Indian J. Plant Prot. 28(1) : 51-56, 2000
ISSN 0253-4355

Detection of *Pseudomonas solanacearum* in Wild *Arachis* Spp Imported from Brazil

R.D.V.J. Prasasda Rao, G.A. Gunjotikar*, S.K. Chakrabarty, B. Sarath Babu, K.S. Varaprasad, S.D. Singh*, L.J. Reddy* and Paula Bramel-Cox*

National Bureau of Plant Genetic Resources - Regional Station, Rajendranagar, Hyderabad - 500 030, Andhra Pradesh, India.

** International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru - 500 030, Andhra Pradesh, India.*

Abstract

Groundnut bacterial wilt (*Pseudomonas solanacearum*) (=*Ralstonia solanacearum*) was intercepted on wild *Arachis* spp. when these were grown for quarantine clearance in the Post Entry Quarantine Isolation Area (PEQIA) at ICRISAT center, Patancheru, Andhra Pradesh, India. These accessions were imported from Brazil. Also, there is no record of groundnut bacterial wilt occurrence in Andhra Pradesh. The symptoms of the disease expressed at temperatures more than 30°C. Occurrence of bacterial wilt on accessions from Brazil where the disease has not yet been reported indicates that the pathogen is possibly transmitted in "latent form" from one generation to the next in groundnuts grown under temperate conditions.

Introduction

Groundnut bacterial wilt caused by *Pseudomonas solanacearum* (Smith) (=*Ralstonia solanacearum*) is considered a potential threat to groundnut production in several parts of the world particularly in warm, humid areas. It is reported to cause severe yield losses in Java and Indonesia (Machmud, 1993), Malaysia (Hamidah and Lum, 1993), and Central and North western Uganda (Busolo Bulafu, 1993). The bacterium has a wide host range. It is transmitted through seed in groundnut (Palm, 1922; Machmud and Middleton, 1990). The disease is of quarantine importance to India, inspite of sporadic reports of its occurrence, because of the existence of pathotypes in other parts of the world. Groundnut germplasm is imported into India both by national and international organizations for crop improvement. The imports are channelised through the National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Hyderabad, for quarantine clearance particularly for seed transmitted viruses, scab, and bacterial wilt.

ICRISAT and NBPGR have developed protocols for the safe import of seeds of ICRISAT mandate crops. Since its inception in 1972 until 1997, ICRISAT has safely imported 20, 154 germplasm/breeding material, both wild and cultivated types, from 51 countries. Unfortunately, in 1998, many accessions in a consignment of wild *Arachis* spp. from Brazil produced symptoms typical of bacterial wilt in the Post Entry Quarantine Isolation Area (PEQIA). This note describes details of a study that led to the identification of this disease as bacterial wilt of groundnut caused by the *Pseudomonas solanacearum.*

Materials and Methods

ELISA Test

The seeds were ELISA tested for seed transmitted viruses by non-destructive procedure (Demski and Warwick, 1986). A 50 mg tissue sample, from cotyledon opposite to empryo, was removed from each seed using a sterilized blade. These tissue samples were used in ELISA tests to identify the virus-carrying seeds (peanut stripe and peanut mottle viruses). The seeds that were

free from the viruses were used for grow-out tests.

Grow-out test I

Five ELISA tested seeds of each accession were sown in 10 cm diameter plastic pots, one seed per pot. The pots were filled with sterilized soil + manure mixture (2 : 1 ratio). Before sowing, the seeds were treated with thiram (tetramethyl thiuram disulfide) @ 3g Kg^{-1} seed. The treated seeds were also sprayed with ethrel (2-chlroethyl phosphoric acid) at 3.5 ml/L^{-1} distilled water to improve germination. The pots were maintained in a quarantine greenhouse at NBPGR for four weeks. The plants were regularly observed for the appearance of virus symptoms. Then the plants were maintained in a climatically controlled polyhouse at 25°C until harvest.

Grow-out test II

In another test, five ELISA tested seeds of each accession, treated with thiram and ethrel as for grow-out test, were transferred on to sterilized nutrient agar (NA) medium in 10-cm diameter petri plate. The plates were maintained at 25°C and 12 hr light cycle in an incubator. Ten days old seedlings were carefully removed from the petriplates and transplanted in 10-cm diameter plastic pots that were maintained in a quarantine greenhouse at NBPGR at 25± 3°C for four weeks. The plants were then transplanted in the PEQIA at the ICRISAT Center, Patancheru.

