Effects of Chitosan with or without Nitrogen Treatments on Seedling Growth in *Eustoma grandiflorum* (Raf.) Shinn. cv. Kairyō Wakamurasaki

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Summary

Effects of applying chitosan [poly-(1 → 4)-β-D-glucosamine] and water soluble inorganic fertilizer to soils on seedling growth of *Eustoma grandiflorum* (Raf.) Shinn. cv. Kairyō Wakamurasaki were investigated. The treatment of 1.0% chitosan soil mixture (w/w), combined with a fertilizer containing the same amount of nitrogen as in 1.0% chitosan, significantly promoted plant growth, compared to the treatments of 1.0% chitosan soil mixture and of fertilizer with equal or double amounts of inorganic nitrogen contained in the 1.0% chitosan, when measured 7 weeks after sowing. Nitrogen and phosphorus concentrations in the shoot and root in the fertilizer or chitosan combined treatment were higher than those in the untreated control.

Chitosan supplementation into *in vitro* culture of seedlings also promoted plant growth regardless of chitosan concentration, compared with chitosan-free, half or full strength of Murashige–Skoog medium.

Key Words: chitosan [poly-(1 → 4)-β-D-glucosamine], culture experiment, *Eustoma grandiflorum* (Raf.) Shinn., *in vitro* culture.

Introduction

The period of growth of *Eustoma grandiflorum* (Raf.) Shinn. seedlings is very long. That is, the pre-transplantation period of seedlings ranges from 50 to 140 days. Matsuo and Shirasaki (1990) reported that the dry weight of the plant increased with fertilizer concentration, whereas we found that seedlings of *E. grandiflorum* grew faster with the chitosan treatment (Ohta et al., 1995).

Chitosan promotes plant and root growth of the radish (Tsugita et al., 1993). When combined with a nitrogen-rich fertilizer, chitosan also advanced flowering time, fruit yield, and fruit weight and increased the number of flowers in purple passionfruit (Utsunomiya et al., 1998). These reports suggest that chitosan which contains about 8.7% nitrogen may promote both vegetative and reproductive growth of some plants.

The objective of this study was to investigate whether promotion of early plant growth in chitosan-treated seedlings of *E. grandiflorum* is merely due to the nitrogen-rich effect of the medium or whether it involves an elicitor-like effect.

Materials and Methods

Culture experiment in greenhouse

Seeds of *E. grandiflorum* cv. Kairyō Wakamurasaki were used in this experiment. Chitosan [poly-(1 → 4)-β-D-glucosamine] (Sigma Co Ltd., USA) milled to a fine powder by an electrical mill which was mixed with sandy loam soil to yield a 1.0% chitosan (w/w) medium. For the fertilizer treatment, 300 ml of a water-soluble fertilizer (N:P₂O₅:K₂O=15:8:17) was applied twice a week for 7 weeks to each tray at equal to or double the amount of nitrogen (0.2 or 0.4 M over the total period) contained in the chitosan. A combined treatment of 1.0% chitosan soil mixture and nitrogen fertilizer (0.2 M) was also used. All seeds were sown in the medium in 144-cell polystyrene foam trays. About 10 seeds were sown into each cell (32 mm diameter, 50 mm depth). Three trays per treatment were placed in a greenhouse kept at a minimum of about 18 °C. Seedlings were thinned to two per cell in the tray at the two-pair leaf stage.

At 7 weeks after sowing, 15 plants per treatment were measured for leaf length and width, maximum root length and dry weights of the shoot and root.

The shoots and roots of plants were analyzed for N, P, K, Ca, and Mg. N was analyzed by the Kjeldahl method and P was determined by the vanadomolybdophosphoric colorimetric method at 430 nm. K, Ca, and Mg were analyzed by atomic-absorption spectrophotometry.

In vitro culture

Seeds were sterilized in a solution of sodium hypochlorite (0.7% active chlorine) for 5 min., then rinsed

twice with sterile water and sown. Thirty seeds (3 seeds $\times$ 10) per treatment were put into a glass bottle (80 mm diameter, 130 mm height) that contained agar-solidified medium. The basal medium consisted of MS major elements and Fe-EDTA (Murashige and Skoog, 1962) plus Ringe and Nitch micro-elements and vitamins (Ringe and Nitch, 1968), 2% sucrose, and 0.8% agar at pH 5.6. The cultures were placed under 16-hr illumination of $52 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of cool-white fluorescent light at $20 \pm 1^\circ\text{C}$.

The treatments utilized half-strength (1/2MS) or full strength of MS (1/1MS) basal medium or 0.001%, 0.01% or 0.02% chitosan-supplemented 1/2 MS medium. Lactic acid at 0.0025% was also added to the 1/2 MS medium to dissolve the powdered chitosan. Three bottles were used for each treatment; all treatments

were replicated thrice. Nine weeks after treatment, leaf length and width, maximum root length, and dry weights of shoot and root were recorded.

Results and Discussion

Culture experiment in greenhouse

At 7 weeks after sowing in the greenhouse, leaf size at the 1st node in the chitosan soil mixture treatment, fertilizer treatment or the combination of the two was greater than that of untreated control (Table 1). The leaf at the 3rd node in the combined treatment was larger than that of any other treatment. The same tendency was observed in the leaf at the 5th node. Ohta et al. (1995) reported that leaves of *E. grandiflorum* grew better than on the chitosan-free one. The growth promotion of *E.*

Table 1. Effects of chitosan and fertilizer treatments on plant growth of *Eustoma grandiflorum* cv. Kairyoku Wakamurasaki seedlings for 7 weeks.

