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BRIEF COMMUNICATION

Meristem culture of interspecific hybrids of groundnut

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Abstract

The shoot apices of ten groundnut interspecific hybrids, showing peanut stripe virus symptoms were used as explants. Murashige and Skoog (MS) medium containing 1 mg dm^{-3} each of naphthaleneacetic acid (NAA) and benzylaminopurine (BAP) supported maximum callusing of the hybrids J 11 × *Arachis* sp. PI 30008 as well as J 11 × *A.* sp. Manfredi # 5 (86.3 and 82.6 %, respectively). On MS medium with 3.5 mg dm^{-3} NAA and 0.2 mg dm^{-3} BAP, the meristems of J 11 × *A. stenosperma* as well as J 11 × *A. otavioi* gave good response (79.7 and 77.2 %). The regeneration frequencies ranged from 16.7 to 76.9 % and was more than 30 % in all hybrids except for J 11 × PI 30085. Shoots (5 cm) regenerated from the virus-free (ELISA tested) calli rooted well (60 - 80 %) on MS basal medium supplemented with 0.4 % activated charcoal and 200 mg dm^{-3} casein hydrolysate. In addition, long shoots which failed to produce roots, when transferred to soil during rainy season and protected from direct sun got established.

Additional key words: *Arachis hypogaea*, growth regulators, peanut stripe virus.

Wild relatives of groundnut (*Arachis hypogaea* L.) are bestowed with several desirable traits which can be transferred to the cultivated groundnuts by interspecific hybridization (Stalker and Beute 1993). The interspecific hybrids should be

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Abbreviations: BAP - benzylaminopurine; DAS-ELISA - double antibody sandwich - enzyme-linked immunosorbent assay, NAA - naphthaleneacetic acid; PStV - peanut stripe virus.
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maintained free from pests and diseases. PStV is one of the major pathogens affecting the productivity of groundnut in South East Asian countries, especially China. Recently this disease was reported from India, too. The seed transmission rate of PStV is up to 37 % (Demski *et al.* 1984).

Meristem culture finds a special application in the regeneration of virus-free material. Chen and Sherwood (1990) obtained peanut mottle virus-free plantlets by a combination of meristem culture, thermotherapy, and chemotherapy. This experiment was taken up to standardize a protocol for the meristem culture of interspecific hybrids to eliminate the systemic infection of PStV from invaluable interspecific hybrids for their further utilization.

Ten field-grown interspecific hybrids (Table 1) showed symptoms of PStV on their foliage. The leaflets collected from these interspecific hybrids were tested by DAS-ELISA. Apical (shoot tip) meristems collected from the PStV infected interspecific hybrids were used as explants. The shoot apices were surface-sterilized by 70 % ethanol for 30 s followed by 0.1 % $HgCl_2$ for 2 min. The apical domes were dissected out under a zoom stereo microscope aseptically and 30 explants each were cultured on MS medium with vitamins of B5 containing either 1 mg dm^{-3} each of NAA and BAP or 3.5 mg dm^{-3} NAA and 0.2 mg dm^{-3} BAP. The cultures were incubated at 26 ± 1 °C and 16-h photoperiod. The initial observations of callusing were taken after 2 weeks in culture. Representative portions of these calli were tested by DAS-ELISA for the presence of PStV (Table 1). All the PStV positive calli were discarded. The PStV negative calli were further multiplied by subculturing until they regenerated. The meristems/calli were recultured at an interval of 28 d in jam bottles so as to have maximum amount of the callus and the non-regenerating calli were maintained for 120 d in culture. When the shoots attained a height of 5 cm, were transferred into two sets of MS basal medium, one supplemented with 0.4 % activated charcoal and 200 mg dm^{-3} casein hydrolysate, and the other with 3 mg dm^{-3} NAA and 0.5 mg dm^{-3} BAP for rooting.

Table 1. Selection of calli by DAS-ELISA for regeneration.

