## IN VITRO PLANTLET REGENERATION FROM EMBRYOS OF INTER-SPECIFIC HYBRIDS OF CAPE GOOSEBERRY (PHYSALIS PERUVIANA L.)

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Hybrids were successfully obtained from crosses involving *Physalis minima* L. and *Physalis peruviana* L. by culturing the excised embroys *in vitro* after 20-25 days from pollination on MS medium supplemented with Kinetin (0.2 mg/1) and  $\alpha$ -naphthalene acetic acid (0.1 mg/l). The crossed fruits possessed same colour as that of wild male parent but with a varied intensity. The regenerated plantlets were transferred to pots and watered with nutrient solution. Sixty four per cent of the transferred plantlets established well.

Key words: Cape gooseberry, Physalis peruviana, in vitro hybrids

Cape-gooseberry (Physalis peruviana L. syn. P. edulis), native of Peru and Chile is recent introduction in India. The fruits are delicious, rich source of carotene, vitamin C, pectins and minerals (phosphorus and iron) and seeds have abundance of flavourless oil (Mazumdar, 1979). Ripe berries are also used in preparation of enriched meal and curries. Being an annual crop, cape gooseberry can be grown as intercrop in the orchards. This crop is highly susceptible to leaf curl mosaic virus (LCMV) having more than 60 per cent of disease incidence (Pal et al., 1993) and no resistant source to LCMV among the existing cultivated species is available. During a survey, it was observed that the wild type Bhambola (Physalis minima L.), characterized by fruits with purple patches, possesses resistance against the LCMV. The LCMV free free material is reported to increase the yield by 26.7 per cent (Pal et al., 1993). Hence, the present attempt was made to introgress the gene(s) for resistance against LCMV into the local cultivated variety of Cape gooseberry using embryo rescue technique. Embryo culture has been used to rescue the hybrid embryos in a number of plant species which are otherwise incompatible (Khush and Brar, 1986).

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## MATERIALS AND METHODS

Interspecific hybridization was carried out by using wild species, *Physalis minima* as male parent and cultivated *P. peruviana* as female parent. Crosses were made during October-November by emasculating the flower buds; which were about to bloom within 2-3 days. The emasculated pistils were pollinated by rubbing pollen on receptive sticky stigma using camel hair brush. The pollinated pistils were bagged thereafter for a week. The resultant fruits from the crosses were harvested after 20-25 days from pollination and the embryos were excised aseptically. The F<sub>1</sub> embryos were then cultured on MS<sub>1</sub>, MS<sub>2</sub> and MS<sub>3</sub> medium (Table 1) having nutrient salts of MS medium (Murashige and Skoog, 1962). The culturing was performed under aseptic conditions in a laminar-flow cabinet and the cultures were incubated at 25 ±2°C and 3000 Lux light intensity. The regenerated plantlets were washed with water to remove *agar agar* and transferred to soil.

Table 1: In vitro regeneration of cultured hybrid embryos of cape gooseberry

Medium		Embryos cultured (No.)	Regeneration (%)	Survival in pots (%)	Remarks
MS <sub>1</sub>	NAA (0.1 mg/l) + Kin. (0.2 mg/l)	126	92.8		Normal rooting and shooting
MS <sub>2</sub>	NAA (1.0 mg/l)	50	70.0	0.0	Embryo derived shoots showed callusing at their base and rooting therefrom
MS <sub>3</sub>	Kin. (0.5 mg/l)	50	70.2	0.0	No rooting of shoots, some callusing

## **RESULTS AND DISCUSSION**

Interspecific hybrids were successfully obtained involving wild *P. minima* and cultivated *P. peruviana*. No fruit setting was observed in the emasculated and unpollinated flowers indicating that pollination is prerequisite for fruit setting in gooseberry. The open-pollinated flowers showed higher fruit setting (77.0%) as compared to hand cross pollinated (26.8%). Similar observations pertaining to higher fruit set in open pollinated flowers as compared to hand cross pollinated flowers has been made in *Citrus* (Singh and Joomer, 1949; Raman, 1990). It was interesting to note that crossed fruits possessed the metaxenia effect but with varying magnitude. Some fruits were dark purple to blackish, while the others had a mosaic pattern of purple black with greenish tinge. No such pattern on fruits was observed from open pollinated flowers.

