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Chitosan for Improving Orchid Production and Quality

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ABSTRACT

Chitosan is a deacetylated derivative of chitin that is derived from the cell walls of fungi, crustacean exoskeletons, cuticles of insects and some algae. It is considered environmental friendly for agricultural uses as it is easily degradable and non toxic to humans. Chitosan and its derivatives have been reported to elicit natural defence responses in plants and it has been used as a natural compound to control pre- and postharvest pathogenic diseases. Chitosan application has also been shown to increase yields of some agricultural crops. Chitosan has recently been reported to act as a plant growth promotor in some species including orchids. The degree of deacetylation and concentrations of chitosan have varying effects on the growth and development of orchid cultured *in vitro*. Spraying with chitosan has been shown to significantly reduce the severity of leaf spot disease in orchids. Also, it has been shown that application of chitosan to *Dendrobium* orchid plants tended to increase the size of open florets and length of the inflorescences, but did not affect the display-life of cut orchids.

Keywords: development, displayed-life, growth, inflorescence

CONTENTS

INTRODUCTION

Orchids are members of the family Orchidaceae, which is one of the largest families of flowering plants. The orchid industry is one of the most important agricultural industries in Thailand and it is based mainly on *Dendrobium* hybrids. However, other important orchid genera including *Vanda*, *Mokara*, *Ascocenda*, *Paphiopedilum* and *Cattleya* are also grown. The increase in orchid production is leading to higher public demand for technical assistance and information in order to improve orchid growth, development, production and quality. Chitosan is used in many industries including waste water treatment, membrane technology, pulp and paper, cosmetics, food industry, medical, biotechnology and agriculture (Imeri and Knorr 1998). In agriculture, there is a worldwide trend to use chitosan as an alternative compound because of its fungicidal effects and elicitation of defence mechanisms in many plant tissues. It is also useful in other ways including used as a coating material for prolonging postharvest life and limit fungal decay on strawberry and bell pepper (Terry and Joyce 2004). Moreover, the use of culture medium supplemented with 1.75 % (v/v) chitosan solution (chitogel) enhanced root and shoot biomass, photosynthesis and related parameters of grapevine plantlets *in vitro* culture (Barka *et al*. 2004). Because chitosan is biodegradable, has low potential toxicity and is common in the environment, the USA Environmental Protection Agency (EPA) concluded that chitosan is not harmful to people, pets, wildlife, or the natural environment, and also it was found to be non-toxic when fed to mice, rats, and rabbits (EPA 1995). However, chitosan is registered as a pesticide, which implies a potential for misuse or improper disposal.

This article reviews the effects of chitosan on orchid growth and development.

APPLICATION OF CHITOSAN

Chitosan is one of the most common natural polymers that can be obtained from various species, particularly from the exoskeletons of crustaceans. It is also found in cuticles of insects as well as in the cell walls of fungi and some algae (Sanford and Hutchings 1987; Sandford 1989; EPA 1995). Chitosan is a polysaccharide derived from a low acetyl form of chitin, mainly composed of glucosamine and *N*-acetylglucosamine. Its structure and composition is similar to both cellulose and chitin (Freepons 1991; Hadwiger and McBride 2006). Chitosan has a strong positive charge and it attracts negatively charged molecules. The unique physiological and biological properties of chitosan have led to its use in vari-

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ous industries for removal of metal ions from wastewater, removal of dyes, addition to animal feed and as a preservative in the food industries, for the controlled release of drugs, control of blood cholesterol, and as an additive to cosmetic products such as moisturizers, bath lotions, and face, hand and body creams (Roller and Covill 1999; Benjakul *et al*. 2000; Shahidi *et al*. 2001). Chitosan has been used in agriculture as a coating material for fruits, seeds and vegetables (Zhang and Quantick 1998; Jiang and Li 2001; Lee *et al*. 2005; Photchanachai *et al*. 2006), for controlled agrochemical release of fertilizers (Sukwattanasinitt *et al*. 2001), to stimulate plant immune systems, plant growth and plant production and also to protect plants against attack by microorganisms (El Ghaouth 1994; Hadwiger *et al*. 2002; Nge *et al*. 2006).

