www.gbtrp.com
International Journal of Biological Technology (2011) 2(2):35-41.
ISSN: 0976 – 4313

Original Article

In vitro seed germination of an epiphytic orchid *Xenikophyton smeeanum* (Reichb. f.) by using smoke-saturated-water as a natural growth promoter

Ravindra B. Malabadi[1]*, S. Vijaykumar[3], Jaime A. Teixeira da Silva[2], Gangadhar S. Mulgund[1], K. Nataraja[1]
[1]Division of Plant Biotechnology, Department of Botany, Karnatak University, Pavate nagar, Dharwad-580003, Karnataka State, India (* Corresponding author E-mail: mlbd712@rediffmail.com)
[2]Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan
[3]Department of Biotechnology, Madanpalle Institute of Technology and Science, Madanpalle-517325, Chitoor District, Andhra Pradesh State, India.
Received: 04.07.2011; Revised:.22.08.2011; Accepted:14.08.2011; Published:15.08.2011.

Abstract

This study highlights the role of smoke saturated water (SSW) on asymbiotic seed germination and plant regeneration of an epiphytic orchid *Xenikophyton smeeanum* (Reichb. f.). High percentage germination (75%) and high percentage of plantlet recovery (43%) were achieved by culturing seeds on Mitra *et al*.,(1976) basal medium supplemented with 10% (v/v) SSW. These results suggest that the germination stimulatory activity of SSW at 10% (v/v) could be used as a low cost method for the conservation and propagation of orchids. This will fulfill market demand making production a cost-efficient strategy.

Key words: Conservation, orchid, semi-dry grasses, Western Ghats regions

Abbreviations: butenolide, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one, PLB, protocorm-like body; TRIA, triacontanol, SSW, smoke saturated water

Introduction

Orchids are commercially important plant species known for their medicinal properties as well as horticultural cut flowers. There is a growing demand for orchid cut flowers in national and international markets with high commercial requirements. Orchids are well known for their highly colorful and attractive flowers whose long shelf life and varied shapes and sizes have great value in the floriculture industry as cut flower and potted plants. In nature, seed germination of both epiphytic and terrestrial orchid species is very slow and utilizes fungi as the carbon source. This fungal association provides the seed with essential growth nutrients for the effective germination and plant development of orchids (Arditti, 1968; Arditti *et al*., 1981; Kauth *et al*., 2008). Therefore *in vitro* seed germination has been utilized for the micropropagation of many orchids to meet commercial requirements. *In vitro* propagation of orchids is a powerful tool for conservation and management of orchid resources (Morel, 1964; Rao, 1977; Sharma *et al*., 1991; Lakshmanan *et al*., 1995; Ichihashi, 1997, 1998; Kanjilal *et al*., 1999; Malabadi *et al*., 2004, 2005; Teixeira da Silva *et al*., 2006; Das *et al*., 2007; Malabadi and Nataraja, 2007a, 2007b; Malabadi *et al*., 2008a, 2008b, 2008c; Malabadi *et al*., 2009a, 2009b). Plant tissue culture techniques have become a unique tool in plant biotechnology. As a result, orchid seed germination through plant tissue culture would be a viable alternative for the production of large number of seedlings. Commercial orchid tissue culture holds value only if large numbers of plantlets are produced in a short time frame and with minimum input expenses. Hence, the time taken for plant regeneration becomes a crucial factor as far as the economy of production for orchid commerce is concerned. The use of expensive plant growth regulators can be cut down by replacing them with low cost natural additives such as smoke saturated water (SSW) which is a natural source of plant growth hormone. In this investigation, the effect of SSW on the seeds and shoots and formation of *Xenikophyton smeeanum* (Reichb. f.) plants in a simple and cost-effective manner. *X. smeeanum* is a native epiphytic orchid from the Western Ghat Forests of Karnataka State, India (Krishnaswamy *et al.*, 2004). Although this species is facing rapid destruction, no one has paid attention to its conservation to date. Since vegetative propagation methods are not available, development of *in vitro* methods are essential for conservation and commercialization of this

species. Keeping these limitations in mind, the present preliminary studies were conducted to develop an efficient protocol for the rapid propagation of *X. smeeanum* (Reichb. f.) *via in vitro* seed germination. Our present study constitutes the first report of a successful and efficient *in vitro* propagation protocol for large-scale production of *X. smeeanum* (Reichb. f.).

