Journal of Tropical Agriculture 49 (1-2) : 118-120, 2011

Short communication

In vitro clonal shoot morphogenesis of commercial *Dendrobium* orchid cultivars in polyamines supplemented medium

I. Priya Kumari* and T. Sabina George

Department of Pomology and Floriculture, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram 695 522, Kerala, India.

Received 8 June 2011; received in revised form 23 August 2011; accepted 7 September 2011.

Abstract

The effects of polyamines, spermine and spermidine in enhancing shoot morphogenesis were evaluated at 0.25, 0.5, and 1 mM in three commercial *Dendrobium* cultivars: 'Rungnappa Red', 'Miss Snow White' and 'Earsakul'. The response was variable among the varieties. *In vitro* shoot initiation hastened as spermine and spermidine concentration increased from 0.25 to 1 mM in 'Rungnappa Red'. For example, shoot numbers increased by 116% with spermine and 3 to 100% with spermidine. In 'Miss Snow White' also, up to 200% increase in shoot number over the control was noted for spermine 0.5 mM.

Keywords: Shoot proliferation, Spermine, Spermidine.

Commercial cut flower orchids are propagated through *in vitro* techniques, which are specific to the cultivars. Such protocols need to be developed and refined for enhancing the propagation rate of shoots. Clonal propagation using stem nodal explants from young shoots and kiekies (offshoots on spikes or stem), has been a reliable and efficient method for orchid micropropagation. This method utilizes the enhanced release of axillary buds for shoot multiplication without destroying the shoot tip and mother plants. Only limited investigations were carried out on the effect of polyamines on shoot multiplication in commercial orchids. Saiprasad et al. (2004) observed that the polyamines, spermine, spermidine, and putrescine at 0.2, 0.4, and 1.0 mM, resulted in the production of protocorm-like bodies. However, in *Phalaenopsis amabilis* (L.) Blume and *P. nebula* Bl., the polyamines spermine, spermidine, and putrescine inhibited direct embryo formation from leaf explants (Gow et al., 2008). The present study was undertaken to assess the effects of two standard polyamines, spermine and spermidine in improving *in vitro* shoot morphogenesis in three commercial *Dendrobium* cultivars, viz., 'Rungnappa Red', 'Miss Snow White', and 'Earsakul'.

Stem nodes from 2 to 3 week-old greenhouse grown mother plants were used as explants. Shoots (8 to 12 cm long with 3 to 5 nodes) were harvested and wiped with 100% alcohol. The basal root portion and leaves were removed and the shoot portion cut in to single-node segments (1 to 2 cm). Such stem segments were immersed in 'Labolene' solution diluted 1000 times for 30 min, washed thoroughly in running tap water followed by glass distilled water. Surface sterilization was done inside laminar airflow chamber with mercuric chloride (0.1%) for 5 min, followed by washing with sterile distilled water 3 to 4 times. The basal medium used for the study was ½ MS (Murashige and Skoog, 1962) fortified with sucrose (3%), coconut water (200 ml L^{-1}), and agar (6.2 %). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C and 1.06 kg cm^{-2} for 20 min. The regenerated shoots from culture establishment media were transferred to the multiplication media comprising kinetin (2.0 mg L^{-1} for

*Author for correspondences: Phone 09743719290; Email <priyarenjith@gmail.com>.

'Rungnappa Red', 'and 'Earsakul' and 1.0 mg L^{-1} for 'Miss Snow White') and α-naphthalene acetic acid (NAA: 0.1 mg L^{-1}). The cultures were incubated at 26 ±2°C and photoperiod of 15 h at light intensity of 3000 lux provided by cool white fluorescent tubes. The multiplied shoots were subjected to spermine and spermidine treatments (polyamines) of 0.25, 0.5, and 1 mM. Each treatment was replicated six times and each replication involved single shoots (2 to 3 cm) with 3 to 4 leaves. Observations were recorded on the number of days required for shoot initiation, number of shoots per culture four weeks after subculture, and the nature of shoots developed. Completely randomized design was adopted and the data were analyzed using ANOVA.

In all three *Dendrobium* cultivars, spermine and spermidine hastened shoot initiation. However, the nature of the relationship was divergent among the cultivars. While both spermine and spermidine at 1.0 mM induced early bud initiation in 'Rungnappa Red', there was no such trend in the other cultivars. As polyamine concentration increased from 0 to 1 mM, days for shoot initiation decreased gradually. Both spermine and spermidine combined with kinetin and NAA, stimulated shoot multiplication, which generally exceeded the response that was obtained with kinetin and NAA alone (Tables 1 and 2).

