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Effects of Nitrogenous Organic Compounds on Growth and Flowering in *Eustoma grandiflorum* (Raf.) Shinn.

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***Abstract*. We previously reported that growth of *lisianthus* [*Eustoma grandiflorum* (Raf.) Shinn.] seedlings is accelerated by amending the growing medium with 1% (w/w) chitosan. This finding prompted us to search for organic nitrogenous other substances like chitosan which could accelerate seedling growth. Seeds of *E. grandiflorum* 'Peter blue line 2' were sown in a sandy loam growing medium containing 1% (w/w) chitosan, tryptone, casein, collagen or gelatin. At eleven weeks after sowing, leaf length and width, fresh and dry weights of the shoots and roots of twelve plants were determined for each treatment. Eleven weeks after sowing, the leaves at the fifth node had expanded in the chitosan, tryptone and collagen treatments while the leaves of the third node had not yet expanded in control plants. Fresh and dry weights of shoots and roots were significantly greater for plants grown in media amended with chitosan or tryptone. Percent nitrogen (N) and potassium (K) in the shoots and roots and percent phosphorus (P) in the shoots was greater only in the N side dressing treatment. The nitrate nitrogen (NO_3-N) concentration was significantly greater in media amended with tryptone or collagen compared to the other treatments.**

In our previous study (Ohta et al., 1999, 2001), it was found that amending a soil-based medium with 1% (w/w) chitosan greatly accelerated seedling growth and improved cut flower quality in *E. grandiflorum*. Chitosan has also been reported to increase plant growth in several other crops including *Raphanus satives* L. (Chibu et al., 1999), *Brasica oleracea* L. (Hirano, 1988) and *Passiflora edulis* Sims (Utsunomiya et al., 1998). These improvements in growth caused by chitosan are possibly not due solely to nitrogen (N) (Ohta et al., 2000). We speculated that chitosan had an elicitor-like effect which stimulated plant growth, subsequently increasing flower yield in *E. grandiflorum*. Hasegawa (1987) reported that tryptone increased plant growth in cymbidium (*Cymbidium kanran* Makino). Kobayasi et al. (1995) reported that organic N promoted the growth of snap beans (*Phaseolus vulgaris* L.). The objective of this study was to search for other organic nitrogenous substances which promotes seedling growth like chitosan, and to examine the feasibility of using them to enhance cut flower production in *E. grandiflorum.*

Materials and Methods

Seeds of *E. grandiflorum* 'Peter blue line 2' were sown in sandy loam medium containing 1% (w/w) chitosan, tryptone, casein, collagen or gelatin using 72-cell (32-mm-diameter, 50-mm-deep) polystyrene trays. The total N content and source of each amendment is given in Table 1. About 10 seeds were placed in each cell and three trays (replicates) were used for each treatment. The chitosan and other organic N substances were ground to a fine powder in an electrical mill (TI-100; CMT Co., Ltd., Tokyo, Japan). Two additional treatments were used. In one treatment, a commercial water-soluble fertilizer (15N–8P–17K) was applied twice a week with N at 300mg·L^{-1} ; plants were fertilized for 8 weeks commencing at 3 weeks after sowing at equal the amount of N contained in the chitosan over the total growing period. In another treatment, plants were grown in sandy loam not amended with an organic N source ; plants were not fertilized with water-soluble fertilizer. Trays were kept in a greenhouse in which air temperature was 20 to 30 and irradiance ranged from 200 to 1500µmol sec^{-1}m^{-2}. All the seedlings were watered as needed and fertilized weekly with 300 mg·L^{-1} N using 15N–8P–17K water-soluble fertilizer. The seedlings were thinned to two per cell when two pairs of leaves had expanded.

At 11 weeks after sowing, leaf length and width, maximum root length, and fresh and dry weights of the shoots and roots were determined using 12 plants per treatment. N in the shoots and roots was analyzed by the Kjeldahl method, and P was determined by vanadomolybdophosphoric colorimetric method (Jones and Case, 1990) at 430 nm. K, Ca, and Mg were analyzed by atomic-absorption spectrophotometry (Jones and Case, 1990). The NO_3-N concentration in the medium was measured at 11 weeks after sowing using a compact NO_3^- ion meter (C-141; Horiba Co. Ltd., Kyoto, Japan). All treatments were replicated three times (nine trays total per treatment).

