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SMOKE-INDUCED SEED GERMINATION AND SOMATIC EMBRYOGENESIS

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Smoke from the combustion of plant material stimulates both seed germination and somatic embryogenesis in a wide range of species, providing a potential new class of phyto-active compounds for *in vitro* culture. Smoke saturated water (SSW) promoted asymbiotic seed germination and an early differentiation of protocorms and plant regeneration of *Vanda parviflora* Lindl. High percentage germination (95.0±2.6) followed by high percentage plantlets recovery (93.0±3.4) was achieved by culturing seeds on Mitra *et al.* basal medium supplemented with 10% v/v SSW in *Vanda parviflora* Lindl. The recent identification of the germination cue, butenolide from smoke will now allow for research into the physiological action of smoke on seed germination. Smoke extracts are also able to stimulate other growth processes such as somatic embryogenesis using vegetative shoot apices of mature trees of *P. wallichiana* (Himalayan blue or Bhutan pine) and geranium, flowering, and rooting. As all these physiological effects are in part controlled by plant growth regulator, indications are that the smoke extracts interact in same way with endogenous PGRs.

Keywords: Fire; Orchids; Seed germination; Smoke; Somatic embryogenesis.

Introduction

Wildfires are a natural and widespread feature of temperate ecosystems, and many plant species have seedling recruitment restricted to habitats created by such disturbances. Fire triggered germination is the result of either heat shock or chemical products of combustion, and species appear to utilize one or other of these modes. Heat shock-stimulated germination is widespread in the *Fabaceae*, *Rhamnaceae*, *Convolvuaceae*, *Malvaceae*, *Cisteraceae*, and *Sterculiaceae*, and is found in many ecosystems [1-3]. Smoke is an important chemical stimulant for germination of many fire type species being demonstrated first by de Lange and Boucher for a South African fynbos shrub, and later for many other fynbos species, a *savannah grass*, a Great Basin annual, and a large number of Australian heath shrubs [1-5]. Smoke-stimulated germination has been reported for the *California chaparral* annual, *Emmenanthe penduliflora*. Smoke can effectively break dormancy by directly penetrating the seed, or indirectly by adsorption onto soil particles and later release chemicals in vapors or aqueous leachate. The mechanism of smoke-induced germination is distinctly different from that of heat-shock-stimulated germination, typical of chaparral species in the *Fabaceae* and *Rhamnaceae*. Nitrogen- di-oxide is a significant component of wood smoke and appears to be an important ecological trigger in the germination of *Emmenanthe* and *Silene multinervia* and to lesser extent of *Caulanthus* and *Phacelia grandiflora*. Smoke is highly effective, often inducing 100% germination in deeply dormant seed population with 0% control germination. Smoke-stimulated species differed substantially in the duration of smoke exposure required to induce germination, and this was inversely correlated with tolerance to smoke exposure [5-9]. This review highlights about the role of smoke and aqueous smoke on seed germination and somatic embryogenesis in a wide variety of plants.

