In Vitro Culture to Eliminate Peanut Stripe Virus from Peanut Seed

R.D.V.J. Prasada Rao[2], G. Pio-Ribeiro[3], R. Pittman[4], D.V.R. Reddy[5] and J.W. Demski[4]*

ABSTRACT

When excised seed axes of peanut seed, which tested serological positive for peanut stripe virus (PStV), were grown *in vitro*, 37.5% of the developing plants were PStV-negative. When plants were regenerated from shoot-tips (1 cm long) taken from *in vitro*-grown, virus-infected plants, 29.4% of them were virus-negative. PStV-positive plants obtained from virus-infected seed were grown for 12 wk on Murashige and Skoog (MS) medium supplemented with 40 mg/L ribavirin (MSR) resulted in 78.6% which tested negative for PStV. All plants from seed which tested positive for PStV were 100% identified as negative if grown on MSR for 16 wk. Similarly all plants were PStV negative when obtained from 1 cm long infected shoot tips (from plants grown on MS or MSR medium) if subsequently grown for 12 wk on MSR medium.

Key Words: *Arachis hypogaea,* excised seed axes, shoot tip, ribavirin, virus-free.

One of the major risks of international exchange of peanut (*Arachis hypogaea* L.) germplasm is the introduction of seed-borne viruses. For safe movement of germplasm, techniques for the detection and elimination of seed-borne viruses are essential. Peanut mottle (PMV), peanut stripe (PStV), peanut stunt (PSV), and cucumber mosaic viruses (CMV) are transmitted through peanut seed (Reddy, 1991; Sreenivasulu *et al.*, 1991). PStV is considered to be the most important of these viruses because it may be found in as many as 30% of the seed from an infected plant in certain genotypes (Demski *et al.*, 1984; Prasada Rao *et al.*, 1989). Techniques have been developed for the detection of PStV in infected peanut seed (Demski and Warwick, 1986). However, methods for elimination of PStV from such seed have not been reported. Virus elimination from seed is critical in quarantine and germplasm collections where only a few seeds are available for release.

Meristem culture, thermotherapy, and chemotherapy have been used either separately or in various combinations to obtain virus-free plants in tissue culture (Hakkaart and Versluijs, 1988; Griffiths and Slack, 1990; Conci and Nome, 1991). Chen and Sherwood (1991) observed that a combination of shoot tip culture, thermotherapy, and chemotherapy eliminated PMV from peanut plants. Dunbar *et al.* (1993) also observed that PMV was eliminated from interspecific *Arachis* hybrids by meristem culture in combination with thermotherapy and chemotherapy. However, they found that PStV and tomato spotted wilt virus (TSWV) could be eliminated from interspecific hybrids by shoot tip culture alone. The objective of the present investigation was to evaluate shoot-tip cultures, obtained from in *vitro*-grown PStV-infected seed, either alone or following treatment with ribavirin, for the elimination of PStV.

Materials and Methods

Pods were collected from field-grown, Florunner peanut plants that were infected with PStV early in the season. After drying for 2 wk, the seeds were individually tested by direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) (Demski and Warwick, 1986; Hobbs *et al.*, 1987). Approximately 20 mg of cotyledonary tissue located opposite the radicle was pulverized in 1 mL of 0.05 M sodium carbonate buffer at pH 9.6. The PStV antiserum was cross-adsorbed with extracts from healthy cotyledonary tissue (50 g/L), and employed at a dilution of 1:5000. Anti-rabbit IgG conjugated to alkaline phosphatase enzyme (Sigma No. 8025) was used at a dilution of 1:5000. *p*-Nitrophenyl phosphate was used at 0.25 mg/mL and incubated for 30 min at room temperature. The reaction was stopped by adding 50 mL of 3 M NaOH per well. Absorbencies were recorded at 405 nm using a Dynatek Mini Reader II (Dynatech Laboratory, Alexandria, VA).

Excised seed axes from PStV-infected peanut seeds were washed in 70% ethanol for approximately 1 min and then

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[2]Visiting Scientist, NBPGR campus, Rajendranagar, Hyderabad, India.
[3]Visiting Scientist, Univ. of Pernambuco, Recife, Brazil.
[4]Peanut Curator, USDA-ARS and Res. Leader, Dept. of Plant Pathology, Univ. of Georgia, Georgia Stn., Griffin, GA. 30223.
[5]Visiting Scientist, ICRISAT, Patancheru-P.O., India.
*Corresponding author.

soaked for 3 min in 1% sodium hypochlorite. The axes were rinsed five times with sterile water and placed on a medium containing MS salts (Murashige and Skoog, 1962), B_5 vitamins (Gamborg *et al.*, 1968), 30 g/L sucrose, adjusted to pH 5.8, solidified with 8 g/L agar, and autoclaved for 15 min at 120 C in 25 x 150-mm culture tubes (15 mL medium per tube). The medium was cooled before adding filter-sterilized ribavirin (1,2,4-triazole 3-carboxamole) (Viratek, Costa Mesa, CA) to a final concentration of 40 mg/L. Controls were cultured on MS medium without ribavirin. Cultures were incubated at 25 C with 16-hr photoperiod at 100 mE/m^2/sec provided by cool white fluorescent lamps.