When the seedlings had produced three pairs of true leaves, 15 seedlings from each treatment were selected at random and transplanted into plastic containers (53 cm long, 33 cm wide, 19 cm deep, ≈27 L) filled with 1 sandy loam : 1 bark (v/v) potting mix. For each container, 10 g of a granular fertilizer (6N–40P–6K) was incorporated into the medium. Each treatment was replicated three times. The date of first flowering of each plant was recorded. At anthesis of the third flower, 15 plants per treatment were harvested and number of nodes, shoot length, stem diameter, flower weight and number of flowers were determined. A randomized block design was used and data were analyzed by analysis of variance, using Duncan's multiple range test at $P \leq 0.05$ for mean separation.

Results and Discussion

At 11 weeks after sowing, the leaf length and width at the 1st node in plants growing in media amended with chitosan became larger significantly compared to the other treatments. The leaf length at the 2nd node in plants growing in media amended with chitosan and tryptone became larger significantly compared to the other treatments. The leaf length at the 3rd node in plants growing in media amended with chitosan, trypton, casein, collagen and gelatin became larger significantly compared to the control and N side dressing treatments. The leaf length at the 4th node in plants growing in media amended with chitosan and collagen became larger compared to the other treatments. In the 5th node, the leaves of chitosan, tryptone and collagen soil mix treatments only had expanded but not in other treatments (Table 2). However, there were no differences

Table 1. Total N contents in medium contained each amendment and their source.

Soil mix treatment	Total N content (%)	Source
Control	---	Kasahara Ind. Ltd., Tokyo, Japan
Chitosan	7.0	Sigma, St Louis, U.S.
Tryptone	13.3	Wako pure chemical Ind. Co., Osaka, Japan
Casein	14.5	Wako pure chemical Ind. Co., Osaka, Japan
Collagen	8.0	Wako pure chemical Ind. Co., Osaka, Japan
Gelatin	18.0	Wako pure chemical Ind. Co., Osaka, Japan
N side dressing	15.0	Otsuka chemical Co., Ltd., Osaka, Japan[z]

[z]Liquid fertilizer.

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Table 2. Effects of treatments on leaf length and width in *Eustoma grandiflorum*'Peter blue line 2'seedlings at 11 weeks after sowing.

	1st node		2nd node		3rd node		4th node		5th node	
Treatment	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)	Leaf length (mm)	Leaf width (mm)
Control	18.3 a[z]	9.8 a	26.2 a	14.7 ab	19.3 a	8.3 a	---[y]	---	---	---
Chitosan	25.3 b	13.9 c	36.6 c	15.8 b	29.9 c	12.2 b	24.0 ± 1.1[x]	10.3 ± 0.6	12.2 ± 2.1	6.3 ± 1.3
Tryptone	16.8 a	9.9 a	34.1 c	18.2 b	28.3 c	11.5 b	18.7 ± 1.9	6.9 ± 0.7	7.3 ± 1.0	2.5 ± 0.3
Casein	20.1 a	12.5 bc	30.3 b	15.4 b	31.8 c	13.9 c	15.6 ± 1.8	6.8 ± 0.6	---	---
Collagen	20.0 a	10.6 a	29.5 ab	13.8 a	30.0 c	12.4 bc	21.4 ± 2.2	8.8 ± 0.9	12.6 ± 2.0	6.2 ± 1.1
Gelatin	17.8 a	10.6 a	26.7 a	15.3 ab	28.3 c	12.0 b	12.0 ± 1.2	5.5 ± 0.4	---	---
N side dressing[w]	20.8 a	10.8 ab	29.1 ab	16.2 b	24.0 b	12.0 b	11.6 ± 2.1	5.2 ± 0.9	---	---

[z]Mean separation within columns and times by Duncan's multiple range test, $P < 0.05$.
[y]Seedlings did not form nodes.
[x]Mean ± SE.
[w]Fertilizer with an equal amount of total nitrogen as the chitosan.

Table 3. Effects of substances on maximum root length and fresh and dry weight of shoots and roots in *Eustoma grandiflorum* 'Peter blue line 2'seedlings at 11 weeks after sowing.