Butenolide activity and effects on seed germination- Smoke water is capable of stimulating germination of a range of smoke-response plants. Recently, a highly bioactive molecule, 3-methyl 2H-furo (2, 3-C) pyran-2-one (a butenolide compound), has been isolated from smoke fractions and identified as a key factor for stimulation of seed germination. Butenolide significantly stimulates germination in a range of species at ppb concentrations, indicating a very high biological activity comparable to that of plant growth regulators[3-9]. The mode of action of butenolide as indicated by its effect on seed germination would appear to be that of a germination inducer, mimicking the effects demonstrated by other known germination enhancers such as gibberellic acid. A *butenolide* (3-methyl 2H-furo (2, 3-C) pyran-2-one (a *butenolide* compound) was tested for its effect on somatic embryogenesis, and enhanced development of growth-competent somatic embryos. Smoke contains several thousand compounds. A highly active germination promoting compound has recently been identified as a water soluble butenolide, 3-methyl 2H-furo (2, 3-C) pyran-2-one, from the smoke of burnt fynbos *Passerina vulgaris* Thoday and the grass *Themeda triandra* L. as well as from the combustion of cellulose[5-11]. Smoke is an important factor involved in fire and post-fire germination cues. Farmers have traditionally used fire and smoke in grain drying practices. Fire is a major environmental selective force that influences plant communities in many parts of the world. Smoke from the combustion of plant material stimulates seed germination in a wide range of species[11-15]. Smoke influences not only seed germination but also there is increasing evidence that smoke also has post-germination effects. Depending upon 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. A commercial maize cultivar, rice, vegetables such as tomatoes, okra and beans, grasses and woody *Acacia* species[2-16]. Smoke extracts are also able to stimulate other growth processes such as somatic embryogenesis using vegetative shoot apices of mature trees of *P. wallichiana* (Himalayan blue or Bhutan pine), and geranium, flowering, and rooting[16-23]. Smoke-saturated water and arosol smoke by slow burning of mixture of semi-dry grasses *Aristida setacea* and *Cymbopogon martini* (Graminiaceae) improved the seed germination and seedling vigour of four Indian indigenous medicinal plants such as *Terminalia chebula*, *Holorrhina antidysentrica*, *Clitoria ternatea* and *Gymnema sylvestr*[19]. In another recent study by Malabadi and Vijaya Kumar[19], the overall germination percentage was very high when seeds were treated with different concentrations of smoke saturated water solutions including aerosol smoke against control. The vigour index of all the medicinal plants viz. *Acacia pennata* (Mimosaceae) *Basella alba* (Basellaceae), *Celastrus asiatica* (Celastraceae), and *Cleome gynandra* (Cleomaceae) increased with the applicability of dry smoke and smoke solutions at 10% (v\v) [24-29]. Therefore, from the above results it is clear that active compound(s) of smoke-saturated water plays regulatory role in plant development. As all these physiological effects are in part controlled by plant growth regulator, indications are that the smoke extracts interact in same way with endogenous PGRs [24-29].

Effect of smoke on somatic embryogenesis- On the other hand the addition of smoke saturated water (SSW) at a concentration of 10% in the medium (pre-culture, initiation, maintenance, maturation and germination) has increased the percentage of somatic embryogenesis in all the three genotypes of *P. wallichiana* as compared against control. Lower concentrations of SSW do not have any effect on embryogenesis in *Pinus wallichiana* [18]. Higher concentrations (15 and 20% v\v) of SSW inhibited somatic embryogenesis in all the three genotypes of *P. wallichiana*. Therefore, addition of 10% v\v of SSW in the DCR basal medium was found to be the optimum concentration for the entire process of embryogenesis in *P. wallichiana*[18]. At high concentrations, smoke extracts are known to inhibit seed germination and more dilute solutions improved the germination in dormant seeds of *Syncarpha vestita* (L.) B. Nord. The highest percentage of embryogenic cultures (27%) was recorded in the *P. wallichiana* genotype PW120. Lowest percentage of embryogenic cultures (13%) was recorded in the *P. wallichiana* genotype PW10 as compared against control. The presence of SSW at 10% (v\v) in maturation medium increased the rate of embryo development as evidenced by the occurrence of more number of well matured somatic embryos (PW-37; PW-34;PW120-39) recovered per gram fresh-weight of embryogenic tissue as compared against control in *P. wallichiana* [18]. In the best treatment of SSW at 10% (v\v), 39 somatic embryos of PW120 genotype were at cotyledonary stage compared to 11 in untreated controls in *P. wallichiana.* The germination of somatic embryos in all the three genotypes of *P. wallichiana* was promoted by the presence of SSW at 10% (v\v) in the germination medium as compared against control in *P. wallichiana.* Maximum number of somatic embryos germinated successfully and resulted in the recovery of maximum seedlings compared against control in *P. wallichiana.* SSW at 10% (v\v) treated somatic seedlings showed higher percentage of seedling survival. The physiological mechanism resulting in improved vigour is unknown.

