Identification of in Vitro Resistance of Pigeon Peas [Cajanus Cajan (L.) Millsp.] to *Phytophthora* Stem Canker.[1]

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ABSTRACT

A total of 617 accessions and 40 pigeon pea lines were screened in vitro for their resistance to *Phytophthora parasitica*. Several entries were found resistant to the pathogen in stem-piece inoculations. Even after injury accessions 394-014, 396-383, 396-356, 396-731, 396-295 and lines 8 AB-2 and 731BD did not show lesions from *P. parasitica* even when injured.

INTRODUCTION

Pigeon pea stem canker caused by *P. parasitica* was first reported in Puerto Rico by Kaiser and Meléndez in 1978 (1). The disease is characterized by sunken dark brown to black lesions which may be present on the main stem as well as on the branches of infected plants. Losses result from poor stands because affected stems are girdled or become debilitated and break at canker sites or wilt above infection sites.

Pat et al. (3) and Williams et al. (5, 6) have reported a similar disease which is serious in Indian pigeon pea growing areas. In the northwestern region of Puerto Rico a greater disease incidence occurs in August, thus attacking the plants almost in the middle of their growing cycle (2).

One effective method to control *Phytophthora* stem canker will be the use of resistant varieties. To meet with this objective, local advanced lines, introductions and accessions from the germplasm collection were evaluated for their resistance to *Phytophthora* stem canker.

MATERIALS AND METHODS

The 617 accessions and 40 advanced lines were screened as follows: six 10-cm stem pieces were collected from the main axis of 4-month old plants grown in the greenhouse. Half of these were wounded artificially; the other half were left undisturbed. All stem-pieces were washed with 10% Lysol and the detergent Alconox. After washing in tap water, they were immersed for 5 min in each of the following solutions, 20% Clorox, 70% ethyl alcohol and finally rinsed in sterile distilled water. The stem-pieces were placed in petri dishes (150 × 20 mm) containing sterile moist filter paper and exposed to PDA discs (6 mm) with hyphal tips obtained from a 15-day-old culture of *P. parasitica* isolated from cankered pigeon

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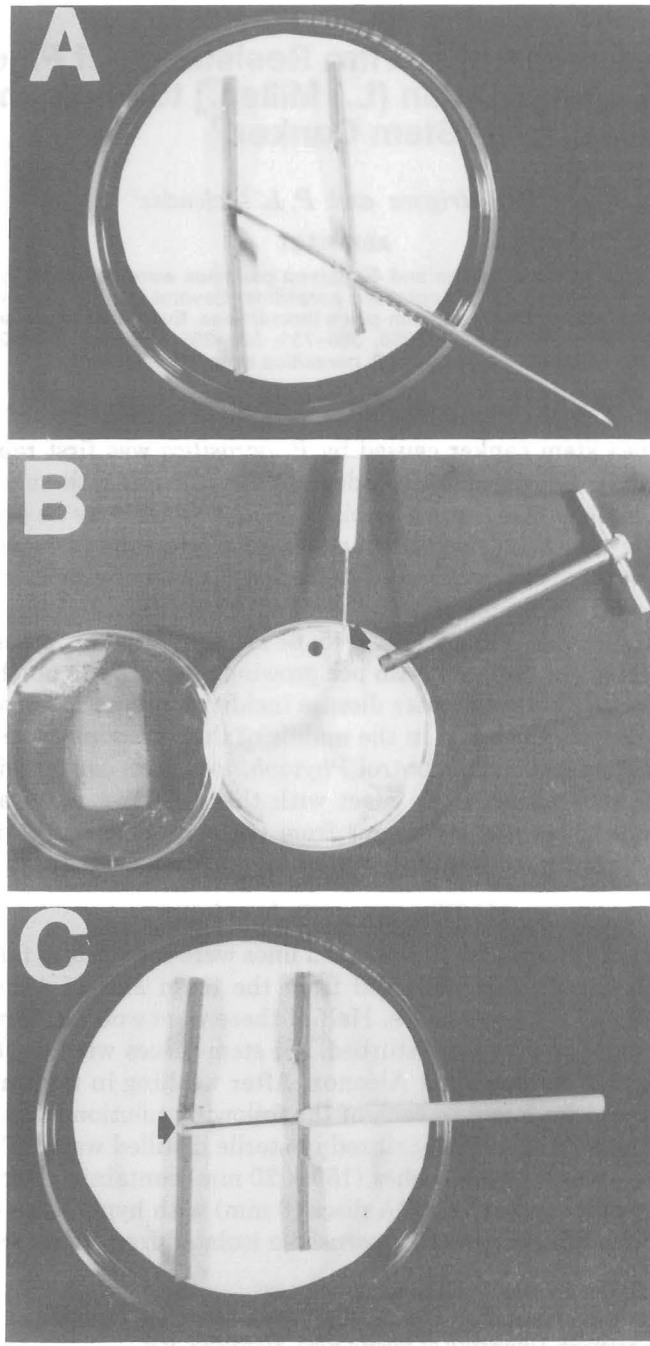