	Maximum root	Fresh wt (mg)		Dry wt (mg)	
Treatment	length (cm)	Shoot	Root	Shoot	Root
Control	7.8	390.6 a[z]	358.6 ab	44.3 a	24.7 a
Chitosan	7.7	937.0 d	629.6 d	121.8 d	72.3 c
Tryptone	8.0	885.1 cd	749.0 d	113.3 d	64.3 c
Casein	7.1	756.3 c	473.9 bc	96.8 c	51.7 b
Collagen	7.0	750.0 c	555.0 cd	95.8 c	51.4 b
Gelatin	7.3	566.0 b	419.7 abc	70.0 b	38.0 ab
N side dressingy	7.2[NS]	553.1 b	330.2 a	65.3 b	25.9 a

[z]Mean separation within columns and times by Duncan's multiple range test, $P \leq 0.05$.
[y]Fertilizer with an equal amount of total nitrogen as the chitosan.
[NS]Nonssignificant at $P < 0.05$.

Table 4. Effects of some nitrogenous organic compounds on N, P, K, Ca, and Mg percentage on the shoots and roots of *Eustoma grandiflorum* 'Peter blue line 2'seedlings at 11 weeks after sowing.

		N	P	K	Mg	Ca
Tissue	Treatment	(%/dry wt)				
Shoot	Control	2.44 ab[z]	0.28 b	2.11 b	0.28 b	0.07 ab
	Chitosan	1.87 a	0.27 b	1.67 a	0.28 b	0.04 a
	Tryptone	2.29 ab	0.24 b	2.17 b	0.35 b	0.09 b
	Casein	2.09 a	0.17 a	1.49 a	0.19 a	0.04 a
	Collagen	1.98 a	0.18 a	1.69 a	0.26 b	0.05 a
	Gelatin	2.66 b	0.19 a	1.58 a	0.28 b	0.09 b
	N side dressing[y]	3.20 c	0.36 c	2.43 b	0.29 b	0.10 b
Root	Control	1.22 ab	0.19 a	2.19 b	0.11 a	0.19 a
	Chitosan	0.88 a	0.18 a	0.80 a	0.12 a	0.18 a
	Tryptone	0.97 a	0.16 a	1.62 ab	0.16 ab	0.20 a
	Casein	0.85 a	0.23 ab	0.79 a	0.17 ab	0.20 a
	Collagen	1.02 a	0.26 ab	1.03 a	0.18 ab	0.23 a
	Gelatin	0.91 a	0.36 b	1.32 ab	0.25 b	0.33 b
	N side dressing	1.98 b	0.23 ab	2.63 b	0.16 ab	0.23 a

[z]Mean separation within colums and times by Duncan's multiple range test, $P \leq 0.05$.
[y]Fertilizer with an equal amount of nitrogen as in the chitosan.

Table 5. Effects of substances on first flowering date and cut flower quality in *Eustoma grandiflorum* 'Peter blue line 2'.

Soil mix treatment	First flowering date (month/day)	No. node (pair)	Stem length (cm)	Cut-flower wt (g)	No. of flowers
Control	9/12.5 c[z]	7.4 ab	33.3 a	21.9 a	7.5 a
Chitosan	8/20.5 a	8.1 c	47.3 b	38.2 c	11.9 c
Tryptone	8/19.7 a	7.8 bc	49.1 bc	39.3 c	13.5 c
Casein	8/21.6 a	7.4 ab	51.0 c	31.7 bc	13.2 c
Collagen	8/21.9 a	7.5 ab	43.9 b	31.3 bc	13.2 c
Gelatin	8/25.7 ab	6.9 a	45.2 b	29.8 bc	10.6 b
N side dressing[y]	8/30.8 b	7.0 a	31.4 a	24.5 ab	10.1 ab

[z]Mean separation within colums and times by Duncan's multiple range test, $P \leq 0.05$.
[y]Fertilizer with an equal amount of nitrogen as in the chitosan.

in maximum root length among the treatments (Table 3). Fresh and dry weights of shoots and roots increased markedly when chitosan or tryptone were added to the medium.

In shoots, N and P contents increased significantly in the N side dressing treatment compared to the chitosan treatment. K and Ca contents increased significantly in the tryptone and N side dressing treatments compared to the chitosan treatment. In root, only N and K contents increased significantly in the N side dressing treatment compared to the chitosan treatment (Table 4). From these data, chitosan, tryptone, casein, collagen and gelatin treatments did not influence almost the inorganic nutrient percentage except for N side dressing treatment in the plants. Thus, in the chitosan, tryptone, and collagen soil mixtures, we found that growth promotion of seedlings is not proportionally correlated with absorbed N. There was not a significant correlation ($r = -0.107$) between N percentage in plant and total N percentage in each amendment. These data correspond with our previous findings (Ohta et al., 2000).

