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Smoke-Saturated Water Influences *in Vitro* Seed Germination of *Vanda parviflora* Lindl.

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ABSTRACT

This study for the first time reports the influence of smoke saturated water (SSW) on asymbiotic seed germination and an early differentiation of protocorms and plant regeneration of *Vanda parviflora* Lindl. High percentage germination (95%) and high percentage of plantlet recovery (93%) was achieved by culturing seeds on Mitra *et al*. (1976) basal medium supplemented with 10% (v/v) SSW. Rapid regeneration was observed within 60-70 days of culture on this medium where the majority (93%) of propagules developed leaves and roots. Well-rooted shoots were transferred to pots containing charcoal chips, coconut husk and broken tiles (2:2:1) and 90% survived. This study emphasizes the role of SSW as a natural additive at different stages of development from seed germination to plant regeneration. These results also suggest that the germination stimulatory activity of SSW at 10% (v/v) could be applied for micropropagation of other orchids as a low cost method. The added benefit of this protocol is that it can be transferred to a rural community surrounded by rich orchid diversity in the Western Ghat Forests of Karnataka state with less technical know-how where small-scale tissue culture units can be set up to generate employment as well as to conserve biodiversity.

Keywords: Belgaum, India, micropropagation, semi-dry grasses, Western Ghat Forests
Abbreviations: butenolide, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one, **BAP**, benzyl amino adenine, **2,4-D**, 2-4-dichlorophenoxy acetic acid; **24-epiBL**, 24-epibrassinolide; **IAA**, indole-3-acetic-acid; **IBA**, indole-3-butyric acid; **NAA**, α-naphthalene acetic acid; **PLB**, protocorm-like body; **TRIA**, triacontanol, **SSW**, smoke saturated water

INTRODUCTION

Fire is a major environmental selective force that influences plant communities in many parts of the world (Brown and van Staden 1997). Smoke is an important factor involved in fire and post-fire germination cues (Brown and van Staden 1997). Farmers have traditionally used fire and smoke in grain drying practices (van Staden *et al*. 2000) and smoke from the combustion of plant material stimulates seed germination in a wide range of species (e.g. Brown *et al*. 2003; Light and van Staden 2004; Daws *et al*. 2008). The ability of plant-derived smoke to break dormancy and stimulate germination was first reported by de Lange and Boucher (1990) for *Audouinia capitata*, a fynbos species growing in a fire-prone habitat. Since this discovery, the application of smoke and aqueous smoke extracts to improve seed germination has been shown in a wide range of plants from many families, irrespective of their fire sensitivity (Dixon *et al*. 1995; Pierce *et al*. 1995; Roche *et al*. 1997; Brown and Botha 2004). This phenomenon and its applications in seed technology have been extensively discussed in a number of reviews (Brown and van Staden 1997; van Staden *et al*. 2000; Light and van Staden 2004). Smoke contains several thousand compounds (Maga 1988). A highly active germination promoting compound has recently 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).

Current interest in propagation of native orchids has created a need to develop practical propagation methods. The increased popularity of native orchids has lead to a major increase in their production and sales, which have slowly increased. A major obstacle to native orchid production is the difficulty in seed germination. Orchids are produced through seed germination, but seedling development can be a long process and flowering plants are often produced only after 3-5 years of growth. *Vanda parviflora* Lindl. is one of the native epiphytic orchids from the Western Ghat forests of Karnataka known for its beautiful flowers, and there is a growing demand in the commercial market as a popular potted floriculture crop. There is also a growing perception that *V. parviflora* can be profitable for commercial growers, segmentation of the market, and a reduction in price of individual potted plants. Tissue culture techniques have been widely used for the *in vitro* mass multiplication of several commercially important orchids (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*. 2008). Orchid seeds are often referred to as *dust seeds*, as they are tiny and contain few food reserves. The seeds contain a small embryo and lack enzymes to metabolize polysaccharides, but utilize lipids as a major nutrient source. The embryo also lacks enzymes to convert lipids to soluble sugars, which cause a requirement by orchid seeds for a symbiotic relationship with a mycorrhizal fungus in order to germinate under natural conditions (Arditti 1968; Arditti *et al*. 1981). This fungal association provides the seed with

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carbohydrates, nutrients, minerals and water. In this paper we report for the first time an efficient multiplication method for *V. parviflora* through *in vitro* seed germination culture by incorporation of smoke-saturated water (SSW) in the nutrient medium. The main objective of this work is to study the effect of different concentrations of SSW on the *in vitro* seed germination of *V. parviflora*, and extend this low cost technology for the conservation of native orchids. We also propose that SSW, which is readily available, is easy to use and inexpensive to produce, and thus can help in rapid plant regeneration without causing any deformation in plant growth. Our results for the first time demonstrate that SSW influences the seed germination of *V. parviflora* and might be effectively used in the micropropagation of other orchids.

