.D. (0.05)[2]		11			66	

[1]Least significant difference (0.05) to compare treatment means.
[2]Least significant difference (0.05) to compare genotype means within a treatment.

units that received the stem inoculation treatment did not differ significantly from the control on any of the reading dates.

There was a significant difference among genotypes for ashy stem blight incidence. The least significant difference values used to distinguish differences among genotypes were smaller toward the end of the growing season, when percentage infection was higher. If the incidence of ashy stem blight is used to evaluate bean genotypes for resistance, the most appropriate time for disease evaluation appears to be at or near senescence. In 1984, Pompadour checa, XAN-149, and CC4462-3-1-6 had the lowest percentage infection at the end of the growing season. In 1985, Talamanca, ICA Pijao, XAN-149, and PC-157 were among those with the lowest levels of infection. Genotypes with the highest percentage infection were A-475, PAI-43, Venezuela 44, BAT 240 and CC4462-12 in 1984, and A-475, RIZ-30, BAT 240, and H-270 in 1985.

There was no significant difference in seed yield between the seed and stem inoculation treatments and the control. Seed yield differences

among genotypes were more of a reflection of differences in seed type and growth habit rather than of differences in the incidence of infection with ashy stem blight.

Results of percentage germination readings agreed well with the percentage infection readings taken near harvest maturity. Spearman rank correlations between percentage germination, ranked from lowest to highest, and percentage infection of the seed inoculated genotypes, ranked from highest to lowest, were significant in both 1984 (0.54 **) and 1985 (0.51 **). These results indicate that seed inoculation combined with percentage germination counts might be a useful technique in the preliminary screening of bean for resistance to ashy stem blight. With a germination test, a large number of lines could be screened in a relatively small area over a short period of time. A germination test would also permit the rapid screening of bean genotypes for different isolates of the fungus.

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