Thirty-five seed axes were cultured on MS medium and another 35 on MS medium supplemented with ribavirin (MSR). After 4 wk each axis produced one to three shoots. Each shoot was assayed for PStV. Shoot tips (1 cm long) grown on MS medium containing PStV antigens were subdivided and subcultured on MS or MSR. Similarly, shoot tips grown on MSR containing PStV antigens were subdivided and cultured on MS and MSR. Tissues were assayed for virus after 16 wk.

Results and Discussion

Of 973 peanut seeds harvested from early infected plants, only 71 (7.3%) were positive for PStV in ELISA tests. When seed axes from virus-infected seed were cultured on MS and MSR media, 37.5 and 56.7% of the resulting seedlings, respectively, were identified as free of PStV antigens (Table 1). Previous research demonstrated a high correlation between PStV in cotyledons and transmission to seedlings in grow-out tests (Demski and Warwick, 1986). Because some of the seed axes excised from cotyledons that were PStV-positive by ELISA gave rise to healthy seedlings, it is assumed that virus was not present in some seed axes although the viral antigens were detected in cotyledons. However, this assumption requires confirmation through experiments aimed at inducing shoots by tissue culture techniques from seed axes and cotyledons of seed having PStV antigens in cotyledons.

Seed axes which gave rise to plants containing PStV after 4 wk were subsequently transferred to MS medium and maintained for 12 wk. All plants were positive for PStV in ELISA (Table 2). However, 29.4% of plants regenerated on MS medium from 1-cm long shoot tips were negative for PStV in ELISA. Dunbar *et al.* (1993) reported that every plant regenerated from 1 cm long shoot tips having high temperature pretreatment were free from PStV. Without high temperature pretreatment we detected only 29.4% of regenerated plants were identified as negative for PStV. Shoot tips derived from PStV-infected plants grown on MS medium, when subsequently grown for 12 wk on MSR medium, resulted in 78.6% plants identified as negative for PStV. Similarly, shoot tips derived from PStV-infected plants grown on MSR medium, when subsequently grown on MS and MSR media, 36.4 and 100%, respectively, were negative in ELISA for PStV. PStV plants grown continously for 16 wk on MSR medium were negative for PStV.

Ribavirin in culture media at concentrations higher than 20 mg/L was toxic to shoot tips of *A. hypogaea* (Chen *et al.*, 1990). There was no significant affect on growth and shoot regeneration in the plants grown in ribavirin-supplemented medium at the concentrations used. Similar results were reported also by Dunbar *et al.* (1993) when interspecific *Arachis* hybrids were tested in medium containing ribavirin. The medium worked well with cv. Florunner. Dunbar *et al.* (1993) have shown that five interspecific hybrids performed well under similar conditions used in this study. Therefore we presume that results reported will be applicable for other peanut genotypes.

Our results demonstrate that utilizing seed axes and shoot tip culture along with the incorporation of ribavirin in the growth medium, virus-free plants can be produced from seed that were PStV-infected. The use of this procedure can prevent contaminated seed from being exchanged.

Table 1. Enzyme-linked immunosorbent assays on *in vitro* plants grown from seed axes obtained from peanut seed cotyledons containing peanut stripe virus (PStV) antigens.

Tissue type	MS medium	MS medium supplemented with ribavirin
No. seed axes grown	35	35
No. seed axes germinated[a]	32	30
% Plants negative for PStV[a,b]	38	57

[a]Four weeks after germination.
[b]At ELISA detectable levels.

Table 2. Results of enzyme-linked immunosorbent assays (ELISA) after 16 wk on PStV-positive seedlings subjected to shoot tip culture and ribavirin treatment.

Medium for seed axes	Seed axes grown	PStV+ plants after 4 wk[a]	Shoot tips grown	ELISA after 16 wk[b] PStV-	ELISA after 16 wk[b] PStV+	PStV- plants
	no.					%
MS[c]	35	20	—	0	9	0.0
MS			19	5	12	29.4
MSR			19	11	3	78.6
MSR[d]	35	13	—	7	0	100.0
MS			12	4	7	36.4
MSR			13	12	0	100.0

[a]Plants containing PStV antigens (detected at 4th week), after removing shoot tips, grown for 12 wk on same medium.
[b]Fungal/bacterial contaminated plants or shoot tips were not tested and hence the missing values.
[c]MS = Murashige and Skoog growth medium.
[d]MSR = Murashige and Skoog growth medium plus ribavirin.

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