NO_3-N in the growing medium increased significantly in tryptone and collagen treatments at 11 weeks after sowing compared to other treatments (Fig. 1). In the chitosan soil treatment, however, NO_3-N in the soil did not increase. Therefore, there is not a significant correlation ($r = 0.086$) between total N percentage in the amendment and NO_3-N percentage in soil. Thus, NO_3-N percentage in the growing medium was not significantly correlated ($r = 0.170$) with seedling growth in this study. Thus, plant growth applied NO_3-N was promoted without increasing N percentage, but with increasing biomass.

The date of first flowering was accelerated significantly in chitosan, tryptone, casein and collagen treatments compared to the control (Table 5). The number of nodes and the weight of cut flowers increased in chitosan and tryptone treatments compared to the other treatments. The flower number also increased in these treatments. The stem length of casein treatment increased significantly compared to chitosan treatment. Thus, flower quality of both the chitosan and tryptone soil mix treatments compared to the treatments increased compared to the other treatments. These results on chitosan treatment were almost the same as those published previously (Ohta et al., 1999, 2001).

These results demonstrate that tryptone as chitosan in the growth of seedlings regardless of that the N percentage of tryptone in shoot and root is higher compared to that of chitosan. Furthermore, tryptone, casein, and collagen like chitosan promote the growth and increases flower quality of *E. grandiflorum* compared to the untreated control and N side dressing. Tryptone, casein and collagen were as effective

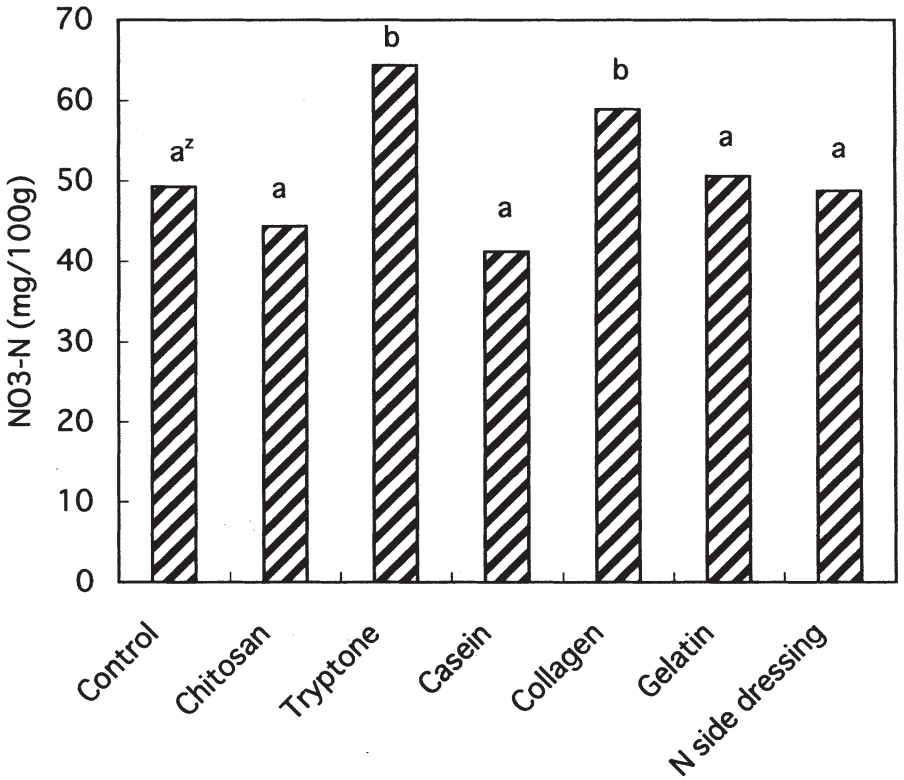

Fig. 1. Effects of media amendments on concentration of NO_3-N in the medium at 11 weeks after sowing seeds of *Eustoma grandiflorum* 'Peter blue line 2'. Mean separation by Duncan's multiple range test, $P \leq 0.05$. [z]The N side dressing treatment contained fertilizer with an equal amount of nitrogen as in the chitosan.

as chitosan on enhancing growth and flowering of *E. grandiflorum.* Thus, these organic substances may be alternative of chitosan.

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