

Reference

Hong, N.X., Nghia, T., and Chinh, N.T. 1990. Main foliar diseases of groundnut in Vietnam and varieties' resistance. *In* Tropical information on leguminous plants. Information Centre, Ministry of Agriculture and Food Industry, Vietnam. 42 pp. (In Vietnamese with summaries in English).

Characterization of a Necrosis Strain of Peanut Stripe Virus Infecting Beggerweed and Groundnut in Georgia, USA

P. Sreenivasulu, J.W. Demski, C.W. Kuhn, and R.G. Christie (The University of Georgia College of Agriculture, Department of Plant Pathology, Georgia Experiment Station, Griffin, GA 30223, USA)

Beggerweed (*Desmodium canum*) is a leguminous weed common in southeastern USA. Edwardson et al. (1970) isolated a virus from this plant in Florida and designated it as desmodium mosaic virus (DMV). The virus has the properties of the potyvirus group. We isolated a virus from beggerweed with mosaic symptoms that was growing in a groundnut field in Tift county, Georgia, in 1985. Initial isolation was made to groundnut (*Arachis hypogaea* cv. Florunner) in the greenhouse by sap inoculation. The foliar symptoms of sap-inoculated groundnut mimicked the symptoms incited by the necrotic strain of peanut mottle virus (PMV-N) (Paguio and Kuhn 1973) and tomato spotted wilt virus (TSWV) (Halliwell and Philley 1974) at certain stages of disease development. In 1990 we isolated a virus from groundnut in Decatur county, Georgia with foliar necrosis that was indistinguishable from the beggerweed isolate based on serology, host range, and symptoms in groundnut.

The virus cultures were maintained in groundnut and white lupine (*Lupinus albus*). We made sap inoculations using the juice from infected leaves triturated in 0.05 M potassium phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol (PBM). The host range was determined by sap-inoculating 6 to 10 plants of each test species. The dilution end point (DEP), thermal inactivation point (TIP) and the longevity *in vitro* of the virus in sap extracted from groundnut with PBM were determined by using *Chenopodium amaranticolor* as a local lesion assay host. Aphid transmission was determined using populations of *Aphis craccivora* maintained on cowpea (*Vigna*

unquiculata Subsp. *unquiculata* cv California Blackeye) and *Myzus persicae* maintained on pepper (*Capsicum annuum*) in a growth chamber. The aphids were starved overnight, given a 1-min acquisition-access period on virus-infected detached groundnut leaves, and then transferred to healthy groundnut plants (1 aphid/plant) and given a 1-2 h inoculation-access period. Florunner groundnut seeds were planted on an experimental farm in Spalding county, Georgia and the seedlings in the center of five rows were mechanically inoculated at the fourth leaf stage to determine the effect on seed yield and seed transmission. The virus was purified from white lupine leaves and an antiserum produced essentially as described by Demski et al. (1984). Virus coat protein molecular weight was determined by the procedure of Laemmli (1970) and of virus nucleic acid by the procedure described by Vance and Beachy (1984). Viral inclusion bodies were examined in infected groundnut and white lupine epidermal tissues under a light microscope using tissue preparation and staining techniques of Christie and Edwardson (1986). Serological relationships with other potyviruses were determined using the indirect enzyme-linked immunosorbent assay (ELISA) as previously described by Hobbs et al. (1987). Symptoms induced in beggerweed take the form of a general mosaic of light and darker green areas characteristic of those induced by many potyviruses. In groundnut, the first few newly developed leaves showed chlorotic spots and necrotic patches, followed by drooping of whole leaves with downward rolling of leaf margins. Newly developed leaves were small and the whole plant appeared stunted when infected early.

The virus produced necrotic local lesions on *C. amaranticolor*; systemic symptoms on *Nicotiana benthamiana*, *N. clevelandii*, *Sesamum indicum*; and both local and systemic symptoms on *Canavalia ensiformis*, *Glycine max* cv Bragg, *Lupinus albus* and cowpea (cv California blackeye). *Gomphrena globosa* and *Petunia hybrida* were infected locally without visual symptoms. The following plant species neither showed symptoms nor was the virus recovered from them: *Capsicum annuum*, *Cucurbita pepo* cv melopepo, *Cucumis melo*, *Datura stramonium*, *G. max* cv Davis, *Lycopersicon esculentum*, *Medicago sativa*, *N. glutinosa*, *N. tabacum* cv Burley 21, *Phaseolus vulgaris* cvs Pinto and Topcrop, *Pisum sativum* cvs Little Marvel and Alaska, *Vinca rosea*, and cowpea cv Clay.

The DEP of the virus was between 10^{-4} to 10^{-5} , and the TIP between 50° to 55°C. The virus retained infectivity for 8 days but not for 10 days at 25°C.