FIG. 1.—Inoculation steps for stem-piece technique. A. Artificial wounds. B. PDA discs with hyphal tips. C. Inoculation of the stem-pieces with fungus hyphal tips in direct contact with epidermal tissues.

pea in the field (fig. 1.). Checks were similarly treated and exposed to PDA-discs free of the fungus. To test pathogen virulence, we included in each assay an additional check consisting of stem pieces of susceptible cv. Kaki. After 4 days of incubation at 28° C, the inoculated stem-pieces were examined on the basis of appearance of symptoms, and the extent of fungal invasion was measured and recorded.

RESULTS AND DISCUSSION

Pigeon pea accessions 394-014, 396–295, 396–356, 396–383 and 396–731 did not show evidence of invasion by *P. parasitica* even when they were wounded. One hundred sixty-four accessions showed no external disease symptoms on unwounded inoculated stems; however, a range of lesion sizes was found with wound inoculations. Less than 20% of invasion was observed in 70 accessions and less than 40% was found in 41 others. Stem-pieces of 48 accessions showed invasion in more than 40% of their exposed surfaces (table 1). Two of the *P. parasitica* resistant accessions, 394–002 and 394–005, have also been found resistant to foliar diseases in Puerto Rico (4).

Several of the accessions evaluated reacted differently from the accessions mentioned above (table 2). Some of these lines showed symptoms when unwounded, but invasion was somewhat arrested compared with that showed on susceptible cv. Kaki. Conversely, accessions 394–279, 394–409, 394–675 394–712, 394–724, 394–768, 394–769 and 394–777 succumbed entirely to *P. parasitica* attack, showing cankers all over their exposed surfaces even when unwounded.

Tables 3 and 4 show the results on the evaluation of advanced lines and introductions. Line 8 AB-2 and 731BD did not show *Phytophthora* stem lesions even when wound inoculated. Twenty-five lines were not invaded by the pathogen when wounded, but they showed various extensions of lesions with wound inoculations. Lines 1 and 7221 particularly showed outstanding resistance to the pathogen even when wounded (table 3), whereas 7018 and 7172 were found susceptible to the disease, showing symptoms in more than 50% of their stem-pieces (table 4). Line 64-16A, which possesses a certain degree of resistance to *P. parasitica*, has also been resistant to foliar diseases (4). Some advanced lines and introductions showed good levels of resistance to *P. parasitica* judging by the degree of infection under both wounded and unwounded conditions. Symptoms on unwounded and wounded stem-pieces from lines Kaki × GI 27–4A, 2624, 28, 6520, 6997 and 7181 covered less than 10% and 20% of their surfaces, respectively.

This screening has identified sources of resistance to *P. parasitica*, which can be used to improve commercial cultivars. Accessions and

TABLE 1.—*Percentage of surface cankered on wounded stem pieces from pigeon pea accessions after in vitro exposure to* Phytophthora parasitica[1]

Accession number[2]						
0%	1–10%	11–20%	21–30%	31–40%	41–50%	51–100%
394-014	394-002	394-028	394-003	394-001	394-013	394-008
396-295	394-005	394-031	394-007	394-021	394-128	394-039
396-356	394-012	394-065	394-059	394-033	394-170	394-089
396-383	394-056	394-069	394-206	394-121	394-280	394-113
396-731	394-152	394-086	394-220	394-138	394-306	394-123
	394-198	394-096	394-223	394-174	394-498	394-237
	394-208	394-103	394-253	394-260	394-660	394-275
	394-216	394-114	394-277	394-273	394-782	394-706
	394-255	394-125	394-294	394-342	394-783	394-738
	394-274	394-136	394-301	394-764	394-784	394-785
	394-313	394-156	394-302	394-778	394-788	394-786
	394-314	394-163	394-401	394-855	396-023	394-808
	396-240	394-177	394-490	394-877	396-046	394-856
	396-313	394-181	394-656	394-878	396-159	394-857
	396-745	394-183	394-725	394-907	396-180	394-881
	396-784	394-186	394-766	396-139	396-278	396-015
	396-851	394-194	394-826	396-282	396-287	396-042
	396-939	394-195	394-937	396-863	396-338	396-128
		394-202	396-013	396-982	396-565	396-203
		394-204	396-036		396-945	396-318
		394-205	396-832		396-997	396-840
		394-212	396-835			397-158
		394-215				397-273
		394-219				397-341
		394-225				397-534
		394-226				397-985
		394-256				397-998
		394-258				
		394-262				
		394-272				
		394-276				
		394-282				
		394-286				
		394-290				
		394-315				
		394-321				
		394-353				
		394-494				
		394-528				
		396-005				
		396-026				
		396-073				
		396-082				
		396-100				
		396-156				
		396-193				
		396-211				
		396-303				
		396-341				
		396-635				
		396-699				
		396-946				