In cv. 'Rungnappa Red', shoot production increased exponentially with increase in spermine concentration from 0.25 to 1.0 mM. Likewise, in 'Miss Snow White' and 'Earsakul' the number of shoots increased as the concentration of spermine increased up to 0.5 mM and 0.25 mM respectively, but declined thereafter. A similar enhancement of shoot production in polyamine supplemented media was reported in *Achras sapota* (Purohit et al., 2007), probably due to the stimulatory effect on cell division and/or to the inhibitory effect on ethylene production (Bais et al., 2000). The differential response of cultivars to a particular growth regulator/

Table 1. In vitro shoot morphogenesis in *Dendrobium* cvs. in spermine supplemented ½ MS medium at four weeks culture period.

Spermine (mM)	Days for shoot initiation			Number of shoots			% increase in shoot number		
	'Rungnappa Red'	'Miss Snow White'	'Earsakul'	'Rungnappa Red'	'Miss Snow White'	'Earsakul'	'Rungnappa Red'	'Miss Snow White'	'Earsakul'
Control	16.7	23.5	19.7	1.2	1.0	2.17	–		–
0.25	15.5	15.0	17.7	1.7	2.2	3.83	44	117	77
0.5	14.0	20.2	16.7	2.0	3.0	2.17	72.4	200	–
1.0	11.0	17.0	14.2	2.5	1.7	1.67	116	67	–77
F	7.4**	10.1**	7.1**	8.7**	13.8**	NS			–
CD (0.01)	2.7	3.5	2.5	0.6	0.7				–

** Significant at 1% level; data represents mean value of six replications.

Table 2. In vitro shoot morphogenesis in *Dendrobium* cvs. in spermidine supplemented ½ MS medium at four weeks culture period.

Spermidine (mM)	Days for shoot initiation			Number of shoots			% increase in shoot numbers		
	'Rungnappa Red'	'Miss Snow White'	'Earsakul'	'Rungnappa Red'	'Miss Snow White'	'Earsakul'	'Rungnappa Red'	'Miss Snow White'	'Earsakul'
Control	16.7	23.5	19.7	1.1	1.0	2.2	–	–	–
0.25	19.5	20.5	18.3	1.2	1.3	1.8	3.4	33	–15.7
0.5	17.8	19.7	18.5	1.3	1.3	1.8	12.1	33	–15.7
1.0	10.0	18.8	18.7	2.3	1.5	2.0	100.9	50	–7.8
F	38.8**	5.2**	NS	8.7**	NS	NS			
CD (0.01)	1.9	2.6	–	0.6	–	–			

** Significant at 1% level; data represents mean value of six replications.

media supplement may be due to the variations in the endogenous balance of the plant growth regulators (Prakash et al., 1996) and amino acids (Saiprasad et al., 2004). The regenerated shoots in polyamine supplemented cultures were also greener with more leaves and roots than the control. In addition, the polyamines supplemented cultures were free from the phenolic browning which was prevalent in the activated charcoal-free culture medium, as reported by Tang et al. (2004). Overall, the present study highlights the positive effect of polyamines on shoot morphogenesis in the commercial propagation of *Dendrobium* 'Rungnappa Red' and 'Miss Snow White'.

Acknowledgements

The present work forms a part of the PhD (Hort) thesis submitted by the first author to Kerala Agricultural University in 2009.

References

Bais, H.P., Sudha G., and Ravishankar, G.A. 2000. Putrescine and silver nitrate influence shoot multiplication, *in vitro* flowering and endogenous titers of polyamines in *Cichorium intybus* L. cv. Lucknow Local. J. Plant Growth Regul., 19: 238–248.

Gow, W.P., Chen, J.T., and Chang, W.C. 2008. Influence of growth regulators on direct embryo formation from leaf explants of *Phalaenopsis* orchids. Acta Physiol. Pl., 30(4): 507–512.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays of tobacco tissue cultures. Physiol. Pl., 15: 473–497.

Prakash, L., Lee, C.L., and Goh, C.J. 1996. *In vitro* propagation of commercial orchids: An assessment of current methodologies and development of a novel approach – Thin section culture. J. Orchid Soc. India, 10: 31–41.

Purohit, S.D., Singhvi, A., Nagori, R., and Vyas, S. 2007. Polyamines stimulate shoot bud proliferation and multiplication in *Achras sapota* grown in culture. Indian J. Biotech., 6: 85–90.

Saiprasad, G.V.S., Raghuveer, P., Khetarpal, S., and Chandra, R. 2004. Effect of various polyamines on production of protocorm-like bodies in orchid - *Dendrobium* 'Sonia' Sci. Hortic. 100(1-4): 161–168.

Tang, W., Newton, R.J., and Outhavong, V. 2004. Exogenously added polyamines recover browning tissues into normal callus cultures and improve plant regeneration in pine. Physiol. Pl., 122(3): 386–395.