Reaction of *Arachis* Germplasm to Peanut Stripe, Peanut Mottle and Tomato Spotted Wilt Viruses[1]

R.D.V.J. Prasada Rao[2], G.P. Ribeiro[2], R. Pittman[3], D.V.R. Reddy[2], and J.W. Demski*[4]

ABSTRACT

Arachis spp. PI 262794, 262817, 421707, 468141, 468144, 468170, 468174, 468345, 468363, 468366, 468370 and 468371 were resistant to mechanical infection with peanut mottle (PMV), peanut stripe (PStV) and tomato spotted wilt viruses(TSWV). PI 262817, 421707 and 468363 (all in the section *Rhizomatosae*) did not show infection after graft inoculation with tissue singly infected with either PMV, PStV or TSWV. When three scions, each infected with one of the viruses, were grafted on the same plant, only PI 262817 remained virus free.

Key Words: Peanut viruses, virus resistance, groundnut

Peanut (*Arachis hypogaea* L.) is infected by numerous viruses (Sreenivasulu *et al.*, 1991; Reddy *et al.*, 1991) of which peanut mottle (PMV), peanut stripe (PStV) and tomato spotted wilt (TSWV) viruses are widespread and economically important (Kuhn and Demski, 1975; Demski and Lovell, 1985; Reddy *et al.*, 1991). Although various methods for managing these virus diseases have been proposed (Reddy, 1991), use of resistant cultivars appears to be the most practical method.

Resistance to PMV, PStV or TSWV has not been reported in *A. hypogaea*. However, lines without seed transmission (Nc Ac 17090, EC76446 (292) and Nc Ac 17133 (RF)) and lines with tolerance or field resistance (Nc Ac 2240, Nc Ac 2243) to PMV (Bharathan *et al.*, 1984; D.V.R. Reddy, unpublished), PStV (Kuhn *et al.*, 1978) and TSWV (Culbreath *et al.*, 1992) were reported. This tolerance or field resistance was based on no apparent effects on yield or on reduced incidence of infected plants. It was not based on plant susceptibility.

Resistance to PStV and PMV has been identified in some wild *Arachis* species. PMV resistance was identified in PI 468141, 468142, 468169, 468171, 468174, 468363, 468366 and 468371 (Melouk *et al.*, 1984) and also in 262794, 262817, 262818, 172223, 421707 and AM 3867 (Demski & Sowell, 1981). PStV resistance was identified in PI 276235, 468170, 468176, 476998, 476004, 476012 and 476013 (Prasada Rao *et al.*, 1991). Culver & Sherwood (1987) reported that many of the PMV resistant accessions (Melouk *et al.*, 1984) were also resistant to PStV. Resistance to TSWV has not been identified in any of the wild species. To determine if the wild *Arachis* species carrying resistance to PMV and PStV could also carry TSWV resistance, genotypes of section *Arachis* *Erectoides* and *Rhizomatosae* were selected for testing resistance to PStV, PMV and TSWV by mechanical and graft inoculation.

Materials and Methods

The taxonomy and origin of the *Arachis* germplasm lines used in this study are given in Table 1. The selected wild peanut entries were obtained from the USDA-ARS germplasm collections at Griffin, GA. PI 468150, 468154, 468159, 475998 and 497578 were obtained by seed and the remainder were maintained through vegetative propagation. Accessions PI 476004, 476012 and 497581 were obtained as seed from International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad, India.

PStV, PMV and TSWV isolates used for mechanical inoculations were maintained in *Lupinus albus* L., *Pisum sativum* cv. Little Marvel and *Nicotiana tabacum* L. cv. Burley-21 respectively. For the purpose of graft inoculation, all viruses were maintained in peanut cv. Florunner.

At least five test plants of each genotype were mechanically inoculated with extracts from young leaves showing typical symptoms of PStV, PMV or TSWV. Inoculum was prepared by grinding infected leaf tissues in chilled 0.025M potassium phosphate buffer pH 7.0 containing 0.01M sodium sulfite. Young leaves on each of the test plants were dusted with 400-mesh carborundum and inoculum was applied to the leaf surfaces with a cheesecloth pads. Each test plant was inoculated three times with a three day interval between each inoculation. Symptoms were recorded 3 weeks after final inoculation. Both inoculated and a subsequently formed leaflet from every plant were tested for the viruses by direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) (Hobbs *et al.*, 1987). Crude antisera of PStV, PMV and TSWV, cross absorbed with healthy plant extracts, were used at 1:5000, 1:5000 and 1:2500 dilution, respectively. Anti-rabbit IgG conjugated to alkaline phosphatase (Sigma No. A-8025) was used at a dilution of 1:5000. Extracts from healthy Florunner peanut leaves were used as negative controls and extracts from infected lupine, pea and tobacco leaves were used as positive controls for PStV, PMV and TSWV, respectively. Absorbance values (410 nm) of at least 10X those of comparable healthy controls were considered positive for the virus. Typically optical density readings for healthy controls ranged from 0.00 to 0.03; values ranging from 0.30 to 1.93 were regarded as positive for the virus.