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TABLE 2.—*Selection of pigeon pea accessions based on percentage of surface cankered by* Phytophthora parasitica

Accession number[1]				
Less than 10% when unwounded and less than 20% when wounded		More than 50% of invasion under both conditions		
394-094	394-363	394-004	394-409*[2]	397-224
394-030	394-410	394-036	394-414	397-277
394-048	394-446	394-038	394-531	397-287
394-175	394-472	394-067	394-552	397-294
394-179	394-574	394-072	394-562	397-298
394-191	396-107	394-073	394-675*	397-300
394-213	396-117	394-080	394-699	397-400
394-222	398-137	394-085	394-712*	397-408
394-232	396-187	394-088	394-724*	397-446
394-245	396-209	394-098	394-734	397-477
394-263	396-313	394-105	394-762	397-537
394-268	396-863	394-145	394-768*	397-556
394-270	398-002	394-162	394-769*	397-576
394-316	398-007	394-203	394-776	397-838
394-328		394-227	394-777*	397-848
		394-279*	397-896	397-949
		394-281	397-057	398-003
		394-295	397-162	398-004
		394-329	397-167	398-018
		394-380	397-190	398-020

[1] USDA-PIN. Accessions received through S-9 Regional Project.
[2] Almost 100% of invasion in both conditions.

TABLE 3.—*Percentage of surface cankered on wounded stem pieces from pigeon pea advanced lines and introductions after in vitro exposure to* P. parasitica[1]

Line					
0%	1–10%	11–20%	21–30%	31–40%	41–50%
		64-16 A	Am × Kaki	16 AIrr	69-52
8 AB-2	1	69-68	69-58	142 A	Pant-A-2
73-1BD	7221	1641	Cv. 2-B-Bushy	4779	
		3D-8102	64-21 B-2	OP62	
			Pusa Ageti	T21	
			3D-8103	B51	
			OP61	3D-8125	
			UPA 120		

[1] Unwounded stem pieces from each line were not invaded by the pathogen.

advanced lines which have shown no *P. parasitica* lesions, even when wound inoculated are particularly promising. However, lines which resisted invasion when unwounded may not be as valuable for increasing disease resistance because in the field, crops are subjected to a wide

number of factors which might affect the integrity of epidermal stem tissues. Consequently, lines and accessions with certain levels of resistance when injured should be considered in breeding for resistance to *Phytophthora* stem canker disease, particularly if they also have superior agronomic traits. Accessions and advanced lines have been identified with combined resistance to *Phytophthora* stem canker, rust and foliar spots in Puerto Rico. These lines can be important sources of multiple disease resistance.

The stem-piece technique is a useful tool in screening for resistance. It provides conditions for rapid lesion development by permitting early detection of disease differences among pigeon pea lines. Heterogenicity of test materials complicates their classification. Thus, accessions selected under these conditions should be further evaluated to confirm their disease reactions under field conditions.

TABLE 4.—*Distribution of advanced lines and introductions based on percentage of surface cankered by* P. parasitica *on wounded and unwounded stem pieces*

Less than 10% when unwounded and less than 50% when wounded	Less than 20% when unwounded and less than 50% when wounded	More than 50% of invasion under both conditions
7	7119	7018
Kaki × GI27-4A	7135	7172
12	7229	
2624	7035	
28		
6520		
6997		
7181		

RESUMEN

So evaluó un total de 617 líneas de la colección de germoplasma y 40 líneas introducidas y desarrolladas por el programa de fitomejoramiento de gandul para resistencia al cancro causado por *Phytophthora*. La evaluación de llevó a cabo *in vitro* utilizando el método de pedazos de tallos. Algunos de los materiales evaluados mostraron poseer resistencia al patógeno. Las líneas 394-014, 396–383, 396–356, 396–731, 396–295, 8AB-2 y 731BD no mostraron síntomas de la enfermedad aun en presencia de heridas. Diferentes grados de resistencia fueron detectados entre la población de plantas examinadas.

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