PHYSIOLOGICAL RESPONSES TO CHITOSAN

Role of chitosan in plant resistance to pathogens and defence mechanisms

Chitosan is an exogenous elicitor of response mechanisms and has been demonstrated to induce plant defences in tomato (Benhamou and Thériault 1992; Benhamou *et al*. 1994), cucumber (Ben-Shalom *et al*. 2003), chili seeds (Photchanachai *et al*. 2006), strawberry fruits (El Ghaouth *et al*. 1992) and rose shrubs (Wojdyla 2004). Several studies have shown that chitosan stimulates other systems involved in resistance of plants to infection (Bohland *et al*. 1997; Creelman and Mullet 1997; Vander *et al*. 1998; Kim *et al*. 2005). Chitosan induces the accumulation of phytoalexins resulting in antifungal responses and enhanced protection from further infections (Hadwiger and Beckman 1980; Vasyukova *et al*. 2001). The increase in phenolic substances following chitosan application has been reviewed (Bautista-Baños 2006). Application of chitosan and chitin oligomers increased the activities of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), the key enzymes of the phenylpropanoid pathway, in soybean leaves (Khan 2003), and sweet basil (*Ocimum basilicum* L.) (Kim *et al*. 2005). The products of PAL and TAL are modified via the phenylpropanoid pathways to produce precursors of secondary metabolites, including lignin, flavonoid pigments, and phytoalexins, which play an important role in plant-pathogen interactions (Morrison and Buxton 1993; Taiz and Zeiger 2002). Chitosan treatment increases polyphenol oxidase (PPO) activity in disease resistant cultivars (Thipyapong *et al*. 2004; Raj *et al*. 2006). Oxidation of phenolic compounds associated with enhanced resistance to pathogens may involve PPO, which could generate reactive oxygen species (ROS) (Mayer 2006). Kim (2005) reported that chitosan and methyl jasmonate increased antioxidant activity (DPPH) 3.5- and 2.3-fold in sweet basil, respectively. Pre-incubation of suspension-cultured wheat cells in a growth medium of *Pantoea agglomerans* with chitin or chitosan led to a strong increase in extracellular peroxidase activity (Ortmann and Moerschbacher 2006). Application of the soluble derivatives of chitin and chitosan to wounds of wheat plants has been shown to elicit lignification (Pearce and Ride 1982). Chitosan enhances phytoalexin production in germinating peanut (Cuero *et al*. 1991) and also induces the formation of phytoalexins in legumes and solanaceous plants (Cote and Hahn 1994). Thus, chitosan may be involved in the signalling pathway for the biosynthesis of phenolics. It has been shown that chitosan can induce chitinase and chitosanase, which are members of a group of plant pathogenesis-related (PR) proteins (Collinge *et al*. 1993; van Loon *et al*. 1994). These PR proteins can degrade the cell walls of some phytopathogens and consequently may play a role in host plant defence systems (Dixon *et al*. 1994; Graham and Sticklen 1994). Moreover, chitosan can also induce plant immune systems (systemic acquired resistance or SAR), which is long lasting and often confers broad-based resistance to different pathogens. SAR develops in uninfected parts of the plant. As a result the entire plant is more resistant to a secondary infection. Representative proteins of SAR include antifungal chitinases, β-1,3-glucanases, PR-1 and PR-5 (Sathiyaba and Balasubramaman 1998). Further studies have shown that chitosan induces the expression of various genes involved in plant defence responses such as a gene encoding PAL and protease inhibitors (Notsu *et al*. 1994; Doares *et al*. 1995; Vander *et al*. 1998). Chitosan has been shown to elicit defence genes in several species such as rice (Rakwal *et al*. 2002), slash pine (Mason and Davis 1997), and tomato (Ben-Shalom *et al*. 2000). These genetic studies implied that chitosan may involve jasmonic acid (JA) pathways since transcription activation of genes encoding PAL and protease inhibitors are induced by both JA and chitosan (Walker-Simmons *et al*. 1983; Farmer and Ryan 1992; Doares *et al*. 1995). Therefore, the antifungal action of chitosan seems to comprise more than one mode of action by which chitosan affects fungal cell wall biosynthesis and/or alteration of the ability of pathogens to infect and/or its ability to increase plant resistance.

Chitosan enhances the production of secondary metabolites

The use of biotic or abiotic elicitors is one way to increase the yields of secondary metabolites in *in vitro* cultures (Eilert 1987; Bohlmann and Eilert 1994). Supplementation of hairy root cultures of *Brugmansia candida* with chitosan at certain concentrations was found to increase the content of root scopolamine and hyoscyamine, which are valuable anticholinergic drugs employed as antispasmodics and in the treatment of motion sickness. Both are members of the tropane group of alkaloids (Hashimoto *et al*. 1993; Yamada *et al*. 1994). Hairy root cultures of *Hyoscyamus muticus* supplemented with chitosan produced 5-fold more hyoscyamine than the control (Sevón *et al*. 1992). Chitosan added to suspension cell cultures of parsley (*Petroselinum crispum*) elicited a rapid deposition of the β-1,3-glucan, callose in the cell walls and a slower formation of coumarins (Conrath *et al*. 1989). The addition of chitosan to the culture media has been shown to enhance the production of hernandulcin, a minor constituent (0.004% dry wt) of the essential oil obtained from the aerial parts of *Lippia dulcis* Trev (Sauerwein *et al*. 1991). Treating hairy root cultures of *Trigonella foenum-graecum* L. with 40 $mg.L^{-1}$ chitosan induced a three fold increase in diosgenin, a spirostanol important for the synthesis of steroid hormones (Merkli *et al*. 1997).