Materials and Methods

The protocol used in this study has been adopted from one of our previous reports on the *in vitro* seed germination of *Vanda parviflora* Lindl., using SSW as a natural additive (Malabadi *et al*., 2008a).

Preparation of SSW

SSW was prepared according to the procedure described by Thomas and van Staden (1995) and Dixon *et al*. (1995). This was achieved by slow burning of a mixture of two local (Indian) semi-dry grasses *Aristida setacea* and *Cymbopogon martini* (Graminiaceae) (Malabadi and Vijaykumar, 2006, 2007d; Malabadi and Nataraja, 2007c; Malabadi *et al.,* 2008a). The resulting smoke was first passed into a metal drum connected to a flask containing 500 ml of distilled water through a pipe (Malabadi *et al.,* 2008a). The smoke was forced to pass through the water by blowing air using a fan or compressed air for 1 to 2 h at a rate of 50 to 60 psi continuously (Malabadi *et al*., 2008a). The SSW was collected and stored at 2°C until further use. Different concentrations of SSW were used in the following *in vitro* seed germination experiments.

In vitro seed germination using SSW

Green capsules (approx. 3 to 5 cm in length) of *X. smeeanum*, which were collected from the Western Ghat Forests of Karnataka State near Khanapur, Belgaum, India, were carefully washed in sterilized double distilled water. They were surface decontaminated sequentially with 0.1% streptomycin (1min), 70% (v/v) ethanol (5 min) and 0.1% (w/v) $HgCl_2$ (2 min) (Sigma, USA), and thoroughly rinsed with sterilized double distilled water. After sterilization, the capsules were dried and dissected longitudinally with a surgical blade under aseptic conditions. The seeds were scooped out from sterilized capsules and sown by spreading as thinly as possible over the surface of Mitra *et al*., (1976) basal medium containing 3.0% sucrose, 0.7% agar, 0.5 gl^{-1} *myo*-inositol, 1.0 gl^{-1} casein hydrosylate, 0.5 gl^{-1} L-glutamine, 250 mgl^{-1} peptone, 0.2 gl^{-1} *p*-aminobenzoic acid, and 0.1 gl^{-1} biotin (all reagents from Sigma), the control medium, in 250-ml conical flasks (3 conical flasks per capsule; one capsule per treatment; experiments were repeated 3 times). The effect of SSW was studied on seed germination of *X. smeeanum* by incorporating different concentrations (5, 10, 15 and 20%) into the control medium. The pH of the medium was adjusted to 5.8 with 1 N NaOH or HCl before agar was added. The medium was then sterilized by autoclaving at 121°C and 1.05 kg/cm^2 for 15 min. L-glutamine and casein hydrolysate were filter sterilized (Whatman filter paper, pore size = 0.45 µm; diameter of paper = 25 mm), and added to the medium after it had cooled to below 50°C. All cultures were maintained in the dark at 25 ± 2°C. The percentage germination was calculated by dividing the number of germinating seeds by the total number of seeds in the sample, as observed under a microscope (Malabadi *et al*., 2008a). Various developmental stages of seed germination of *X. smeeanum* were adopted from Kauth *et al*., (2006, 2008) and Johnson and Kane, (2007). These stages are (stage 0 = ungerminated seed with embryo; stage 1 = enlarged embryo, testa ruptured (= germination); stage 2 = appearance of protomeristem or rhizoids; stage 3 = emergence and elongation of first leaf; stage 4 = protocorm with developing leaves and rhizoids; stage 5 = two leaves and one or more roots present; stage 6 = presence of two or more leaves, roots present (= seedling) (Malabadi *et al*., 2008a). The protocorms (60-70), in various stages of development, were subcultured on fresh medium for 30 days. The percentage of propagules in each stage was calculated by dividing the number of propagules in that stage by the total number of propagules × 100 (Malabadi *et al*., 2008a). The cultures were maintained for 6-10 weeks to initiate protocorm-like bodies (PLBs) or proliferating shoot buds. The freshly initiated individual PLBs were transferred (~5-10 PLBs per conical flask) to basal medium containing 10% SSW (this is the optimum concentration for growth and development) (Senaratna *et al*., 1999; Malabadi and Nataraja, 2007c). Healthy shoots with 2-3 leaves, which developed within 10-12 weeks (Malabadi *et al*., 2008a), were subcultured on the same medium for another 2 weeks for further shoot development.