Isolation of the pathogen

The pathogen was isolated from the wilting groundnut plant. The infected stem was cut into 2 cm long pieces, thoroughly washed in sterile water, dried on sterile blotting paper and placed in a test tube containing 5ml of sterile distilled water for 15 min for bacterial oozing. A loopful of bacterial suspension was streaked on to Tetrazolium Chloride Agar (TZCA) medium (Kelman, 1954) (containing peptone 10g; casein hydrolysate 1 g; glucose 5 g; 2,3,5- triphenyl tetrazolium chloride (TZC) 0.05 g and agar 15g). One millilitre of a filter sterilized TZC was added to 100 ml of sterilized medium before pouring into petriplates. The petriplates were incubated at 30°C for 72 hr.

Pathogenicity test

The bacterium was multiplied on nutrient agar. Three-day old bacterial culture was washed in 5 ml sterile distilled water. Twenty-five seeds of groundnut cultivar JL-24, were soaked in the bacterial suspension for 30 min. The seeds were then sown in pots containing sterile soil and manure mixture (the soil manure mixture was sterilized at 121.6°C and 15 lbs pressure for 3 consecutive days). The pots were maintained at 32-35°C for the development of the disease in a greenhouse at ICRISAT Center. The bacterium from the wilted plants was isolated, cultured on TZCA medium and compared with the original cultures. (Mehan *et al.*, 1994).

Results and Discussion

Germination

Seeds in all accessions plated on nutrient agar showed about 48% germination whereas germination/emergence from directly sown seed was only 28%.

Wilt appearance and development

The plants grown in polyhouse under controlled temperature conditions did not develop wilt until harvest. Also, seedlings transplanted in PEQIA in November 1997, did not show any bacterial wilt symptoms until about mid February 1998. However, during the last week of February, wilt apppeared in few plants of nine accessions when the day temperatures rose beyond

Table 1 : Wild groundnut accessions that developed bacterial wilt (*Pseudomonas solanacearum*) in Post Entry Quarantine Isolation Area (PEQIA) at ICRISAT during 1998 summer.

S.No.	Accession	Species	Section	Location	PEQIA	QGH
1.	V 13962	*A.benthamii*	ER	Campua/3.5km E MS-436	+	
2.	W 866	*A.triseminata*	TR	Xique-Xique	-	-
3.	V 13710	*A.simpsonii*	AR	Prota Esperidiao	-	
4.	V 13202 (W 201)	*A.giacomettii*	HE	Montalvaria (8.4km/NW)	+	
5.	V 9060	*A. appressipila*	PR	corumba	-	-
6.	V 14021	*A. stenophylla*	ER	Bonito/3km Nrio Prata	+	-
7.	V 8816	*A. villosulicarpa*	EX	Comodoro (P.I. Aroeira)	-	-
8.	Md 1022	*A. villosulicarpa*	EX	Comodoro	+	-
9.	V 7632	*A. major*	ER	Anastacio	-	
10.	V 8530	*A. major*	ER	Anastacio	-	
11.	Bi 664	*A. sylvestris*	HE	Com Pedro II	+	
12.	V 13102	*A. sylvestris*	HE	Coronel Murta	+	
13.	V 13107	*A. sylvestris*	HE	Januaria	-	-
14.	V 13306	*A. sylvestris*	HE	Laciara	+	
15.	V 6727	*A. pintoi*	CA	Aracui (Rio Aracuai)	+	
16.	V 6728	*A. pintoi*	CA	Aracuai (estr. P/Badaro)	+	
17.	V 13162	*A. pintoi*	CA	Unai (Rib. Forquilha)	-	
18.	V 13468	*A. pintoi*	CA	Divinopolis (Rio Manso)	-	-
19.	VKAP 13641	*A. pintoi*	CA	Formosa (estr.ltiquira)	-	-
20.	V 13643	*A. pintoi*	CA	Brasilla (SQN 407)	-	-
21.	W 34	*A. pintoi*	CA	Frabcisco Badaro	+	
22.	W 153	*A. pintoi*	CA	F1. de Goias (CanaBrava)	+	
23.	W 224	*A. pintoi*	CA	Brasilandia	-	
24.	W 646	*A. pintoi*	CA	Buritinopolis	-	-
25.	W 647	*A. pintoi*	CA	Buritinopolis	-	-
26.	V 7669	*A. paraguariensis*	ER	Bela Vista	+	
27.	V 7677	*A. paraguariensis*	ER	Bela Vista	-	-
28.	V 13546	*A. paraguariensis*	ER	Bela Vista	-	
29.	V 14024	*A. paraguariensis*	ER	Caracol/Ridovia	-	-
30.	Bi 676	*A. dardani*	HE	Crateus	+	
31.	V 13322	*A. dardani*	HE	Campina Grande	+	
32.	V 13383	*A. dardani*	HE	Taquaritinga	+	
33.	V 13393	*A. dardani*	HE	Amparo do S. Franscisco	+	
34.	V 13400	*A. dardani*	HE	Porto Real do Colegio	+	
35.	V 7863	*A. burchellii*	EX	Pium	+	
36.	V 9955	*A. decora*	AR	Campos Belos	+	
37.	V 13430	*A. decora*	AR	Flores de Goias	-	-