Effect of chitosan on plant growth

It has been reported that chitosan increased the growth rates of roots and shoots of daikon radish (*Raphanus sativus* L.) (Tsugita *et al*. 1993). Utsunomiya and Kinai (1994) applied chitosan-oligosaccharides to soil used for cultivating passionfruit (*Passiflora edulis* Sims). They showed that chitosan-oligosaccharides advance flowering time and increased flower numbers (Utsunomiya and Kinai 1994). The effect of chitosan on the growth of gerbera plants has been studied. The results showed that chitosan significantly enhanced growth factors in terms of the average values of flower-stem length, the number of growing leaves, including leaf width and length as well as the number of flowers per bush (Wanichpongpan *et al*. 2000). Chitosan also promoted growth of various crops such as cabbage (*Brassica oleracea* L. var. 'Capitata') (Hirano 1988), soybean sprouts (Lee *et al*. 2005) and sweet basil (Kim 2005). Chitogel, a derivative of chitosan, was found to improve vegetative growth of grapevine plantlets (Ait Barka *et al*. 2004). This study showed that the average O_2 production of plantlets cultured on medium supplemented with 1.75% chitogel increased 2-fold, whereas CO_2 fixation increased only 1.5-fold, indicating that chitogel had a beneficial effect on net photosynthesis in plantlets and confirmed its positive effects on grapevine physiology (Ait Barka *et al*. 2004). It

has also been shown that chitosan promotes vegetative growth and enhances various processes in developing flower buds including induction of flowering of lisianthus (*Eustoma grandiflorum*) (Ohta *et al*. 1999; Uddin *et al*. 2004). Several experiments on the effects of concentration and frequency of chitosan application were conducted in various crops such as chilli, Chinese cabbage, celery, bitter cucumber and rice (Chandrkrachang *et al*. 2003; Boonlertnirun *et al*. 2005). The data showed that chitosan concentration and frequency of application significantly increased growth rates of chilli and the harvest yield of Chinese cabbage (Chandrkrachang *et al*. 2003). However, the application of chitosan had no effects on rice. In contrast, Boonlertnirun *et al*. (2005) found that four foliar sprays with chitosan at the rate of 20 ppm gave a small increase in yield of rice. It has been reported that the addition of chitosan to a liquid culture medium also enhances shoot growth of *L. dulcis* Trev. (Sauerwein *et al*. 1991). Lee *et al*. (1999) found that chitosan treatment increases the yield and marketability of soybean sprouts. However, the mechanism of action of chitosan on plant growth remains unclear.

Other effects of chitosan on plant responses

Chitosan was found to reduce plant transpiration in pepper plants resulting in a 26-43% reduction in water use while maintaining biomass production and yield. These results suggested that chitosan might be an effective antitranspirant to conserve water use in agriculture (Bittelli *et al*. 2001). Increasing levels of abscissic acid (ABA), which plays a key role in the regulation of water use by plant resulted in closure of stomata and decreased transpiration (Willmer and Pricker 1996). Sembdner and Parthier (1993) reported that ABA exhibited some activities similar to jasmonic acid. Therefore, stomata closure induced by foliar application of chitosan may influence pathways involving jasmonic acid or ABA. Azian *et al*. (2004) reported that vase solutions containing chitosan at 25 and 50 mg.L^{-1} increased the vase life of cut chrysanthemum by 13 and 15 days, respectively, compared to control flowers that had a vase life of 6.8 days. The application of chitosan combined with ammonium carbonate offers a commercially acceptable, economically viable and effective alternative for postharvest control of anthracnose in stored papaya. Dipping papaya in chitosan plus ammonium carbonate, significantly (P<0.005) retarded colour development of skin and flesh, increased fruit firmness and reduced weight loss (Sivakumar *et al*. 2005).