www.gbtrp.com
International Journal of Biological Technology (2011) 2(2):35-41.
ISSN: 0976 – 4313

Plantlet hardening and acclimatization

The well-developed shoots were further transferred to fresh basal medium supplemented with or without (control) 2.0 µM triacontanol (TRIA) for improving rooting (Malabadi *et al*., 2008a). The shoots with well developed roots on TRIA-supplemented basal medium were washed thoroughly under running tap water and transplanted into 15-cm diameter pots containing a potting mixture of charcoal chips, coconut husks and broken tiles (2: 2: 1) (Malabadi *et al.,* 2008a). Three to four plantlets were planted in each pot, watered daily and fertilized weekly with a foliar spray of a mixture of commercial DAP (di-ammonium phosphate) and NPK (nitrogen 20: phosphorous 10: potassium 10) (Malabadi *et al*., 2004, 2005; Malabadi and Nataraja, 2007a; Malabadi *et al*., 2008a).

Statistical analyses

All experiments contained 15 cultures per replicate, with four replicates (60 cultures) per experimental treatment, and each treatment was repeated three times (60 × 3 = 180). Data presented in the tables were arcsine transformed before being analyzed for significance using ANOVA, and the differences contrasted using Duncan's multiple range test. All statistical analyses were performed at the 5% level using the SPSS (Microsoft Windows v. 13.0.1.1) statistical software package.

Results and Discussion

In the present investigation, the germination of *X. smeeanum* was marked by swelling and emergence of embryo from the testa. Seeds started to swell by imbibing water and nutrients. During germination, the undifferentiated embryos formed an irregular shaped cell mass, termed the spherules, which turned green and formed round structures, the protocorms (Fig.1A). These swollen protocorms started to develop a vegetative apex at the upper part and rhizoids at the lower part within 8-10 weeks. Furthermore, an increase in percentage germination as well as early differentiation of protocorms into seedlings (stages 3, 4) was observed on 10% (v/v) SSW-supplemented Mitra *et al*., (1976) basal medium compared to the control (Table-1). Maximum percentage germination (75%) was observed on this medium and seed germination percentage was greatly inhibited at higher concentrations of SSW (15 and 20%) compared to the control; in this case, most seeds failed to germinate and died (Table-1). This higher percentage of seed germination also corresponds to the highest percentage recovery of seedlings (43%) with well developed roots (Fig.1B) (stage 5) (Table-1). The plants were normal and showed healthy growth with an 80% survival rate, i.e. SSW at 10% (v/v) aids in rapid regeneration of *X. smeeanum*. This enhanced effect of differentiation of protocorms to form plantlets (i.e. leaves and roots) by the presence of SSW in basal medium indicated that