Upon culturing the  $F_1$  embryos, green and healthy plantlets were regenerated *in vitro*. The radical began to emerge within 3-5 days after culturing followed by opening of green and/or partly etiolated cotyledons. The primary leaves were initiated after 10-12 days followed by elongation of epicotyl. The immature embryos excised from younger fruits (20-25 DAP) showed lower regeneration (22%) as compared to mature fruits (99 %), 45-50 days after planting.

Among the different media tried (Table 1), MS<sub>1</sub> was found to be the best for high plantlet regeneration (92.8%). The regenerated plantlets had normal shoot and roots capable of supporting further plant growth (Fig. 1A).

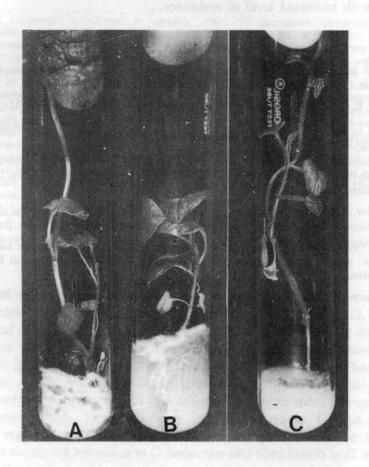


Fig. 1.A-C. In-vitro culture of F1 hybrid embryos

- (A) Showing normal plantlet regeneration on MS medium supplemented with NAA (0.1 mg/l) and Kin (0.2 mg/l)
- (B) Showing extensive rooting but from callused regions on MS medium supplemented with NAA (1 mg/l)
- (C) In-vitro raised shoot from cultured embryo showing callusing but no rooting on MS medium supplemented with Kin (0.5 mg/l)

These plantlets showed the maximum survival (64.2%) under field conditions. Though shoot regeneration frequency was also high on  $MS_2$  and  $MS_3$  but these media did not support a root system capable of successful transfer to field. It was seen that on  $MS_2$  medium, there was extensive rooting but from the callusing area of the shoot (Fig. 1B). On  $MS_3$  medium, the embryo derived shoot did not root, but some callusing was observed at the base of shoot (Fig. 1C). This may be due to higher level of auxin-cytokinin ratio. The embryo derived plants ( $F_1$ ) have been transferred to the field and are being further evaluated. The screening of hybrids for LCMV and other desirable traits alongwith backrossing programme is underway to develop better cape gooseberry types with increased level of resistance.

## **REFERENCES**

- Khush, G. S. and D. S. Brar. 1986. Wide hybridization and chromosomal manipulations in cereals. *In*: Handbook of Plant Cell, Tissue and Organ Culture, Vol. VI.
- Mazumdar, B. C. 1979. Cape gooseberry The jam fruit of India. World Crops 31: 19-23.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15**: 437-479.
- Pal, Barjinder. 1991. Studies on adaptation of cape-goosberry (*Physalis peruviana* L.) under Punjab conditions. A Ph. D. Dissertation submited to Punjab Agricultural University, Ludhiana (India).
- Pal, Barjinder, A. S. Bindra and H. Raman. 1993. Morphological and biochemical alternations associated with leaf curl mosaic virus in cape-gooseberry. *Plant Dis. Res.* (In press).
- Raman, H. 1990. Somaclonal variation and distant hybridization for canker resistance in Citrus species. Ph. D. dissertation submitted to Punjab Agricultural University, Ludhiana.
- Singh, S. N. and B. S. Joomer. 1949. Bearing habit of Kagzi lime. Indian Fmg 10: 532-540.