EFFECT OF CHITOSAN ON ORCHIDS

Chitosan sprays (10 mg.L^{-1}) significantly increased growth of young orchid plants (Chandrkrachang 2002). Limpanavech *et al*. (2003) studied the effects of concentration, degree of deacetylation, and polymerisation of chitosan at deflasking, on growth and development of *Dendrobium* Sonia Jo 'Eiskul', the major cut flower orchid of Thailand. The oligomeric and polymeric forms of chitosan with 70, 80, and 90% deacetylation (%DD) at concentrations of 1, 10, 50 and 100 mg.L^{-1} had no effect on vegetative growth, but chitosan induced early flowering in *Dendrobium* Sonia Jo 'Eiskul'. Further experiments were conducted *in vitro* to determine the effects of concentrations, deacetylation rate, and polymer size of chitosan on growth of *Dendrobium* Sonia Jo 'Eiskul' protocorm-like bodies (PLBs). Six types of chitosan were used; polymer (P) and oligomer (O) with degrees of deacetylation of 70%, 80% and 90%, and molecular weight (MW) of P = 400,000-500,000 and O = 30,000-100,000 at 6 different concentrations, 0, 10, 20, 40, 80, and 160 mg.L^{-1}. The results showed that PLBs were bleached and killed when treated with 160 mg.L^{-1} of any chitosan type. Chitosan concentration of 80 mg.L^{-1} inhibited growth of PLBs. However, all molecule types of chitosan in this experiment significantly enhanced PLB growth at concentrations of 10 and 20 mg.L^{-1} of P70 and P90 respectively (Pornpeanpakdee *et al*. 2006). Nge *et al*. (2006) studied the effects of chitosan of various molecular weights on the growth of *Dendrobium phalaenopsis* PLBs *in vitro*. It was found that application of shrimp chitosan with a molecular weight of 1 kDa accelerated the growth and development of the meristematic tissue of orchid from explants to PLBs in liquid media, more than treatment with 10 and 100 kDa chitosan respectively. Fungal chitosan (10 kDa) at 15 mg.L^{-1} was more effective than shrimp oligomer chitosan at 1 and 10 kDa for increasing growth of orchid PLBs after six weeks of cultivation. Moreover, application of fungal chitosan generated the highest number of orchid plantlets (Nge *et al*. 2006). Chandrkrachang (2002) reported that chitosan sprays at 2.5-40 mg.L^{-1} increased leaf length of *Paphiopedilum* orchid. Kliangkeaw *et al*. (2003) showed that chitosan increased the growth of *Paphiopedium bellatulum* x *Paph*. Angthong in tissue culture. Encapsulating seeds of *Spathoglottis plicata* with alginate-chitosan has been reported to minimize infections by mycorrhizal fungi (Tan *et al*. 2004). Limpanavech (2003) reported that chitosan at 10 mg.L^{-1} enhanced the growth and the number of PLBs of *Dendrobium formosum* Roxb. *ex* Lindl, and *Paphiopedilum sanderianum* (Rchb. f.). The induction of root and leaf growth in *D. formosum* by treatment with chitosan depended on the composition of the growth medium (Limpanavech 2003). Pichyangkura (2004) demonstrated that the effectiveness of chitosan depended on molecular weight, ratio of sugar carbons to glucosamine and *N*-acetyl-glucosamine, and the concentration and frequency of applications. Spraying with chitosan has been shown to significantly reduce severity leaf spot disease and increase the length of inflorescences in *Dendrobium* Missteen (three and half years old), however there was no effect on floret size and new shoot growth (Win *et al*. 2005). Chitosan-treated orchid plants (*Dendrobium* Sensational 'Purple') had more flower shoots and yields tended to be higher on extra grade compared to the control plants (Chandrkrachang *et al*. 2005). *Dendrobium* Sonia 'No. 17' with immature inflorescences were sprayed six times with chitosan at 0 (water), 200, 400, or 600 mg.L^{-1} at weekly intervals. The data showed that florets sprayed with 600 mg.L^{-1} chitosan had significantly higher fresh weights (29 g compared to 26.8 g for the untreated controls). Spraying with chitosan at 600 mg.L^{-1} increased the width of the sepals at harvest (Uthairatanakij *et al*. 2006).

CONCLUSIONS

Chitosan can be use as a plant growth enhancer for orchid production especially for immature plants or in tissue culture. It increases the length of the stalks of *Dendrobium* 'Missteen'. Possibly, chitosan may induce a signal to synthesize plant hormones such as gibberellins. In addition, chitosan may enhance growth and development by some signalling pathway related to auxin biosynthesis via a tryptophan-independent pathway. However, chitosan has inconsistent effects on growth and development of mature orchid plants. Chitosan can reduce disease severity in orchids, possibly by increasing the activity of PAL and PPO, lignification resulting from increased biosynthesis of phenolic compounds or induced secondary metabolites and SAR. Also, increased disease resistance may be mediated in part via an increase in the concentrations of jasmonic acid. Moreover, resistance to disease infections may also involve closure of stomata by ABA.

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