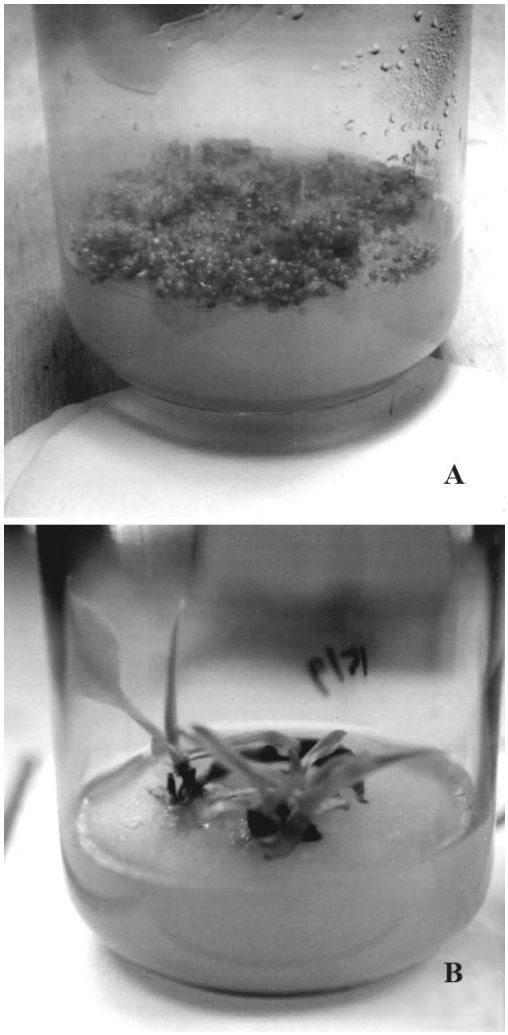

Fig.1: Influence of 10% SSW on seed germination of *X. smeeanum* (Reichb. f.). (A) Seed germination and formation of protocorms (bar = 1.3 cm). (B) Seedlings with well developed roots after 16 weeks and ready for hardening (bar = 0.7 cm).

SSW has some growth-promotive substance(s). This is evidenced by our previous report showing that the highest percentage germination (95%)

and plantlet recovery (93%) was achieved by culturing seeds of *V. parviflora* on Mitra *et al*. (1976) basal medium supplemented with 10% (v/v) SSW (Malabadi *et al.,* 2008a). Furthermore, Smoke influences seed germination and post-germination processes of many plant species (Thomas and van Staden, 1995; Strydom *et al*., 1996; Baxter and van Staden, 1994; Blank and Young, 1998; Brown *et al*., 2003; Sparg *et al*., 2005a, 2005b; Sparg *et al*., 2006; Kulkarni *et al*., 2006; Jain and van Staden, 2006; van Staden *et al*., 2006; Kulkarni *et al*., 2007; van Staden *et al*., 2000; Jain *et al*., 2008; Light and van Staden, 2004; Malabadi and Nataraja, 2007c, 2008; Malabadi et al., 2009c). SSW was also able to stimulate somatic embryogenesis in geranium (Senaratna *et al*. 1999) and in *Pinus wallichiana* (Himalayan blue or Bhutan pine), specifically at 10% using vegetative shoot apices of mature trees of (Malabadi and Nataraja, 2007c), flowering in fire-lily (*Cyrtanthus ventricosus*) (Keeley, 1993) and rooting in *Vigna radiata* (L.) Wilczek hypocotyl cuttings (Taylor and van Staden, 1996). Smoke contains several thousand compounds (Maga, 1988). A highly active germination promoting compound has been identified as a water-soluble butenolide, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one, from the smoke of burnt fynbos *Passerina vulgaris* Thoday and the grass *Themeda triandra* L. (van Staden *et al*., 2004) as well as from the combustion of cellulose (Flematti *et al*., 2004). This compound, which is water-soluble and heat-stable, can stimulate seed germination at very low concentrations (10^{-9} M; Flematti *et al*., 2004; van Staden *et al*., 2004) and can be stored as an aqueous solution for long periods while retaining its activity after autoclaving (van Staden *et al*., 2000; 2004).

Table- 1: Effect of different concentrations of SSW-supplemented Mitra *et al*. (1976) basal medium on seed germination of *X. smeeanum* (Reichb. f.)

SSW concentrations (%, v/v)	No. of protocorms (%)	Time taken for germination (weeks)	№ of protocorms with 2-3 leaves (%)	№ of seedlings with roots (%)
*control	5.0 ± 0.1 b	14-18	2.5 ± 0.1 b	1.0 ± 0.1 b
5	18.0 ± 0.2 b	8-10	8.0 ± 0.2 b	3.0 ± 0.4 b
control	3.0 ± 0.1 b	14-18	1.3 ± 0.2 b	0.0 ± 0.0 c
10	75.0 ± 1.6 a	8-10	57.0 ± 1.8 a	43.0 ± 1.0 a
control	4.0 ± 0.1 b	14-18	1.2 ± 0.1 b	2.0 ± 0.1 b
15	12.0 ± 0.6 b	8-10	6.0 ± 0.1 b	3.0 ± 0.1 b
control	3.0 ± 0.3 b	14-18	1.5 ± 0.1 b	1.0 ± 0.1 b
20	2.0 ± 0.1 b	8-10	0.0 ± 0.0 c	0.0 ± 0.0 c

*Control = Mitra *et al*. (1976) basal medium without SSW

Data scored after 16 weeks and represent the mean ± SE of at least three different experiments. In each column, the values with different letters are significantly different ($P<0.05$) according to DMRT (Duncan's multiple range test).