Aphis craccivora transmitted the virus to 8 of 49 plants and *M. persicae* to 4 of 50 groundnut plants, in a nonpersistent manner.

Table 1. Reciprocal reaction of seven potyviruses with beggerweed isolate and peanut stripe virus (PStV) antisera in indirect enzyme-linked immunosorbent assay.

Antigens ¹	Antigen dilutions ²	Antisera ³		
		Beggerweed isolate	PStV	
BICMV	10 ⁻¹	1.10 ⁴	1.03	
	10 ⁻²	1.46	1.41	
	10 ⁻³	1.00	0.97	
PGMV	10 ⁻¹	0.43	0.40	
	10 ⁻²	0.70	0.72	
	10 ⁻³	0.98	0.87	
PMV	10 ⁻¹	0.02	0.00	
	10 ⁻²	0.07	0.03	
	10 ⁻³	0.09	0.01	
SMV	10 ⁻¹	1.08	0.78	
	10 ⁻²	1.16	0.96	
	10 ⁻³	0.75	0.51	
TEV	10 ⁻¹	0.23	0.19	
	10 ⁻²	0.29	0.26	
	10 ⁻³	0.38	0.35	
WMV 2	10 ⁻¹	0.44	0.36	
	10 ⁻²	0.48	0.51	
	10 ⁻³	0.77	0.76	
Sesame isolate	10 ⁻¹	0.59	0.57	
	10 ⁻²	0.59	0.50	
	10 ⁻³	0.51	0.48	
Virus control	10 ⁻¹	>2.00	1.61	
	10 ⁻²	>2.00	>2.00	
	10 ⁻³	>2.00	>2.00	
Healthy control				
	white lupine	10 ⁻¹	0.01	0.00
	pea	10 ⁻¹	0.00	0.00
	tobacco	10 ⁻¹	0.00	0.00
	soybean	10 ⁻¹	0.01	0.00
	cowpea	10 ⁻¹	0.01	0.00

1. BICMV = blackeye cowpea mosaic, PGMV = peanut green mosaic, PMV = peanut mottle, SMV = soybean mosaic, TEV = tobacco etch, WMV 2 = watermelon mosaic viruses, and a virus isolated from sesame.

2. Antigen leaf tissues extracted at 1 g tissue per 9 mL 0.05 M carbonate buffer + 0.01 M sodium diethyldithiocarbamate (antigen buffer) = 10⁻¹ dilution.

3. Crude antisera used at 1:1000 dilution.

4. Values (A₄₁₀) represent average of two replications read over antigen buffer controls.

The yield of six groups of 10 consecutive infected plants were 331, 382, 363, 329, 339, and 308 g compared to six groups of 10 consecutive healthy plants of 384, 356, 387, 396, 389 and 380 g with an average of 342 g for infected plants and 382 g for healthy plants.

From the 467 germinated seeds from infected parents, one seedling showed visible virus symptoms. This symptomatic plant, tested by ELISA, was positive for virus.

Purified virus was infective on groundnut and *C. araranticolor*. The ultraviolet absorption spectrum of purified virus had a shoulder at 290 nm and the A₂₆₀/A₂₈₀ ratio was 1.27 to 1.29. The A₂₆₀/A₂₄₅ ratio was 1.10 to 1.18. In indirect PTA-ELISA tests the antiserum produced against the purified virus had a titer of 1:60,000 and did not react with crude healthy leaf extracts. Serological reactions of some potyviruses against the antiserum of the beggerweed isolate and peanut stripe virus are given in Table 1.

The virus-protein preparations contained a major polypeptide of 34.5 × 10³ and a minor polypeptide of 31 × 10³ daltons. The virus nucleic acid, assumed to be RNA, migrated as a single band with an estimated molecular weight of 3 × 10⁶ daltons.

Cytoplasmic cylindrical inclusions were observed in epidermal peelings of groundnut and white lupine leaves under the light microscope.

The DMV from beggerweed in Florida is not available and thus could not be directly compared to our isolates from beggerweed and necrotic peanut. However the literature on host range and other characteristics indicate that DMV is a distinctly separate virus.

Comparisons of our beggerweed and necrotic peanut virus isolates with peanut stripe virus (PStV) shows that these viruses are closely related in nearly all aspects and we conclude that our isolates are symptom variants of PStV.

Isolates of PStV that induce necrosis in groundnut have been reported from Thailand (Wongkaew and Dollet 1990) and Taiwan (Chang et al. 1990). We now report a necrosis isolate of PStV naturally infecting groundnut and weeds in the USA.