S.No.	Accession	Species	Section	Location	PEQIA	QGH
38.	V 13478	*A. decora*	AR	Aurora do Tocantins	-	-
39.	V 13884	*A. decora*	AR	Alvorada do Norte	+	-
40.	W 648	*A. decora*	AR	Buritinopolis	+	-
41.	W 674	*A. decora*	AR	Alvorada do Borte	+	-
42.	BW 3663	*A. decora*	AR		-	-
43.	V 6325	*A. helodes*	AR	S. Antonoi do Leverger	+	
44.	V 11022	*A.pusilla*	HE	Itacarambi	+	
45.	V 10932	*A.pusilla*	HE	Missao Velha	+	
46.	V 11022	*A.pusilla*	HE	Piracuruca	-	-
47.	V 13514	*A. valida*	AR	Corumba/Fz.S.S.C.Caranda	+	-
48.	V 13516	*A. valida*	AR	Corumba (Fazendauruba)	-	
49.	V 14031	*A. valida*	AR	Porto Murtinho	-	-
50.	V 13250	*A.kermpffmercadoi*	AR	S.cruz de la Sierra	-	
51.	V 6466	*A.lutescens*	EX	Jaragua	-	
52.	V 13841	*A.prostra*	EX	Araguacu(Rio Pau Seco)	+	
53.	V 9146	*A. hoehnei*	AR	Corumba	+	
54.	V 13985	*A. hoehnei*	AR	Corumba(Er.paraguai)	+	-
55.	V 13777	*A.praecox*		Caceres	-	
56.	V 6344	*A.kuhlmannii*	AR	Caceres/Fz.santo Andre	-	
57.	V 13530	*A.kuhlmannii*	AR	Miranda (Rio Miranda)	-	
58.	V 13770 (V6410)	*A.kuhlmannii*	AR	Caceres (Aeroporto)	+	
59.	W 156	*A.retusa*	EX	Alto paraiso de Goias	+	
60.	SV 3042	*A.stenosperma*	AR	Guiratinga/E de V. Rico	-	
61.	V 7762	*A.stenosperma*	AR	Aranguaiana/Faz.Mocambo	-	-
62.	V 12646	*A.stenosperma*	AR	S.Antonio do Leverger	-	-
63.	V 13672	*A.stenosperma*	AR	General Cameiro	-	-
64.	V 13693	*A.stenosperma*	AR	Guiratinga/E de V.Rico	-	-
65	V 13796	*A.stenosperma*	AR	Araguaiana(Faz.B.Flor)	-	-
66.	V 7821(V12535)	A.macedoi	EX	Porto Alegre de Norte	+	
67.	V 13472	*A.macedoi*	EX	Combinado (?)	+	-
68.	V 13716	*A.simpsonii*	AR	Porto Esperidiao	-	-
69.	V 13728	*A.simpsonii*	AR	San matias	+	-
70.	V 13760	*A.magna*	AR	Vila Bela S.Trindade	+	-
71.	V 13748	*A.magna*	AR	Porto Esperidiao	-	-
72.	SV 3817	*Arachis* spp			+	-
73.	V 14042	*A.microsperma*	AR	Porto Murtinho	+	-
74.	V 13968	*A.archeri*	ER	Porto Murtinho	+	-

ER - Erectroides; TR - Triseminatae; AR - Arachis; HE - Heteranthae; PEQIA - Post Entry Quarantine Isolation Area
EX - EXtranervosae; CA - Caulorrhizae; PR - Procumbentes; QGH - Quarantine Green House
"+" and "-" signs indicate occurrence or non-occurrence of wilt. Blank boxes under the column "QGH" indicate that the corresponding accessions were not grown in the quarantine green house.

30°C and spread rapidly to other plants during March. All the plants in 36 of the 74 accessions were killed. The symptoms appeared as drooping of terminal leaves in some or all the branches followed by slight bending of the tips, drying and complete wilting and death of the plants.