In another study, the effect of butenolide was tested for its effect on somatic embryogenesis with an important species for commercial horticulture, *Baloskion tetraphyllum* (Restionaceae) (Ma *et al*., 2006). SSW and aerosol smoke by slow burning of a mixture of semi-dry grasses *Aristida setacea* and *Cymbopogon martini* (Graminaceae) improved the seed germination and seedling vigour of Indian indigenous medicinal plants (*Terminalia chebula*, *Holorrhina antidysentrica*, *Clitoria ternatea*, *Gymnema sylvestre*, *Acacia pennata*, *Basella alba*, *Celastrus asiatica*, and *Cleome gynandra* (Malabadi and Vijaykumar, 2006, 2007d). The application smoke and smoke solutions enhanced the seedling vigour of all species (Malabadi and Vijaykumar, 2006, 2007d). The identification of the germination cue, butenolide, from smoke will now allow for research into the physiological action of smoke on seed germination.

These observations suggest that the active ingredient(s) in SSW play a regulatory role in plant development and therefore, SSW acts like a growth regulator more than as a nutritional additive. It has been suggested that smoke may have an action similar to cytokinins in breaking celery seed dormancy (de Lange and Boucher, 1990; Dixon *et al*., 1995; Pierce *et al*., 1995; Roche *et al*., 1997; Thomas and van Staden, 1995; van Staden *et al*., 2000; Brown *et al*., 2003; Brown and Botha, 2004; Light and van

Staden, 2004; Daws *et al*., 2008). The bioactivity of butenolide, which is structurally related to butenolides from smoke, was first identified by Pepperman and Cutler, (1991) who conducted bioassays on wheat coleoptiles. The cytokinin and auxin-like activity of smoke-derived butenolide was assessed using soybean (*Glycine max* L. cv. 'Acme') callus and mungbean (*Vigna mungo* L.) rooting bioassays (Jain *et al*., 2008). Similarly, a combination of SSW and cytokinin (BA) was more effective in breaking thermodormancy in lettuce seeds compared to cytokinins applied alone (Strydom *et al*., 1996; Thomas and van Staden, 1995). However, the mode of action of SSW is still unknown even after the identification of butenolide. It has been suggested that the smoke compound acts either by modulating the sensitivity of the tissue to PGRs, activation of enzymes or by modifying the receptor molecules (Thomas and van Staden, 1995; Brown and van Staden, 1997; van Staden *et al*., 2000; Light and van Staden, 2004). Therefore, the use of SSW (10%) in the medium initiates early differentiation of protocorms to form leaves and roots such that advanced stages of development are reached faster than in the control.

References

Arditti, J. 1968. Germination and growth of orchids on banana fruit tissue and some of its extracts. *American Orchid Society Bulletin* 37, 112-116.

Arditti, J, Michaud, J. D. and Oliva, A. P. 1981. Seed germination of North American orchids. I. Native California and related species of *Calypso*, *Epipactis*, *Goodyera*, *Piperia* and *Platanthera*. *Botanical Gazette,* 142: 442-453.

Baxter, B.J.M, van Staden, J, Granger J.E. and Brown, N.A.C.1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra* Forssk. *Environment and Experimental Botany*,34: 217-223.

Baxter, B. J. M. and van Staden, J. 1994. Plant-derived smoke: an effective seed pre-treatment. *Plant Growth Regulation*,14:279-282.

Blank, R.R. and Young, J.A.1998. Heated substrate and smoke:influence on seed emergence and plant growth. *Journal of Range Management,* 51:577-583.

