ABA-Induced Growth Retardation of Kiwifruit (*Actinidia chinensis*) *In Vitro*

Sandhya Gupta
National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012

Key words: Abscisic acid (ABA), *Actinidia chinensis*, *In vitro* conservation, Kiwifruit, Slow growth

Vegetatively propagated crops cannot be conserved as seeds as they are either sterile or are heterozygous. Various biotechnological approaches such as cryopreservation, minimal growth maintenance in *in vitro* and storage of DNA as genomic or DNA libraries (Rao and Riley, 1994) have been suggested for the conservation of such species. However, to date most emphasis has been given on the development and use of *in vitro* minimal growth storage techniques. These techniques are currently being utilized for conservation of various clonally propagated crops and their related species (Ashmore, 1997; Withers and Engelmann, 1997; Reed, 1999). Despite the current applications of minimal growth maintenance strategies, data relative to effectiveness of specific growth retardant is lacking in the scientific literature (Jarret and Gawel, 1991). This paper describes ABA-induced inhibition of shoot growth of *Actinidia chinensis* (kiwifruit or Chinese gooseberry) *in vitro*, and discusses the potential use of ABA to enhance minimal growth for conservation of kiwifruit germplasm.

In vitro cultures of *Actinidia chinensis* cv. Allison (female), procured from Dr YS Parmar University of Horticulture and Forestry, Solan, were multiplied and used for investigation. Nodal segments used as explants were excised from 3-week- old stock cultures maintained on Murashige and Skoog's (1962) medium (MS) containing 3% (w/v) sucrose and solidified with agar (0.8% w/v). Cultures were raised in test tube (25 x 150 mm) containing 10-12 ml of MS supplemented with various concentrations of ABA (SRL) at 0.0, 0.01, 0.1, 1.0 or 10.0 mg/l. MS medium without ABA was treated as control. Twenty-four cultures were raised/treatment. Cultures were incubated at 25±2°C under 8 h photoperiod. About 40 m mol/m^2s^{-1} illumination was provided by 40 watt cool-white fluorescent tubelights (Phillips, India) adjusted 30 cm high from culture tubes. Cultures were maintained in similar culture condition up to 180 days. After 90 and 180 days cultures were subcultured/transferred to ABA-free MS medium. The quantitative data were recorded periodically. Values represent the mean of 24 cultures for each treatment. Statistical Error (SE) was calculated on shoot length using the standard software. Viable nodes were defined as those from which visible shoot development could be observed after 30 days of transfer to MS medium.

ABA inhibited shoot development from axillary buds of kiwifruit at all concentration tested. No significant reduction in growth parameters was observed up to 30 days after culture initiation on low concentrations of ABA (0.01-1 mg/l). The 90-days-old cultures showed significant reduction in shoot length on 0.01-1 mg/l ABA-supplemented medium. The mean values of shoot length at 0.01, 0.1, 1.0 mg/l ABA were 1.4±0.1, 0.7±0.0 and 0.3±0.0, respectively, which are significantly lower than the mean shoot length in control (2.7±0.1). However, ABA at these concentrations did not inhibited number of shoots/culture. Similar trend in shoot growth reduction was observed in 180-day-old cultures also. Effects on growth inhibition by ABA has already been reported *in vitro* in *Solanum tuberosum* (Henshaw and O'Hara, 1983a,b; Westcott *et al.*, 1977), *Ipomea batatas* (Jarret and Gawel, 1991) and *Malus domestica* (Wilkins *et al.* 1988). No shoot development was observed from nodes cultured on media containing 10 mg/l ABA after 90 days of culture initiation. However, nodal segments on this treatment remained green and some swelling of the axillary bud was evident. Although no shoot elongation occurred but viability of nodal segments was about 80% after 90 days from culture. Shoot regeneration from nodes, cultured in MS+10 mg/l ABA for 90 and 180 days, were not significantly different in length as compared to control. Jarret and Gawel (1991) reported that ABA at 10 mg/l completely inhibited axillary shoot development but did not affect the viability of *in vitro* cultures of sweet potato cv. Jewel over a culture period of 365 days. Viability, as determined by the ability to recover shoots from the recultured nodes on MS, exceeded 70% for all the treatments after 180 days from culture. Normal plantlets were recovered after transfer of nodes to MS medium.

