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# Nitrate uptake by nitrogen-starved plants of the red alga *Gracilaria tenuistipitata* var. *liui*

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Nitrate-nitrogen uptake by nitrogen-starved plants of the red alga *Gracilaria tenuistipitata* var. *liui* ZHANG and XIA (formerly called *Gracilaria verrucosa*) was studied under 25 combinations of temperature (10, 15, 20, 25 and 30°C) and salinity (10, 20, 30, 40 and 50‰). The plants assimilated all $NO_3^-$ (200 $\mu$M) in the medium within 24 hours at the combinations of 15°C, 20°C, 10‰ and 20‰, but the uptake was slow at lower temperature (10°C) and at higher salinity (40 and 50‰).

Nitrate uptake by the plants in the "light" and in the "dark" was almost the same during the first 6 hr, but afterwards, the plants in the light assimilated nitrogen much more rapidly than those in the dark. The "light" plants assimilated all of the $NO_3^-$ (200 $\mu$M) in the medium within 20 hr and continued the assimilation in a new medium. In contrast the "dark" plants assimilated $NO_3^-$ slowly during the first 24 hr and stopped the assimilation in a new medium. The basal segments of the N-starved plants absorbed more than 80% of $NO_3^-$ (200 $\mu$M) in the medium within 24 hr, whereas the apical segments absorbed only 40% of it.

*Key Index Words:* Gracilaria—Gracilaria tenuistipitata *var.* liui—*Gracilariaceae—nitrate uptake—Rhodophyta—seaweed cultivation.*

*Gracilaria* is a marine red alga which has been studied extensively because of its commercial value as a source of agar, its importance in diet, and its increasing demand in the cultivation of the sea abalone (CHIANG 1981, EDWARD *et al.* 1982, CORDERO 1984). Previous studies have shown that the growth, agar content and its quality of this alga may be limited by the amount of available nitrogen and the rate of nitrogen uptake also can be limited by a variety of environmental conditions (DEBOER *et al.* 1978, DEBOER 1979, WANG and YANG 1980, BIRD *et al.* 1981, 1982, BIRD 1988, LAPOINTE 1981, LAPOINTE *et al.* 1984a, b, FUJITA and GOLDMAN 1985, ROTEM *et al.* 1986). Those kinds of information are most useful for purpose of *Gracilaria* cultivation. Accordingly, we initiated the present study to examine the effects of salinity, temperature and light on the uptake of $NO_3^-$ of *Gracilaria tenuistipitata* var. *liui* ZHANG and XIA (formerly called *Gracilaria verrucosa*) which has been cultivated extensively in Taiwan (CHIANG 1981).

## Materials and Methods

Vegetative plants of *G. tenuistipitata* var. *liui* were collected from an aquaculture pond at Anpin, Tainan, Taiwan.

Plants of about 5 kg (wet weight) were grown in a flat-bottom concrete tank (2.0 m × 4.0 m × 1.0 m) for 3–4 weeks in unenriched running seawater (about 1.5 volumes of seawater were exchanged per day) until the plants became pale straw-yellow in color, which is indicative of a nitrogen deficiency of the plants (RYTHER *et al.* 1981).

### *Epiphytes removed*

Before each experiment, nitrogen-deficient *Gracilaria* were cleaned to ensure that all of the nitrogen from the medium had been assimilated by the *Gracilaria* plants only. The procedure used for cleaning the plants was based on the technique of BIRD (1976). Hav-

ing removed sand and epiphytic algae, the plants were immersed for 30 seconds in a sodium dodecyl sulfonate seawater solution. The fronds were then transferred to a 0.001% (v/v) formaldehyde solution for 30 seconds, whereupon they were rinsed twice in sterilized seawater.

*Apparatus*

Experiments were carried out in a growth chamber at 10°C. For cultivation of the fronds, 500 m*l* Pyrex flasks were used and they were kept in glass-made aquaria (60 cm × 30 cm × 36 cm) with water of required temperature which was controlled with LAMYCON 500 DX IC Controller thermostat. Water in the aquaria was well mixed by bubbling with compressed air. Irradiance was provided by cool-white 40-W fluorescent lamps at 120 $\mu$E $m^{-2}s^{-1}$.