Treatment	1st node		3rd node		5th node		Maximum root length (cm)	Dry weight	
	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)		Shoot (mg)	Root (mg)
Untreated control	11.4a ^z	4.9a	1.5a	0.8a			4.9a	6a	1a
1.0% chitosan soil mixture	16.0b	7.3b	52.2b	17.1b	2.1 ± 1.42^y	0.7 ± 0.47	6.7c	55b	15c
Fertilizer with an equal amount of nitrogen as in the chitosan	14.6b	6.6b	61.9c	19.7c	8.8 ± 1.34	2.9 ± 0.43	5.9b	67b	11b
Fertilizer with double amount of nitrogen as in the chitosan	12.8a	6.5b	54.1b	19.7c	8.0 ± 1.93	2.6 ± 0.63	5.9b	69b	11b
1.0% chitosan soil mixture combined with an equal amount of nitrogen in the chitosan	15.1b	6.9b	71.3d	22.7d	17.5 ± 1.44	5.9 ± 0.45	5.5ab	87c	12bc

^z Values within each column with different letters differ significantly at $P=0.05$ by Duncan's new multiple range test.

^y Mean $\pm$ standard error.

Table 2. Effects of chitosan treatment on plant growth of *Eustoma grandiflorum* cv. Kairyoku Wakamurasaki seedlings cultured *in vitro* for 9 weeks.

Treatment	1st node		2nd node		3rd node		Maximum root length (cm)	Dry weight	
	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)		Shoot (mg)	Root (mg)
1/2MS	11.7a ^z	4.4	17.3a	5.1a	6.5a	2.7a	3.1a	5.4a	2.1c
1/1MS	12.9ab	4.5	16.5a	4.9a	3.9a	1.7a	3.1a	4.0a	0.7a
0.0025% lactic acid	14.6bc	4.5	18.9a	4.7a	13.6b	4.5b	3.4ab	4.8a	1.4b
0.001% chitosan mixture	17.5d	5.1	27.0b	6.5b	17.0b	5.3bc	6.1c	8.4a	2.0c
0.01% chitosan mixture	16.2cd	4.9	26.0b	6.9b	17.1b	5.8c	4.7c	9.1b	1.9bc
0.02% chitosan mixture	17.0d	4.9	26.5b	6.8b	16.9b	5.5bc	4.0b	8.6b	2.0c
ns									

^z Values within each column with different letters differ significantly at $P=0.05$ by Duncan's new multiple range test.

grandiflorum cv. Miyakomurasaki followed a similar trend as that of the cultivar used in this experiment (data not shown). At 7 weeks after sowing we found that the maximum root length in the 1.0% chitosan soil mixture was greater than in all other treatments. The dry weight of the shoot was increased significantly by the combined chitosan/nitrogen treatment. The dry weights of roots in all plots were heavier than those of the untreated control.

In chrysanthemum, plant growth was promoted by increasing the nitrogen concentration (Kageyama et al., 1991). Our data shows that plant growth was similarly promoted by the fertilizer treatments or the chitosan treatment. The combined treatment promoted plant growth to a greater extent than the individual treatments. Thus, chitosan supplement for growth promotion may be due to a nitrogen effect, but it may involve an elicitor effect as reported in tobacco (Suzuki and Shinshi, 1998) in which oligo chitosan promoted plant cell growth. This hypothesis is also supported by the inorganic analysis of *E. grandiflorum*; nitrogen and phosphorus concentrations in the chitosan-treated plants were almost equal to those in the untreated control plants, whereas those in fertilizer-treated plants were higher than the untreated control (data not shown). The concentration of the other elements did not significantly differ in any of the treatments.

In vitro culture

For *in vitro* culture, the 1st, 2nd, and 3rd node leaf lengths in chitosan mixture treatments were the longest of all the treatments (Table 2), whereas the 1st leaf width did not differ among any of the treatments; the 2nd and 3rd leaf widths in chitosan soil mixture treatments were wider than those in 1/1 MS medium or lactic acid-supplemented 1/2 MS medium. These results suggest that chitosan has an elicitor-like effect.

In conclusion, the chitosan soil mixture treatment combined with N-enriched fertilizer promoted plant growth of *E. grandiflorum* seedlings, as did the chitosan treatment in *in vitro* culture. Thus, chitosan has a stimulative effect that is similar to an elicitor on the growth of *E. grandiflorum*.

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トルコギキョウ ‘改良若紫’ におけるキトサン処理が幼苗の生長に及ぼす影響

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摘 要

トルコギキョウ ‘改良若紫’ においてキトサンの土壌混和処理および水溶性無機肥料の施与を行い、栽培試験により生長量を調査した。その結果、播種7週間後において、1.0% キトサン土壌混和処理はキトサンに含まれている無機窒素含量と等量および倍量区に比べて同等の生育を示した。とりわけ、1.0% キトサンと窒素等量組み合わせ区では最も生長が促進された。地上部と地下部の多量元素を調査した結果、窒素およびリンの含量は肥料処理区またはキトサンと窒素等量組み合わせ区で対照区より多くなった。

また、1/2MS培地に無菌播種した場合、添加したキトサン濃度に関係なくキトサン添加処理で植物体の生長が促進されたことが認められた。