Hybrid	Calli tested	Calli positive
J 11 × *A. villosa*	60	16
J 11 × KSSC 36025-1	90	60
J 11 × PI 30085	48	11
J 11 × PI 30008	37	6
J 11 × *A.* sp. Manfredi #5	88	10
J 11 × *A. helodes*	108	23
J 11 × *A. stenosperma*	141	27
J 11 × *A. otavioi*	128	29
J 11 × *A. khulmanii*	230	41
TMV 2 × *A. chacoense*	120	26

Apical meristems from all the hybrids responded *in vitro* by producing calli in both the growth regulator combinations at varying frequencies (Table 2). The maximum callusing was obtained from the meristem of the hybrid J 11 × *A.* sp. PI 30008 (86.3 %) followed by J 11 × *A.* sp. Manfredi # 5 (82.6 %) in the medium containing 1 mg dm^{-3} each of NAA and BAP. On the other hand, the meristems of J 11 × *A. stenosperma* responded the maximum (79.7 %) in MS with 3.5 mg dm^{-3} NAA and 0.2 mg dm^{-3} BAP. The hybrid of J 11 × *A. otavioi* was responding in both the media almost equally.

Among the samples of calli tested, in average 24.6 % turned out to be PStV positive in ELISA (Table 1). These calli were discarded as they may pass on the infection to the regenerating plantlets also. Among the ten hybrids cultured, six regenerated giving rise to shoots (Table 2). The regeneration frequencies ranged from 16.7 to 76.9 % and was more than 30 % in all the hybrids except for J 11 × PI 30085. The apical meristem from the hybrid J 11 × *A. villosa* regenerated in almost equal frequencies in both the combinations of the medium whereas J 11 × KSSC 36025-1 failed to regenerate in the medium containing 1 mg dm^{-3} each of NAA and BAP. Similarly, the meristems from the hybrid J 11 × PI 30008 and J 11 × *A.* sp. Manfredi # 5 failed to form shoots in the medium containing 3.5 mg dm^{-3} NAA and 0.2 mg dm^{-3} BAP (Table 2).

Table 2. The frequency of callusing and regeneration *in vitro* of nine interspecific hybrids of groundnut as affected by concentration of growth regulators [mg dm^{-3}].

Hybrid	1.0 NAA + 1.0 BAP		3.5 NAA + 0.2 BAP	
	callus [%]	shoot [%]	callus [%]	shoot [%]
J 11 × *A. villosa*	72.9	33.0	25.0	30.0
J 11 × KSSC 36025-1	80.0	-	62.5	50.0
J 11 × PI 30085	64.3	16.7	70.0	76.9
J 11 × PI 30008	86.3	57.1	58.3	-
J 11 × *A.* sp. Manfredi #5	82.6	42.9	55.6	-
J 11 × *A. helodes*	47.2	-	16.7	-
J 11 × *A. stenosperma*	71.9	-	79.7	-
J 11 × *A. otavioi*	82.2	53.2	77.2	66.7
J 11 × *A. khulmanii*	31.1	-	44.4	-
TMV 2 × *A. chacoense*	19.0	-	32.5	-

Roots were observed on 60 - 80 % of the shoots cultured on MS medium containing 0.4 % activated charcoal and 200 mg dm^{-3} casein hydrolysate. On MS medium containing 3 mg dm^{-3} NAA and 0.5 mg dm^{-3} BAP rooting was only sporadic and basal portions of shoots started callusing. When the regenerated shoots were allowed to grow in the regeneration medium itself for long time, the shoots elongated and occasionally rooted. However, long shoots which failed to produce roots in the rooting media, when transferred to soil during rainy season and protected from direct sun by shading, got established in the soil.

In the USA, Dunbar *et al.* (1993) for the first time successfully eliminated tomato spotted wilt virus and PStV in five interspecific hybrids by meristem culture as well as shoot tip culture methods coupled with chemo- and thermotherapy. This is the first report on elimination of PStV from the infected precious groundnut interspecific hybrids through meristem culture. Thus, the ten hybrids which were showing symptoms of PStV infection were cleared of the systemic infection and made available for their further use in the breeding programs. Considering the high rate of seed transmission of the virus (Demski *et al.* 1984), the protocol developed would be highly useful for rescue of valuable material.

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