MATERIALS AND METHODS

Preparation of smoke-saturated-water

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) semidry grasses *Aristida setacea* and *Cymbopogon martini* (Graminiaceae) (Malabadi and Vijaykumar 2006, 2007d; Malabadi and Nataraja 2007c). The resulting smoke was first passed into a metal drum connected to a flask containing 500 ml of distilled water through a pipe. The smoke was forced to pass through the water by blowing air using a fan or compressed air for 1 to 2 h at the rate of 50 to 60 psi continuously. The SSW was collected and stored at 2°C until further use. Different concentrations of SSW (5, 10, 15 and 20%) were used in the following *in vitro* seed germination experiments.

In vitro seed germination using smoke-saturated water

Green capsules (approx. 3 to 5 cm in length) of *V. parviflora* were collected from the Western Ghat Forests of Karnataka near Khanapur, Belgaum, India. These capsules were carefully washed in sterilized double distilled water. They were surface decontaminated sequentially with 0.1% streptomycin (1 min), 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 with 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 Sigma), the control medium, in 250-ml conical flasks (3 conical flasks per capsule and one capsule for each treatment, and experiments were repeated 3 times). The effect of SSW was also studied on the initiation of embryogenic tissue 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 the cultures were maintained in the dark at 25 ± 2°C. Percentage germination was calculated by dividing the number of germinating seeds by total number of seeds in the sample under the microscope. Various developmental stages of seed germination of *V. parviflora* were adopted from Kauth *et al*. (2006) 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). 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. 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 developed within 10-12 weeks. They were subcultured on the same medium for another 2 weeks for further shoot development.

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. 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). 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*. 2008).

Statistical analyses

All experiments contained 25 cultures per replicate, with four replicates (100 cultures) per experimental treatment, and each treatment was repeated three times (100 × 3 = 300). 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

In orchids, protocorm development takes place in a sequence. Protocorm regeneration from seeds has become the favored method for mass production of orchids. Most orchids are produced in this way. Even though double fertilization in orchids takes place as in other angiosperms, the endosperm, however fails to develop. The seeds of all orchids have to be nurtured by their specific mycorrhizal fungi in the initial stages of development because less than 5% of orchid seeds germinate in nature (Rao 1977; reviewed by Kauth *et al*. 2008). On the other hand, mass production of orchids could be achieved asymbiotically in flasks or test tubes.

In the present study, initially the immature embryo of *V. parviflora* elongated and was transparent to white in colour with a covered testa. On being cultured onto the media the seeds turned green and formed swollen structures called protocorms (stages 1, 2) within 7-9 weeks (**Fig. 1A, 1B**). In this study an increase in percentage germination as well as early differentiation of protocorms into seedlings (stages 3, 4) (**Fig. 1C**) was observed on 10% (v/v) SSW-supplemented Mitra *et al*. (1976) basal medium compared to control (**Table 1**). Germination was marked by swelling and emergence of the embryo from the testa. A significant increase in percentage germination (stage 1) was observed as the percentage of SSW in basal medium increased compared to the control after 8 to 10 weeks of culture. Maximum percentage germination (95%) was observed on 10% (v/v) and seed germination percentage was greatly inhibited at higher concentrations of SSW (15 and 20%) compared to the control and most seeds turned brown without germinating (**Table 1**). This higher percentage of seed germination also corresponds to the highest percentage recovery of seedlings (93%) with well developed roots (**Fig. 1D**) (stage 5) (**Table 1**). The mean germination time (8-10 weeks) was affected by the addition of SSW in the medium more than the control (12-16 weeks) (**Table 1**). In this study, the presence of SSW at