At least five plants of each test entry were cleft grafted with scions from PStV-, PMV- and TSWV-infected Florunner peanut. Parafilm strips 1 cm wide were used to secure the stock and scion, and the grafted plants were covered for 5 to 7 days with polyethylene bags. A newly formed leaflet from each graft inoculated plant was assayed by DAC-ELISA.

Plants which were not infected by graft transmission with individual viruses were subsequently graft inoculated with all three viruses simultaneously. These plants were tested by ELISA for viruses at two weeks intervals (for two months) starting one month after graft inoculation.

Results and Discussion

Viruses infecting wild peanuts do not always induce overt symptoms so the presence of virus was determined by ELISA tests. The viruses were not detected in twelve accessions, five in the section *Arachis* [PI 468141 (*A. diogoi*), 468144 (*A. helodes*), 468345 (*Arachis* sp.), 468370 (*Arachis* sp.) and 468371 (*Arachis* sp.)]; one in the section *Erectoides* [PI 468170 (*Arachis* sp.)]; and six in the section *Rhizomatosae* [PI 262794 (*A. glabrata*), 262817 (*Arachis* sp.), 421707 (*A. glabrata*), 468174 (*Arachis* sp.), 468363 (*Arachis* sp.) and 468366 (*Arachis* sp.)] despite repeated sap inoculations (Table 2). In addition, by single graft inoculation (minimum of 5 plants) PI 262817, 421707 and 468363 were not infected with any of the three viruses. Some lines with a resistant reaction to sap inoculation were susceptible in graft

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[2]Visiting Scientist

[3]USDA-ARS

[4]Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA. 30223

*Corresponding author.

Table 1. *Arachis* germplasm lines tested for resistance to peanut stripe, peanut mottle and tomato spotted wilt viruses.

PI	Taxonomic section	Origin (collector)[a]
262794 (*Arachis glabrata* Benth.)	Rhizomatosae	Brazil (GKP 9815)
262817 (*Arachis* sp.)	Rhizomatosae	Paraguay (GKP 9570)
262839 (*A.* sp.)	Rhizomatosae	Paraguay (GKP 9642)
276235 (*A.* sp.)	Arachis	Paraguay (GKP 10601)
421707 (*A. glabrata* benth.)	Rhizomatosae	Selection from Fla
468141 (*A. diogoi* Hoehne)	Arachis	Brazil (GK 30001)
468144 (*A. helodes* Mart ex Hoehne)	Arachis	Brazil (GK 30029)
468150 (*A.* sp.)	Arachis	Brazil (GK 30006)
468152 (*A.* sp.)	Arachis	Brazil (GK 30008)
468154 (*A.* sp.)	Arachis	Brazil (GK 30011)
468159 (*A.* sp.)	Arachis	Brazil (GK 30017)
468170 (*A.* sp.)	Erectoides	Brazil (GKPSc 30126)
468171 (*A.* sp.)	Rhizomatosae	Brazil (GKPSc 30127)
468174 (*A.* sp.)	Rhizomatosae	Brazil (GKPSc 30131)
468345 (*A.* sp.)	Arachis	Bolivia (GKSSc 30102)
468363 (*A.* sp.)	Rhizomatosae	Paraguay (GKPSc 30116)
468366 (*A.* sp.)	Rhizomatosae	Paraguay (GKPSc 30119)
468370 (*A.* sp.)	Arachis	Paraguay (GKPSc 30124)
468371 (*A.* sp.)	Arachis	Paraguay (GKPSc 30125)
475998 (*A.* sp.)	Arachis	Bolivia (KSSc 36019)
476004 (*A.* sp.)	Erectoides	Bolivia (KSSc 36025)
476012 (*A.* sp.)	Arachis	Bolivia (KSSc 36033)
497578 (*A.* sp.)	Arachis	Brazil (VMoGeSv 7377)
497581 (*A.* sp.)	Arachis	Brazil (VSSv 7384)

[a]Collectors initials: G = Gregory, Ge = M.A.N. Gerin, K = A. Krapovickas, Mo = J.P. Moss, P = J. Pietratrelli, S = Simpson, Sc = A. Schinini, Sv = Glocimar P. de Silva, V = Jose F.M. Valls.

Table 2. Reaction of selected *Arachis* germplasm lines to mechanical inoculation with peanut stripe (PStV), peanut mottle (PMV) and tomato spotted wilt virus (TSWV).