However, smoke water may protect the seed and seedlings against microbial attack which can result in higher seedling survival. 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.* Both SSW at 10% (v\v) treated and untreated (control) somatic embryos have taken same days (3 weeks) for the germination. Therefore, SSW at 10% (v\v) has affected the total number of somatic embryo germination but not the germination time. In case of geranium, *Pelargonium hortorum* Bailey cv Elite, 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. 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 explant or induction stage compared to the untreated control. This study suggests that SSW may affect the process of somatic embryogenesis in a manner analogous to a plant growth regulator. Somatic embryogenesis is initiated in response to a chemical signal (s) which generally are growth regulators, which in turn alters the endogenous auxin and cytokinin concentration, with the auxin: cytokinin ratio suggested critical factor in the induction of embryogenic competence. The inductive signal for the initiation of somatic embryogenesis of *P. wallichiana* used in this study were BAP, NAA and 2, 4-D. Smoke-saturated-water (without BAP, NAA and 2, 4-D) did not induce any form of cell proliferation; however SSW appeared to act synergistically with the inductive signal. 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 [18-27].

Effect of smoke on other plant species - In another recent study, the main germination active compound in smoke, 3-methyl-2*H*-furo [2, 3-c] pyran-2-one (butenolide), has 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) [15-29]. Butenolide stimulated germination of both Orobanche minor and Striga hemonthica to levels as the synthetic strigol analogue GR24 and was effective at similar concentrations 10^{-5} to 10^{-11}M. 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 strigolactone. 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 [17-24]. The agricultural application of strigolactones (eg. GR24 and Nijmegan-1) to soil to induce suicidal germination of parasitic weeds has been proposed. 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 smoke saturated water on orchid seed germination. Similarly very recently the cytokinin and auxin-like activity of the smoke-derived butenolide using the soybean (*Glycine max* L. cultivar Acme) callus and mungbean (*Vigna mungo* L.) rooting bioassays. In the soybean bioassay, a concentration-dependent response was recorded for both the fresh and dry weight of calli 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. Cytokinin-like activity of the butenolide (10^{-18} - 10^{-8}) 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 ($10 \mu g^{-1}$) kinetin. 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. 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. 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. This is the report of synergistic effects between the isolated butenolide compound and cytokinins (kinetin) and Auxins (IBA). There are other reports of aqueous smoke extracts having synergistic effects with PGRs[26-30]. When smoke saturated water and gibberellin (GA_3) were applied alone, they were not able to break thermodormancy in lettuce seeds while a combination of smoke water and gibberellins was effective. Similarly, a combination of smoke water and cytokinin (BA) was more effective in breaking thermodormancy in lettuce seeds compared to cytokinins applied alone. Application of gibberellin (GA_3) and smoke-saturated water were also effective in breaking dormancy in celery seeds while smoke saturated water alone could not break this dormancy[20-26]. However, the mode of action of smoke-saturated water is still unknown even after the identification of a compound, 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[26-29].

Effect of smoke on orchid seed germination- The influence of smoke saturated water (SSW) on asymbiotic seed germination and an early differentiation of protocorms and plant regeneration of *Vanda parviflora* Lindl. has been studied[31]. High percentage germination (95.0±2.6) followed by high percentage plantlets recovery (93.0±3.4) was achieved by culturing seeds on Mitra *et al.*[32] basal medium supplemented with 10% v/v SSW. Rapid regeneration was observed within 60-70 days of culture on 10% v/v SSW supplemented Mitra *et al.*[32] medium where maximum percentage propagules (93.0±3.4) showed development of leaves and root formation. Another interesting factor is total duration of the time taken for germination was affected by the addition of SSW in the medium. The addition of SSW at all the concentrations (5, 10, 15 and 20% v\v) has greatly enhanced and decreased the seed germination time for only 8-10 weeks of time as compared against control (12-16)[31]. The well-rooted shoots were transferred to pots containing charcoal chips, coconut husk and broken tiles (2:2:1) and 90% survival rate was achieved. The study emphasizes the role of SSW as a natural additive at different stages of development from seed germination to plant regeneration[31]. These results also suggest that the germination stimulatory activity of SSW could be applied for micropropagation of other orchids as a low cost method[31].

Conclusion

Smoke and aqueous smoke extracts enhance both seed germination and somatic embryogenesis in a wide variety of plants. The butenolide, 3-methyl-2H-furo (2, 3-c)pyran-2-one, has been identified as a highly active germination promoter from plant –derived smoke. Further smoke-derived butenolide has both cytokinin and auxin-like activity when applied at low concentrations as well as synergistic effect when application is combined with either kinetin or IBA, depending on the bioassay. The mode of action of this active compound is still unknown. 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.

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