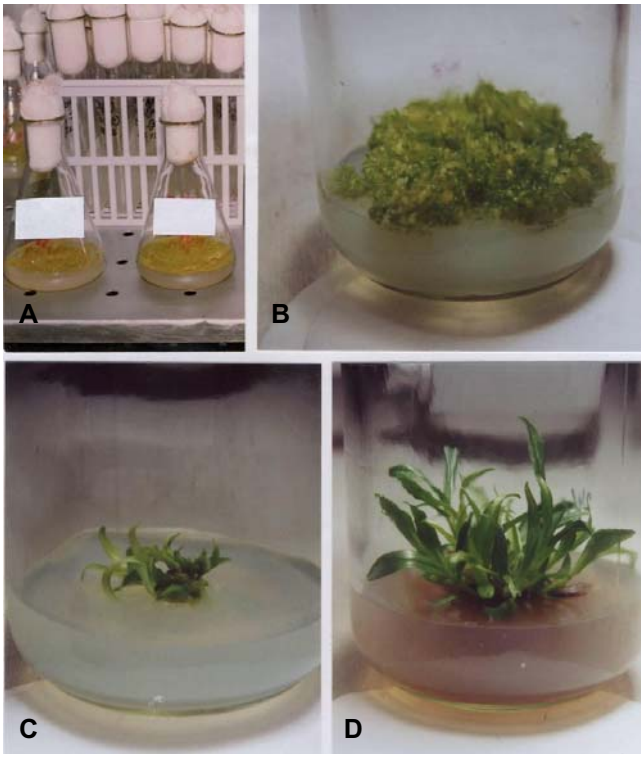

Fig. 1 Influence of 10% SSW on seed germination of *Vanda parviflora* Lindl. (**A**) *In vitro* seed germination on 10% SSW-incorporated Mitra *et al*. (1976) basal medium (bar = 1.0 cm). (**B**) Protocorm formation and growth of shoot buds with leaf primordia within 7-9 weeks (bar = 1.2 cm). (**C**) Well developed protocorms with 2-3 leaves (9-10 weeks) (bar = 0.8 cm). (**D**) Seedlings with well developed roots after 16 weeks and ready for hardening (bar = 0.8 cm).

10% (v/v) in basal medium resulted in faster differentiation of protocorms to form plantlets (i.e. leaves and roots) than the control (**Table 1**). By 60 days of culture, a significantly high percentage of propagules had reached stage 4 on SSW-supplemented basal medium regardless of the concentration compared to the control. Moreover, a higher percentage root formation (stage 6) was also observed on SSW-supplemented basal medium (**Table 1**). The effect of quickening differentiation of protocorms to form plantlets (i.e. leaves and roots) by the presence of SSW in basal medium indicated that SSW has some growth-promotive substance(s). Finally, the well-rooted shoots that regenerated on 10% (v/v) SSW-supplemented basal medium formed plants during hardening that were normal and showed healthy growth with a 90% survival rate, i.e. SSW at 10% (v/v) aids in rapid regeneration of *V. parviflora.*

Smoke influences seed germination and post-germination processes. Depending on the plant species of different geographical locations smoke treatments and butenolide applications are able to improve seedling vigour, and survival rates in some South African indigenous medicinal plants (Sparg *et al*. 2005), a commercial maize cultivar (Sparg *et al*. 2006), rice (Kulkarni *et al*. 2006), vegetables such as tomatoes, okra and beans (Jain and van Staden 2006; van Staden *et al*. 2006), grasses (Baxter and van Staden 1994; Blank and Young 1998) and woody *Acacia* species (Kulkarni *et al*. 2007). SSW was also able to stimulate somatic embryogenesis geranium (Senaratna *et al*. 1999) specifically at 10% using vegetative shoot apices of mature trees of *Pinus wallichiana* (Himalayan blue or Bhutan pine) (Malabadi and Nataraja 2007c) and, flowering in fire-lily *Cyrtanthus ventricosus* (Keeley 1993) and rooting in *Vigna radiata* (L.) Wilczek hypocotyl cuttings (Taylor and van Staden 1996). 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 four Indian indigenous medicinal plants (*Terminalia chebula*, *Holorrhina antidysentrica*, *Clitoria ternatea* and *Gymnema sylvestre*) (Malabadi and Vijay Kumar 2006). Therefore, from the above results it is clear that active compound(s) within SSW play a regulatory role in plant development. As all these physiological effects are in part controlled by plant growth regulators (PGRs), indications are that the smoke extracts interact in same way with endogenous PGRs (van Staden *et al*. 2000; Jain *et al*. 2008).