Results of present investigation indicate that ABA (1-10 mg/l) effectively inhibits growth of kiwifruit *in*

vitro and that this effect does not diminish over a 6 months culture period. The study suggests use of ABA to increase *in vitro* conservation period of kiwifruit.

Acknowledgement: The author is thankful to Dr DR Sharma, Dean, Horticulture, Dr YS Parmar University of Horticulture and Forestry, for providing cultures of kiwifruit. Financial support from Department of Biotechnology, Government of India, is gratefully acknowledged. Technical assistance of Ramesh Chandra from NBPGR is also acknowledged.

References

Ashmore SE (1997) Status Report on the development and application of *in vitro* techniques for the conservation and use of plant genetic resources. International Plant genetic Resources Institute, Rome, Italy, 67 p.

Henshaw GG and JF O'Hara (1983a) Tissue culture methods for the storage utilization of potato germplasm In: DS Ingram and JP Helgeson (ed.) *Tissue Culture for Plant Pathologist.* Blackwell Scientific Publications, Oxford, pp 71-76.

Henshaw GG and JF O'Hara (1983b) *In vitro* approaches to the conservation and utilization of global plant genetic resources. **In:** SH Mantell and H Smith (ed.) *Plant Biotechnology.* Cambridge University Press, Cambridge. pp 219-238.

Jarret R L and N Gawel (1991) Abscisic acid-induced growth inhibition of sweet potato (*Ipomoea batatas* L.) *in vitro. Plant Cell Tiss. Org. Cult.* **24**:13-18.

Murashige T and F Skoog (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.* **15**:473-497.

Rao VR and KW Riley (1994) The use of biotechnology for conservation and utilization of plant genetic resources. *Plant Gen. Res. Newslett.* **97**: 3-20.

Reed BM (1999) *In vitro* storage conditions for mint germplasm. *HortScience.* **34**:350-352.

Westcott R, GG Henshaw and WM Roca (1977) Tissue culture storage of potato germplasm: culture initiation and plant regeneration. *Plant Sci. Lett.* **9**:309-315.

Wilkins CP, HJ Newbury and JH Dodds (1988) Tissue culture conservation of fruit trees. *FAO/IBPGR Plant Gen. Res. Newslett.* **73/74**: 9-20.

Withers LA and F Engelmann (1997) *In vitro* conservation of plant genetic resources. **In:** A Altman (ed.) *Biotechnology in Agriculture.* Marcel Dekker Inc., New York, pp 57-88.

In Vitro Conservation and Encapsulation of *Coleus forskohlii*

Rajasri Bhattacharyya, Bidhisha Mondal and Sabita Bhattacharya
Department of Botany, Bose Research Institute, Calcutta, West Bengal

Key Words: Conservation, *Coleus*, Tissue culture, Encapsulation

Coleus forskohlii Briq. (Lamiaceae) is an important plant in Indian Ayurvedic medicine because its roots produce a labdane diterpenoid, forskolin (Bhat *et al.*, 1977) which has a positive intropic effect on heart action, lowers blood and intra ocular pressure and is an anti-inflammatory (Mukherjee *et al.*, 1996). Forskolin is commonly purified from tuberous roots of wild or cultivated *C. forskohlii* plants. Indiscriminate collection of *C. forskohlii* has led to rapid depletion of wild populations listing it as vulnerable plant in India. Therefore, the conservation of such rare and endangered plant species has become imperative. *C. forskohlii* is mainly propagated vegetatively to maintain clonal genotype. At present, the most common method to preserve the genetic resources of vegetatively propagated plant is, as whole plant in the field. But there are several serious limitations with field genebanks *(Withers and Engels, 1990)* mainly due to attacks by pests and pathogens, exposure to natural disasters, *etc.* In addition distribution and exchange from field genebank is difficult because of the vegetative nature of the material and the greater risks of disease transfer.

The present work aims at detecting the most suitable way of conservation and recovery of true-to-type elite *Coleus forskohlii* plant through the use of *in vitro* culture and encapsulation technique.

Shoot tip (0.5 cm) and nodal explants were collected from micropropagated *C. forskohlii* plants maintained in laboratory condition. Murashige and Skoog (1962) medium (MS) was used as basal medium. Explants were cultured in 25 x 150 mm culture tubes (single explant/ tube) containing 15 ml of semisolid 1/4 MS, 1/2 MS 2/3 ms (where concentration of the macro and micro salts were 1/4, 1/2, 2/3, respectively of the original concentration of MS) and MS medium supplemented