Brown, N.A.C.1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist*,123:575-583.

Brown, N.A.C. and van Staden, J.1997. Smoke as a germination cue: A review. *Plant Growth Regulation*,22:115-124.

Brown, N.A.C., van Staden, J., Daws, M.I. and Johnson, T. 2003. Patterns in the seed germination response to smoke in plants from the Cape Floristic Region, South Africa. *South African Journal of Botany*,69:514-525

Brown, N.A.C. and Botha, P. A. 2004. Smoke seed germination studies and a guide to seed propagation of plants from the major families of Cape Floristic Region, South Africa. *South African Journal of Botany*,70:559-581.

Das, M.C, Kumaria, S. and Tandon, P. 2007. Protocorm regeneration, multiple shoot induction and *ex vitro* establishment of *Cymbidium devonianum* Paxt. *Asian Journal of Plant Sciences,* 6: 349-353.

Daws, M. I., Pritchard, H. W. and van Staden, J. 2008. Butenolide from plant-derived smoke functions as a strigolactone analogue: Evidence from parasitic weed seed germination. *South African Journal of Botany,* 74: 116-120.

de Lange, J. H. and Boucher, C. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). 1. Plant derived smoke as a germination cue. *South African Journal of Botany,* 56:700-703.

Dixon, K.W, Roche, S. and Pate, J. S. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia,* 101: 185-192.

Drewes, F. E., Smith, M. T. and van Staden, J. 1995. The effect of plant-derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation,* 16: 205-209.

Flematti, G. R, Ghisalberti, E. L., Dixon, K. W. and Trengove, R. D.2004. A compound from smoke that promotes seed germination. *Science,* 305: 977

Ichihashi, S. 1997. Research on micropropagation of *Cymbidium, Dendrobium* and *Phalaenopsis* in Japan. In: Arditti J., Pridgeon AM (Eds) *Orchid Biology: Reviews and Perspectives* (Vol VII), Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 285-316.

Ichihashi, S. 1998. Control of growth and development of protocorm-like bodies derived from callus by carbon sources. *Plant Biotechnology,* 15:183-187.

Jain, N. and van Staden, J. 2006. A smoke derived butenolide improves early growth of

tomato seedlings. *Plant Growth Regulation,* 50: 139-145.

Jain, N., Strik, W. A. and van Staden, J. 2008. Cytokinin and auxin-like activity of a butenolide isolated from plant derived smoke. *South African Journal of Botany,*74:327-331.

Johnson, T. E. and Kane, M.E. 2007. Asymbiotic germination of ornamental *Vanda*: *in vitro* germination and development of three hybrids. *Plant Cell Tissue and Organ Culture,* 91:251-261.

Kanjilal, B., Sarkar, D. D., Mitra, J. and Datta, K. B. 1999. Stem disc culture: development of a rapid mass propagation method for *Dendrobium moschatum*, an endangered orchid. *Current Science,* 77: 497-500.

Kauth, P. J., Vendrane, W. A. and Kane, M.E. 2006. *In vitro* seed culture and seedling development of *Calopogon tuberosus*. *Plant Cell, Tissue and Organ Culture,* 85:91-102.

Kauth, P.J,, Dutra, D., Johnson, T. R., Stewart, S. L., Kane, M. E. and Vendrame, W. 2008. Techniques and applications of *in vitro* orchid seed germination. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol V), Global Science Books, Isleworth, UK, pp. 375-391.

Keeley, J.E. and Fotheringham, C.J.1998. Smoke-induced seed germination in California chaparral. *Ecology,* 79:2320-2336.

Keeley, J. E. 1993. Smoke-induced flowering in the fire-lily *Cyrtanthus ventricosus*. *South African Journal of Botany,* 59:638.

Krishnaswamy, K., Krishnakumar, H.N., Ramakrishna, T. M. and Ramaswamy, S. N. 2004. Studies on seed morphometry of epiphytic orchids from Western Ghats of Karnataka. *Taiwania*, 49(2): 124-140.

Kulkarni, M.G., Sparg, S.G., Light, M.E. and van Staden, J. 2006. Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *Journal of Agronomy and Crop Sciences,*192:395-398.