DISCUSSION

In one of our previous studies of the effect of smoke and SSW on seed germination of four medicinal plants (*Acacia pennata* (Mimosaceae), *Basella alba* (Basellaceae), *Celastrus asiatica* (Celastraceae), and *Cleome gynandra* (Cleomaceae) (Malabadi and Vijaykumar 2007), it was noticed that all plants showed a higher rate of germination under 16:8 h light/dark in the control and smoke treatments. By treating the seeds with aerosol smoke, the mean germination time for all the species was reduced. The calculated vigour index (the vigour index of one-week-old seedlings was calculated as VI = (shoot length + root length) × percentage germination) of one week-old-seedlings showed that the application (i.e. aerosol smoke, not SSW) of aerosol smoke and smoke solutions enhanced the seedling vigour of all four species. Furthermore, in most cases aerosol smoke was more effective than aqueous smoke solutions (Malabadi and Vijaykumar 2006, 2007d). At high concentrations (100: 500), smoke extracts of burnt fynbos *Passerina vulgaris* Thoday and the grass *Themeda triandra* L. inhibited seed germination while more dilute solutions (1: 500) improved the germination in dormant seeds of *Syncarpha vestita* (L.) B. Nord (Brown *et al*. 2003). However, SSW may protect seeds and seedlings against microbial attack and thus result in higher seedling survival (Light and van Staden 2004). The recent identification of the germination cue, butenolide from smoke will now allow for research into the physiological action of smoke on seed germination. SSW does not have any significant effect on the germination period of somatic embryos in all the three genotypes of *P. wallichiana* although it did affect the total number of somatic embryos that germinated (Malabadi and Nataraja 2007c). In geranium (*Pelargonium hortorum* Bailey cv. 'Elite'),

Table 1 Effect of different concentrations of SSW-supplemented Mitra *et al*. (1976) basal medium on seed germination of *Vanda parviflora* Lindl.

SSW concentrations (%, v/v)	№ of protocorms	Time taken for germination (weeks)	№ of protocorms with 2-3 leaves (%)	№ of seedlings with roots (%)
*control	12.0 ± 0.3 b	12-16	6.5 ± 0.5 b	4.0 ± 0.1 b
5	38.0 ± 0.6 b	8-10	18.0 ± 1.7 b	21.0 ± 0.4 b
control	10.0 ± 0.3 b	12-16	5.3 ± 0.7 b	8.0 ± 0.3 b
10	95.0 ± 2.6 a	8-10	87.0 ± 3.8 a	93.0 ± 3.4 a
control	11.0 ± 0.3 b	12-16	4.2 ± 0.7 b	3.0 ± 0.1 b
15	22.0 ± 1.6 b	8-10	16.0 ± 1.2 b	12.0 ± 2.3 b
control	10.0 ± 0.3 b	12-16	3.5 ± 0.2 b	5.0 ± 2.1 b
20	5.0 ± 0.2 b	8-10	7.0 ± 0.3 b	8.0 ± 1.0 b

*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).

SSW treatment (10% v/v) of the explant prior to induction, or together with the inductive signal (TDZ) produced the highest number of somatic embryos (Senaratna *et al*. 1999). In another study, the effect of butenolide, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one was tested for its effect on somatic embryogenesis with an important species for commercial horticulture, *Baloskion tetraphyllum* (Restionaceae) (Ma *et al*. 2006). It was observed that when somatic embryos of *B. tetraphyllum* were transferred to basal medium (MS) supplemented with 0.067 µM butenolide, the development of growth-competent somatic embryos was enhanced using different explants such as shoots and coleoptiles (Ma *et al*. 2006). Butenolide resulted in a high frequency of somatic embryos progressing to plantlets, and a higher number of plantlets per explant compared to non-butenolide (control) media for both shoot and coleoptile explants in *B. tetraphyllum* (Ma *et al*. 2006). These observations suggest that the active ingredient(s) in SSW play a regulatory role in plant development. The number of somatic embryos doubled following the addition of SSW at either the explant or induction stage compared to the untreated control. The inductive signals for the initiation of somatic embryogenesis of *P. wallichiana* were benzyl amino adenine BAP, α-naphthalene acetic acid (NAA) and 2-4-dichlorophenoxy acetic acid (2,4-D). SSW without BAP, NAA or 2,4-D did not induce any form of cell proliferation; however, SSW appeared to act synergistically with the inductive signal (Malabadi and Nataraja 2007c). Collectively taken, these observations suggest that SSW acts like a growth regulator than a nutritional additive. It has been suggested that smoke may have an action similar to cytokinins in breaking celery seed dormancy (Thomas and van Staden 1995).