PI	PStV			PMV			TSWV		
	No. plants tested	No. plants with symptoms	ELISA results[a]	No. plants tested	No. plants with symptoms	ELISA results	No. plants tested	No. plants with symptoms	ELISA results
262794	9	0	-	9	0	-	8	0	-
262817	10	0	-	9	0	-	9	0	-
262839	5	0	-	4	0	-	4	2	+
276235	4	0	-	4	0	-	4	3	+
421707	9	0	-	8	0	-	9	0	-
468141	7	0	-	8	0	-	7	0	-
468144	8	0	-	7	0	-	7	0	-
468150	7	2	+	6	0	-	6	3	+
468152	8	5	+	7	2	+	7	2	+
468154	5	3	+	5	1	+	5	4	+
468159	6	2	+	5	3	+	5	2	+
468170	10	0	-	10	0	-	10	0	-
468171	9	2	+	9	0	-	8	3	+
468174	10	0	-	10	0	-	9	0	-
468345	8	0	-	9	0	-	8	0	-
468363	8	0	-	7	0	-	7	0	-
468366	10	0	-	10	0	-	9	0	-
468370	10	0	-	10	0	-	10	0	-
468371	3	0	-	3	0	-	4	0	-
475998	4	0	-	4	0	-	5	2	+
476004	5	0	-	5	2	+	4	3	+
476012	5	0	-	4	1	+	4	2	+
497578	7	4	+	5	1	+	6	1	+
497581	6	2	+	6	3	+	5	4	+
Florunner	39	39	+	40	40	+	40	37	+

[a] - = negative ELISA test on all plants; + = positive ELISA test for plants with symptoms

Table 3. Reactions of selected *Arachis* germplasm lines to graft inoculations with peanut stripe (PStV), peanut mottle (PMV) and tomato spotted wilt viruses (TSWV).

PI	PStV			PMV			TSWV		
	No. plants grafted	No. plants with symptoms	ELISA results[a]	No. plants grafted	No. plants with symptoms	ELISA results	No. plants grafted	No. plants with symptoms	ELISA results
262794	7	0	-	6	0	-	5	4	+
262817	7	0	-	5	0	-	5	0	-
421707	7	0	-	6	0	-	6	0	-
468141	7	0	-	5	0	-	4	1	+
468144	8	0	-	6	1	+	6	2	+
468170	6	0	-	6	2	+	6	3	+
468345	4	0	-	5	1	+	7	3	+
468363	4	0	-	4	0	-	6	0	-
468366	8	0	-	7	0	-	7	1	+
468370	6	0	-	6	0	-	7	2	+
468371	5	0	-	5	0	-	4	2	+

[a] - = negative ELISA test on all plants; + = positive ELISA test for plants with symptoms

inoculation (Table 3).

Resistance previously observed in PI 468141, 468174, 468363, and 468366 for PStV (Culver *et al.*, 1987) and PMV (Melouk *et al.*, 1984) was confirmed in our tests and all but 468174 were also resistant to mechanical inoculation with TSWV. In addition to confirming the results reported by Demski and Sowell (1981) for PMV, we were able to show that PI 262794, 262817 and 421707 were also resistant to PStV and TSWV by mechanical and single graft inoculation. When a single plant of these three PI's was simultaneously graft inoculated with the viruses, PI 262817 was resistant to all three viruses but PI 262794 and 421707 were susceptible to TSWV.

The majority of the wild species having resistance to PStV, PMV and TSWV belong to section *Rhizomatosae*. It is known that the genotypes of the section *Rhizomatosae* are incompatible with *A. hypogaea* (Gregory and Gregory, 1979). Currently, techniques are not available for successful hybridization with *A. hypogaea*. For compatibility of diploid wild species in the section *Arachis*, techniques such as embryo rescue (Tallury *et al.*, 1992) or protoplast fusion (Harius, 1985) could facilitate their use in breeding programs. Protoplast fusion was successful in transforming virus resistance from wild species into cultivated tobacco and potato (Austin *et al.*, 1985; Bates, 1990; and Helgeson *et al.*, 1986). Recent studies in this laboratory detail an efficient method of regenerating protoplasts of *Arachis* (Li *et al.*, 1993). This should permit movement of genes within and between *Arachis* species to greatly expand the genetic diversity.

The peanut germplasm lines selected for this study were wild species earlier reported to be resistant to PStV or PMV (Culver *et al.*, 1987; Demski and Sowell, 1981; Melouk *et al.*, 1984; Prasada Rao *et al.*, 1981 and Reddy, *et al.*, 1983) in addition to some species which were promising against TSWV in field tests. Culver *et al.*, (1987) identified PStV resistance in 5 germplasm lines (PI 468141, 468142, 468174, 468363 and 468366), which were earlier shown to be resistant to PMV (Melouk *et al.*, 1984). The present study confirms the earlier findings except for PI 468142 which was not included in the test. In addition PI 468141, 468363 and 468366 were shown to be resistant to TSWV. PI 262817 was found to carry high degree of resistance to PStV, PMV and TSWV which was confirmed by grafting the scions of all 3 viruses on one plant.

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