Kulkarni, M.G., Sparg, S. G. and van Staden, J. 2006. Germination and post-germination response of Acacia seeds to smoke-water and butenolide, a smoke-derived compound. *Journal of Arid Environments,* 69:177-187.

Light, M. E. and van Staden, J. 2004. The potential of smoke in seed technology. *South African Journal of Botany,* 70: 97-101.

Light, M.E., Gardner, M.J., Jager, A.K. and van Staden, J.2002. Dual regulation of seed germination by smoke solutions. *Plant Growth Regulation,* 37:135-141.

Lakshmanan, P., Lee, C. L., Loh, C. S. and Goh, C. J. 1995. *In vitro* propagation of commercial orchids. An assessment of current methodology and development of a novel approach of thin cross section culture. In: Islam AS (Ed) *Plant Tissue Culture*, Oxford IBH New Delhi, India, pp. 42-49.

Ma, G. H., Bunn, E., Dixon, K. and Flematti, G. 2006. Comparative enhancement of germination and vigor in seed and somatic embryos by the smoke chemical butenolide, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one in *Baloskion tetraphyllum* (Restionaceae). *In Vitro Cellular and Developmental Biology – Plant,* 42: 305-308.

Maga, J. A. 1988. *Smoke in Food Processing*, CRC Press, Boca Raton, USA, pp. 22-34.

Malabadi, R.B, Mulgund, G. S, Nataraja, K. 2004. Efficient regeneration of *Vanda coerulea*, an endangered orchid using thidiazuron. *Plant Cell, Tissue and Organ Culture,* 76: 289-293.

Malabadi, R.B., Mulgund, G.S. and Nataraja, K. 2005. Micropropagation of *Dendrobium nobile* from shoot tip sections. *Journal of Plant Physiology,* 162: 473-478.

Malabadi, R. B. and Vijaya Kumar, S. 2006. Smoke induced germination of some important medicinal plants. *Journal of Phytological Research,* 19: 221-226.

Malabadi, R.B. and Nataraja, K. 2007a. Brassinosteroids influences *in vitro* regeneration using shoot tip sections of *Cymbidium elegans* Lindl. *Asian Journal of Plant Sciences,* 6:308-313.

Malabadi, R. B. and Nataraja, K. 2007b. Genetic transformation of *Vanilla planifolia* by *Agrobacterium tumefaciens* using shoot tip sections. *Research Journal of Botany,* 2: 86-94.

Malabadi, R. B. and Nataraja, K. 2007c. Smoke-saturated water influences somatic embryogenesis using vegetative shoot apices of mature trees of *Pinus wallichiana* A. B. Jacks. *Journal of Plant Sciences,* 2: 45-53

Malabadi, R. B., Teixeira da Silva, J. A. and Mulgund, G.S. 2008a. Smoke-saturated water influences *in vitro* seed germination of *Vanda parviflora* Lindl. *Seed Science and Biotechnology,* 2(2):65-69.

Malabadi, R. B., Teixeira da Silva, J. A., Nataraja, K. and Mulgund, G. S. 2008b. Shoot tip transverse thin cell layers and 24-epibrassinolide in the micropropagation of *Cymbidium bicolor* Lindl. *Floriculture and Ornamental Biotechnology,* 2(2): 44-48.

Malabadi, R. B., Teixeira da Silva, J. A. and Mulgund, G.S. 2008c. Micropropagation of *Eria dalzelli* (Dalz.) Lindl., through TCL *in vitro*

culture. *Floriculture and Ornamental Biotechnology*, 2(2):77-80.

Malabadi, R. B. and Vijaykumar, S. 2008. Effect of smoke on seed vigour response of selected medicinal plants. *Journal of Phytological Research,* 21(1):71-75.

Malabadi, R. B., Teixeira da Silva, J. A. and Mulgund, G. S. 2009a. *In vitro* shoot regeneration by culture of *Liparis elliptica* (Rees) Lindl., shoot tip-derived transverse thin cell layers induced by 24-epi brassinolide. *International Journal of Plant Developmental Biology,* 3(1): 47-51.