In another recent study, the main germination active compound in smoke, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one (butenolide), was shown to have structural similarities with strigolactones that function as germination stimulants for root parasitic plants such as *Orobanche* spp. such as *O. aegyptiaca* Pers., *O. caryophyllacea* Sm., *O. cernua* Loefl., *O. corymbosa* Ferris, *O. minor* L., *O. purpurea*, *O. ramose* L., *O. rapum-genistae* Thuill., *O. uniflora* L., and *Striga* spp. such as *S. hermonthica* (Scrophulariaceae) (Daws *et al*. 2008). Butenolide stimulated germination of both *O. minor* and *S. hermonthica* to levels as the synthetic strigol analogue GR24 and was effective at similar concentrations (10^{-5} to 10^{-11} M) (Daws *et al*. 2008). Both butenolide and GR24 were more effective than the synthetic strigol analogue Nijmegan-1. Across eight further *Orobanche* spp., and for species from the root parasitic genera *Cistanche phelypaea* Cout., *Conopholis alpine* Liebm., and *Lathraea squamaria* L., butenolide also had a similar level of activity and these results suggest that the germination stimulatory activity of butenolide may result from analogy with strigolactones (Daws *et al*. 2008). The bioactivity of butenolide that is structurally related to butenolides from smoke was first identified by Pepperman and Cutler (1991) who conducted bioassays on wheat coleoptiles. These authors attributed the activity of these compounds to their structural similarities to strigolactones (e.g. strigol) which are important germination stimulants for parasitic weed species. The agricultural application of strigolactones (e.g. GR24 and Nijmegan-1) to soil to induce suicidal germination of parasitic weeds was proposed by Daws *et al*. (2008). However, such application may potentially have unwanted negative effects on soil fungi. Similarly, since butenolide is a naturally occurring chemical in fire environments, it would also be of interest to investigate any potential wider role for SSW on orchid seed germination. The cytokinin and auxin-like activity of the smoke-derived butenolide was assessed using soybean (*Glycine max* L. cv. 'Acme') callus and mungbean (*Vigna mungo* L.) rooting bioassays (Jain *et al*. 2008). In the soybean bioassay, a concentration-dependent response was recorded for both the fresh and dry weight of calli after 28 days in culture. The cellular dimensions of calli grown in the various treatments were significant indicating that the increased weight of the callus is due to an increase in cell number rather than a change in cellular dimensions (Jain *et al*. 2008). Cytokinin-like activity of butenolide (10^{-18}-10^{-8} M) was equivalent to 2.5×10^{-8} M kinetin. Butenolide treatments supplemented with 2.5×10^{-8} M kinetin increased the response of the calli with the optimum treatment (10^{-16} M butenolide) having activity equivalent to 2.5×10^{-8} M 10 μg^{-1} kinetin (Jain *et al*. 2008). A similar concentration-dependent response was recorded in the mungbean bioassay. The optimum butenolide concentration (10^{-6} M) for auxin-like activity was equivalent to 10^{-7}-10^{-6} M IBA (Jain *et al*. 2008). The addition of 10^{-7} M IBA to the various butenolide treatments increased the rooting response with the optimum treatment (10^{-18} M butenolide) having activity when applied at low concentrations as well as a synergistic effect when application is combined with either kinetin or IBA, depending on the bioassay (Jain *et al*. 2008). This response is not necessarily due to the butenolide substituting for a PGR. Rather, the observed response may be due to the butenolide interacting with endogenous hormones already present in the bioassay systems (Jain *et al*. 2008). This is the report of synergistic effects between the isolated butenolide compound and cytokinins (kinetin) and auxins (IBA) (Jain *et al*. 2008). There are other reports of aqueous smoke extracts having synergistic effects with PGRs. When SSW and gibberellic acid (GA_3) were applied alone, they were not able to break thermodormancy in lettuce (*Lactuca sativa* L. cv. 'Ruben') seeds while a combination of SSW and gibberellins was effective. 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). Application of GA_3 and SSW were also effective in breaking dormancy in celery (*Apium gravelolens* L.) seeds while SSW alone could not break this dormancy (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).

SSW is an efficient, cheap and easy way to improve the seed germination, *in vitro* development and *ex vitro* establishment of orchids. This has applications in micro-economic businesses and in biodiversity conservation, in particular of the rich orchid diversity in the Western Ghat Forests of Karnataka state.

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