Acknowledgment. This research was supported in part by State funds allocated to the Georgia Experiment Station, and in part by the Peanut CRSP, USAID Grant no. DAN-4048-G-SS-2065-00.

References

Chang, C.A., Purcifull, D.E., and Zettler, F.W. 1990. Comparison of two strains of peanut stripe virus in Taiwan. *Plant Disease* 74:593-596.

Christie, R.G., and Edwardson, J.R. 1986. Light microscopic techniques for detection of plant virus inclusions. *Plant Disease* 70:273-279.

Demski, J.W., Reddy, D.V.R., Sowell, G., Jr., and Bays, D. 1984. Peanut stripe virus - a new seed-borne potyvirus from China infecting groundnut (*Arachis hypogaea*). *Annals of Applied Biology* 105:495-501.

Edwardson, J.R., Purcifull, D.E., Zettler, F.W., Christie, R.G., and Christie, S.R. 1970. A virus isolated from *Desmodium canum*: characterization and electron microscopy. *Plant Disease Reporter* 54:161-164.

Halliwell, R.S., and Philley, G. 1974. Spotted wilt of peanut in Texas. *Plant Disease Reporter* 58:23-25.

Hobbs, H.A., Reddy, D.V.R., Rajeshwari, R., and Reddy, A.S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three plant viruses. *Plant Disease* 71:747-749.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature* 227:680-685.

Paguio, O.R., and Kuhn, C.W. 1973. Strains of peanut mottle virus. *Phytopathology* 73:976-980.

Vance, V.B., and Beach, R.N. 1984. Translation of soybean mosaic virus RNA in vitro: evidence of protein processing. *Virology* 132:271-281.

Wongkaew, S., and Dollet, M. 1990. Comparison of peanut stripe virus isolates using symptomatology on particular hosts and serology. *Oleagineux* 45:267-278.

Identification of Additional Groundnut Sources Resistant to Bacterial Wilt Under Field Conditions in East Java, Indonesia

D. Sharma and B. Soekarno [Malang Research Institute for Food Crops (MARIF) PO Box 66, Malang 65101, East Java, Indonesia]

In Indonesia, occurrence of bacterial wilt of groundnut (*Pseudomonas solanacearum*) is widespread in Sumatra, Java, Bali, Lombok, and Sulawesi. In the past, estimated

crop losses amounted to 25% to 90% (Machmud 1986). Release of the resistant variety Schwartz 21 in 1925 and its derivatives, including Gajah, since 1951 have markedly reduced crop losses due to bacterial wilt and at present the incidence in farmers' fields ranges from 0.8 to 10.1% with an average of 3.5% (Machmud 1986). However, the introduced exotic germplasm is often highly susceptible under field conditions. We introduced germplasm sources resistant to late leaf spot [*Phaeosariopsis personata* (Berk. & H.A. Curtis) Arx] and rust (*Puccinia arachidis* Speg.) from ICRISAT. The promising introduced lines were evaluated for bacterial wilt resistance under field conditions. The paper reports the results, which indicate the possibility of establishing additional sources of resistance to bacterial wilt and multiple resistance to late leaf spot, rust, and bacterial wilt.

A highly uniform naturally wilt-sick field was chosen at the Jambegede Experimental Station of Malang Research Institute for Food Crops (MARIF) in East Java. Three sets of groundnut genotypes introduced from ICRISAT were evaluated in the field during September-December 1988, April-July 1989, and September-January 1990-91. The first set consisted of 14 introduced genotypes and the local cultivar, Gajah. The second set included 50 introduced genotypes and surviving single plant progenies of the introduced genotypes and Gajah from the previous season. The third set consisted of 31 introduced genotypes and single plant progeny family bulks of promising entries from the previous two seasons. Initially test entries were planted in 2-m, 2- to 4-row plots at 40 × 10 cm spacing. The following year single-plant progenies or progeny bulks were planted in 3, 2, and 1 row nonreplicated plots depending on availability of seed. During 1990-91, a replicated trial of 39 entries consisting of 21 Gajah progeny bulks, 16 selected introduced genotypes, and Gajah and J 11, a susceptible cultivar, as the checks were planted in RBD with two replications in 3-row, 2-m length plots. Test entries were planted perpendicular to the susceptible cultivar, chico at the top and J 11 at the bottom of each 2-m broad block. Indicator rows of Gajah (resistant) and J 11 (susceptible) flanked the entries after every 8 or 12 rows. Initial germination was recorded at 15 days after planting. Percent survival at harvest is reported though observations were recorded every 2-3 weeks.

Nonreplicated Plot Observations

Uniformly low survival of the susceptible checks, chico and J 11 (Table 1) indicated uniformly high bacterial wilt incidence in the three seasons. Survival of the resistant