This finding is in conformity with similar greenhouse studies where wilt severity was shown to be most pronounced under diurnal/nocturnal temprature regimes of 35/30°C and 30/25°C and slight or absent under the regimes of 25/20°C and 20/15°C (Susandiyals and Hayward, 1990). The temperature during March 1998 at ICRISAT was above 35°C which might have aggravated the wilt disease in the 36 accessions. The occurrence of bacterial wilt of groundnut in Andhra Pradesh has not been reported although sporadic reports from other parts of the country are available (Baleswar Singh and Hussain, 1991). Also, groundnut bacterial wilt was not observed in any of the 20154 accessions imported from 51 countries in PEQIA during the last 25 years i.e., until March 1998. Since no locally cultivated groundnut accessions are permitted to grow in the PEQIA, the source of inoculum could only be the imported seed. *Pseudomonas solanacearum* is reported to be transmitted through infected seed of groundnut (Machmud and Middleton, 1990). In the absence of the occurence of bacterial wilt caused by *P.solanacearum* in Brazil, the development of wilt in these wild *Arachis* spp indicates the possibility of perpetuation of the pathogen in "latent form" from one generation to the next under the prevailing conditions in Brazil. The seeds obtained from such symptomless-carrier accessions, when grown under favourable temperature conditions (35°C temperature), produced wilt.

The implication of this research is that all the foreign *Arachis* introductions originating from bacterial wilt infested areas or from areas where groundnuts are cultivated at temperatures less than 30°C, must be grown during the period when the temperature remains above 30°C. This will ensure expression of latent infection and will check its spread to new areas.

Keeping in view the quarantine importance of groundnut bacterial wilt in India (Harinath Naidu & Nirule, 1979) the following steps were taken to eliminate the bacterium from the infested area in PEQIA.

i The infected field was solarized during peak summer months in May 1998, and 1999.

ii In 1999, the field was sown with *Sesbania* sp. to suppress weed growth.

iii Groundnut will not be grown in the field for three successive years.

iv The seeds harvested from the surviving accessions in the wilt-affected plots would be grown under constant supervision before they are used for experimental purpose.

References

Baleswara Singh. and Hussain, S.M. 1991. Bacterial wilt of groundnut - a new record for India. *Indian Phytopathology*. **44:** 369-370.

Busolo - Bulafu, C.M. 1993. Present status of groundnut bacterial wilt research in Uganda. *In* : IBID, pp. 11-14

Demski, J.W. and Warwick, D. 1986. Testing peanut seeds for peanut stripe virus. *Peanut Science.* 13: 38-40.

Hamidah, S. and Lum, K.Y. 1993. Bacterial wilt in Malaysia. In: IBID, 6-10 pp.

Harinath Naidu, P. and Nirula, K.K. 1979. Quarantine important diseases of sorghum, pearlmillet, chickpea, pigeonpea and groundnut. *Indian Journal of Plant Protection.* 7: 178-188

Kelman, A. 1954. The relationship of pathogenicity of *Pseudomonas* solanacearum to colony appearance on a tet-

razolium medium. *Phytopathology.* 44: 693-695.

Machmud, M. 1993. Present status of groundnut bacterial wilt research in Indonesia. In: Groundnut bacterial wilt, Proceedings of the second working group meeting 2 Nov 1992. Asian Vegetable Research and Development Center, Taiwan (Mehan, V.K. and Hayward, A.C. eds.), pp. 15-24.

Machmud, M. and Middleton, K.J. 1990. Seed infection and transmission of *Pseudomonas solanacearum* on groundnut. Page 57 in Bacterial wilt of goundnut: *Proceedings of an ACIAR/ICRISAT Collaborative Research Planning Meeting*, 18-19 March 1990, Genting Highlands, Malaysia (Middleton, K.J., and Hayward, A.C., eds.).

Mehan, V.K., Liao, B.S., Tao, Y.J., Robinson-Smith, A., McDonald, D., and Hayward, A.C. 1994. *Bacterial wilt of groundnut.* Information Bulletin No.35. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Palm, B.T. 1922. Aanteekeningen over slijmziekte in *Arachis hypogaea* (Katjang tanah). (In Nl. Summary in En.) Inst. V. Plantenziekten (Dutch East Indies), Medid. 52.

Subandiayah, S., and Hayward, A.C. 1990. The influence of temperature regime on the interaction of some isolates of *Pseudomonas solanecearum* with peanut (*Arachis hypogaea L.*) *In:* Bacterial wilt of groundnut, proceedings of an ACIAR/CRISAT Collaborative Research Planning Meeting, 18-19 March 1990, Genting Highlands, Malaysia (*Middleton, K.J., and Hayward, A.C., eds.*), 56 pp.

Received :7-1-2001 Accepted : 15-6-2001