Malabadi, R. B., Teixeira da Silva, J.A. and Mulgund, G.S. 2009b. TDZ-induced *in vitro* shoot regeneration of *Aerides maculosum* Lindl. From shoot tip thin cell layers. *Floriculture and Ornamental Biotechnology,* 3(1): 35-39.

Malabadi, R. B., Mulgund, G. S. and Vijaykumar, S. 2009c. Smoke induced seed germination and somatic embryogenesis. *Journal of Phytological Research,*22 (2):205-209.

Mitra, G. C., Prasad, R. N. and Choudhury, R. A. 1976. Inorganic salts and differentiation of protocorms in seed callus of orchid correlative changes in its free amino acid content. *Indian Journal of Experimental Biology,* 14: 350-351.

Morel, G. M. 1964. Tissue culture – a new means of clonal propagation of orchids. *American Orchid Society Bulletin,* 33:473-478.

Pierce, S. M., Esler, K. and Cowling, R. M. 1995. Smoke-induced germination of succulents (Mesembryanthernaceae) from fire-prone and fire-free habitats in South Africa. *Oecologia,* 102: 520-522

Rao, A.N. 1977. Tissue culture in orchids industry. In: Bajaj YPS, Reinert J (Eds) *Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture*, Springer, Berlin, pp 44-69.

Roche, S., Dixon, K. W. and Pate, J. S. 1997. Seed aging and smoke: partner cues in the amelioration of seed dormancy in selected Australian native species. *Australian Journal of Botany,* 45:783-815

Senaratna, T., Dixon, K., Bunn, E. and Touchell, D. 1999. Smoke-saturated water promotes somatic embryogenesis in geranium. *Plant Growth Regulation,* 28: 95-99.

Sharma, S. K., Tandon, P. and Mishra, R. R. 1991. Vitamins as related to axenic seed germination and seedling of *Cymbidium elegans* Lindl. and *Coelogyne punctulata* Lindl. *Journal of the Orchid Society of India,* 5(1-2): 25-28.

Sparg, S. G., Kulkarni, M. G., Light, M. E. and van Staden, J. 2005a. Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology,* 96:1323-1330.

Sparg, S. G., Kulkarni, M. G., Light, M. E. and van Staden, J. 2005b. Aerosol smoke and smoke-water stimulation of seedling vigour of a commercial maize cultivar. *Crop Science,* 46: 1336-1340.

Strydom, A., Jager, A. K. and van Staden, J. 1996. Effect of plant-derived smoke extract, N-benzyladenine and gibberellic acid on the thermodormancy of lettuce seeds. *Plant Growth Regulation,* 19: 97-100.

Taylor, J. L. S. and van Staden, J. 1996. Root initiation in *Vigna radiata* (L.) Wilczek hypocotyl cuttings are stimulated by smoke-derived extracts. *Plant Growth Regulation,* 18: 165-168.

Teixeira da Silva, J. A., Chan, M. T., Sanjaya, Chai, M. L. and Tanaka, M. 2006. Priming abiotic factors for optimal hybrid *Cymbidium* (Orchidaceae) PLB and callus induction, plantlet formation, and their subsequent cytogenetic stability analyses. *Scientia Horticulturae,*109: 368-378.

Thomas, T. H. and van Staden, J. 1995. Dormancy break of celery (*Apium gravelolens* L.) seeds by plant derived smoke extract. *Plant Growth Regulation,* 17:195-198.

van Staden, J.1999. Medicinal plants in southern Africa: utilization, sustainability, conservation – can we change the mindsets. *Outlook Agriculture,* 28:75-76.

van Staden, J., Brown, N. A. C., Jager, A. K. and Johnson, T.A.2000. Smoke as a germination cue. *Plant Species Biology*,15: 167-178.

van Staden, J., Jager, A. K., Light, M. E. and Burger, B.V. 2004. Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany,* 70: 654-657.

van Staden, J., Sparg, S. G., Kulkarni, M. G. and Light, M. E. 2006. Post germination effects of the smoke-derived compound 3-methyl-2*H*-furo [2, 3-c] pyran-2-one, and its potential as a preconditioning agent. *Field Crops Research,* 98: 98-105.