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Chitosan as a growth stimulator in orchid tissue culture

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Abstract

The effect of shrimp and fungal chitosan on the growth and development of orchid plant meristemic tissue in culture was investigated in liquid and on solid medium. The growth of meristem explants into protocorm-like bodies in liquid medium was accelerated up to 15 times in the presence of chitosan oligomer, the optimal concentration being 15 ppm. The 1 kDa shrimp oligomer was slightly more effective compared to 10 kDa shrimp chitosan and four times more active compared to high molecular weight 100 kDa shrimp chitosan. The 10 kDa fungal chitosan was more effective compared with 1 kDa oligomer. The development of orchid protocorm into differentiated orchid tissue with primary shoots and roots was studied on solid agar medium. The optimal effect, the generation of 5–7 plantlets in 12 weeks was observed in the presence of 20 ppm using either 10 kDa fungal or 1 kDa oligomer shrimp chitosan. The data are consistent with preliminary results from field experiments and confirm unequivocally that a minor amount of chitosan has a profound effect on the growth and development of orchid plant tissue.

Keywords: Fungal chitosan; Meristematic bud; Orchids; Protocorm; Shrimp chitosan

1. Introduction

Chitin is a natural polysaccharide, which consists of a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine residues, linked by β-1,4 glycosidic bonds. It is present in a variety of species: in shells of crustaceans, in cuticles of insects and in the cell wall of fungi and some algae. The deacetylated form of chitin is chitosan. The chitosan biopolymer has applications in waste water treatment, pulp and paper, in medical and cosmetic products, biotechnology, food and feed and membranes [1]. In agriculture, chitosan has been used in seed, leaf, fruit and vegetable coating [2], as fertilizer and in controlled agrochemical release [3], to increase plant product [4–6], to stimulate the immunity of plants [7], to protect plants against microorganisms [8–10] and to stimulate plant growth. In the latter studies, a positive effect of chitosan was observed on the growth of roots, shoots and leaves of various plants including *Gerbera* [4] and of several crop plants [11]. Orchid roots sprayed with a very diluted chitosan solution show stimulation of growth, renewed flower production and enhanced resistance against fungi and virus [5].

Among the commercially important orchids, *Dendrobium* accounts for about 80% of the total of micropropagated tropical orchids [12]. *Dendrobium* plants are used for the production of orchid flowers [13] and in traditional Chinese medicine [14]. *Dendrobium* is micropropagated in tissue culture by protocorm-like bodies (PLB) but the growth is very slow [15]. In order to stimulate efficient micropropagation of PLB, much effort has been directed to modify the culture media, mainly by the inclusion of plant growth regulators [16,17] such as N^6-benzylaminopurine and α-naphthaleneacetic acid [18], benzyladenine, gibberellic acid and 3-indoleacetic acid [19], various polyamines [20], N^6-benzyladenine and thidiazuron [21,22].

Plant tissue culture is particular suitable to study the effect of chitosan on plants that grow and multiply slowly, like orchids. In the work presented here, the formation of protocorm-like bodies in meristem buds and the growth and differentiation of orchid protocorm have been studied under the controlled conditions of tissue culture in the absence and presence of chitosan. Various chitosan preparations, both of crustacean and fungal origin have been compared in various concentrations.

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The formation of differentiated orchid tissue has been studied in liquid and on solid media. Precise data on the effectiveness of chitosan as growth stimulator are presented.

2. Materials and methods

2.1. Preparation of meristematic auxillary buds and protocorm-like bodies for explants

Young *Dendrobium phalaenopsis* orchid plants were obtained from Orchid Garden, Ratchaburi, Thailand. Shoot segments with one to two meristematic auxillary buds (6–15 mm) were excised. These shoot pieces were washed with soap and tap water three times and immersed in antiseptic solution (5.25% sodium hypochlorite with a few drops of soap) to disinfect the surface. After that the shoot segments were rinsed several times in sterilized distilled water to remove the antiseptic solution and non-attached tissue. Meristematic auxillary buds were excised from these shoot segments and cultured in liquid modified VW medium [23]. *Dendrobium* orchid protocorms three months of age were obtained from the Plant Tissue Culture Laboratory, Chatuchak Weekend Market, Bangkok, Thailand.

2.2. Culture medium and conditions

The VW medium [23] contained 20 g/l sucrose, 150 ml/l coconut water, 0.525 g/l KNO_3, 0.25 g/l KH_2PO_4, 0.5 g/l $(NH_4)_2SO_4$, 0.007 g/l $MnSO_4{\cdot}4H_2O$, 0.025g/l $MgSO_4{\cdot}7H_2O$, 0.028 g/l $FeSO_4{\cdot}7H_2O$ and 0.037 g/l Na_2-EDTA. Coconut water was obtained from local market, Bangkok, Thailand. The media were supplemented with various amounts of chitosan, either chitosan from shrimp shell with a molecular weight of 100 or 10 kDa [24]. Oligomer 1 kDa chitosan was obtained from Kittolife Co. Ltd., Korea. The 10 kDa chitosan was isolated from fungal cell wall [25]. The pH of the medium was adjusted to 4.9 for liquid medium. Solid medium was prepared by adding agar (12 g/l supplied by Sigma) to the complete liquid medium. Liquid medium (50 ml) in the 250 ml Erlenmeyer flasks and 10 ml of solid agar medium in the culture tubes (150 mm × 25 mm) were autoclaved for 20 min at 121 °C.

Meristematic auxillary buds were used as explants in the liquid medium. Protocorm-like bodies (PLB) (0.03–0.04 g fresh weight) were used as explant in liquid and agar medium. Liquid cultures were maintained at 27 °C and 100 rpm. Solid cultures were kept at 27 °C and provided photoperiods of 16 h per day using cool white fluorescent tubes (TLD 18 W/54 ϕ 4A, Daylight 1030 lm, 57 lm/W, Philips, Thailand).

2.3. Data collection and analysis

Visual observations were made every day. Total fresh weight of protocorm-like bodies was recorded at 0 and after 3, 6, 8 and 12 weeks. The number of plantlets generated by the protocorm-like bodies maintained on agar medium were counted after 12 weeks of cultivation. Three replicates were carried out in each experiment. All data were analyzed by SPSS software, version 7.2 using one-way ANOVA analysis. All photographs are representative for the growth condition as described.

3. Results and discussion

3.1. Effect of shrimp chitosan on orchid meristematic bud growth in liquid medium

Meristematic buds of mature orchid plants form initially a protocorm-like body (PLB) when cultivated in VW liquid medium. The growth of meristematic tissue is dependent on size of the inoculum and on the conditions of growth. PLB were grown in liquid media supplemented with 15 ppm of shrimp chitosan of different molecular weights (100, 10 kDa and oligomer 1 kDa). After three weeks, orchid meristem buds cultures supplemented with oligomer chitosan showed more cell propagation than the cultures supplemented with 10 and 100 kDa chitosan ($p < 0.05$). The cell mass in the former culture medium increased to about 15 times after six weeks cultivation. High molecular weight chitosan (100 kDa) had no stimulating effect (Fig. 1).

The effect of chitosan concentration on the growth of PLB's from meristematic buds was investigated in liquid medium supplemented with 0, 5, 10, 15, 20 and 25 ppm of oligomeric chitosan. The medium supplemented with 10 and 15 ppm produced PLB larger in size in comparison to the other treatments. Maximum fresh weight of PLB's was obtained in culture media supplemented with 15 ppm oligomer chitosan (Fig. 2). Under this condition the excised meristem tissue initially increased in size and changed into small round bodies during the first three weeks, after that juvenile leaves appeared after five weeks (Fig. 3). In media with 5, 20 and 25 ppm oligomer chitosan PLB, increase in size was not observed after six weeks of cultivation.

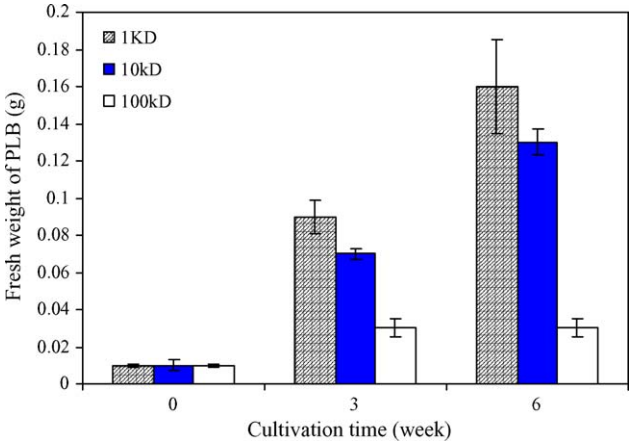

Fig. 1. Effect of shrimp chitosan with different molecular weights. Orchid protocorm-like bodies (PLB's) were cultivated during 0, 3 and 6 weeks on VW liquid medium supplemented with shrimp chitosan with molecular weights of 1 kDa (oligo-chitosan), and of 10 and 100 kDa. Standard deviation was calculated from three independent experiments by one-way ANOVA.