*Preconditioning thallus*

Prior to each experiment, cleaned thalli of 5.0±0.2 g wet weight were preconditioned by incubation in 500 m*l* flasks containing sterilized seawater at the required salinity and temperature under an 11 : $\overline{13}$ hr photoperiod and a light intensity of 120 $\mu$E $m^{-2}s^{-1}$ for 7 days. Each experimental condition was run in duplicate and all experiments were repeated once.

Nitrate was measured according to STRICKLAND and PARSONS (1972). C/N values of the plants before and after each experiment were determined with a Perkin-Elmer 240 elemental analyzer.

A modified growth medium SWM (MCLACHLAN 1973) (lacking soil and liver extract, S-3 vitamins and $NaNO_3$) was used. Salinity was modified by dilutions with distilled water or by concentration of seawater by heating and was corrected for evaporation every other day during the experiments. The pH of the medium was adjusted to 7.5.

*Experiment I*: Effects of temperature and salinity on $NO_3^-$ uptake

Combinations of temperature (10, 15, 20, 25 and 30°C) and salinity (10, 20 30, 40 and 50‰) were used.

Preconditioned thalli of 5.0±0.2 g (wet weight) were inoculated into each flask containing 300 m*l* medium which had 200 $\mu$M $NO_3^-$ and were grown at the required conditions. Every 24 hr, over a 6-day period, water samples of 1 m*l* each were withdrawn from all cultures and analyzed for $NO_3^-$ concentration. In addition, water samples were also taken from a flask without seaweed to determine any other loss of $NO_3^-$ occurring.

*Experiment II*: Effects of light and darkness on $NO_3^-$ uptake

The thalli which had been preconditioned for 7 days under conditions of 15°C, 20‰, 11 : $\overline{13}$ hr photoperiod and 120 $\mu$E $m^{-2}s^{-1}$ were incubated in four flasks each containing 300 m*l* medium with a level of 200 $\mu$M $NO_3^-$. Each flask contained thalli of 5.0±0.2 g (wet weight).

Two of the four flasks were wrapped with aluminum foil and then enclosed in a light-tight container. Then both sets of cultures were returned to the same conditions as those of the precondition, except that 24 hr illumination was provided, instead of 11 : $\overline{13}$ hr photoperiod. Water samples were taken from each culture every 2 hr over the initial 24 hr to determine the level of residual $NO_3^-$.

After 24 hr, the medium of all cultures was replaced with fresh medium, and the experiment was continued for additional 6 days. Every 24 hr, water samples were taken from each culture to determine the $NO_3^-$ concentration.

*Experiment III*: Uptake of $NO_3^-$ by the apical and basal segments

Thalli were treated for 7 days under the same conditions as those in Exp. II and were cut into two portions (apical and basal) of segments, each measuring 3.0 cm and weighing 5.0±0.2 g (wet weight). Then the segments were inoculated into 300 ml medium with 200 $\mu$M $NO_3^-$ and were grown under the same conditons as in the previous culture. Every 2 hr, the concentration of $NO_3^-$ in the culture media was measured.

## Results

*Experiment I*

No measurable amount of $NO_3^-$ was lost from the flasks without plant. Thus any loss from the flasks containing the *Gracilaria* could be assumed to have been due to assimilation by the algae.

As shown in Table 1, the thalli grown in the combinations of 15°C, 20°C, 10‰ and 20‰ assimilated all of $NO_3^-$ in the medium within 24 hr. In general, plants grown in lower salinities (10–30‰) assimilated $NO_3^-$ more rapidly than those grown in higher salinities (40 and 50‰). Those grown at 15–25°C absorbed 85–100% of the $NO_3^-$ within 4 days. However, plants grown at 10 and 30°C assimilated $NO_3^-$ more slowly than those grown at other temperatures regardless of the salinity.

*Experiment II*

The results (Fig. 1) of this experiment showed that $NO_3^-$ uptake in the "light" and in the "dark" was essentially the same within 7 hr after the initiation of the experiment. After 7 hr, however, the plants grown in the "light" began to assimilate more $NO_3^-$ than those in the "dark". For example, after 20 hr the "light" plants had assimilated all $NO_3^-$ in the

Table 1. Uptake (% of initial concentration, 200 μM $NO_3^-$) of nitrate-nitrogen by nitrogen-starved *Gracilaria tenuistipitata* var. *liui* at different temperatures and salinities.

| Temp. (°C) | Sal. (‰) | Uptake | | | | |
|---|---|---|---|---|---|---|
| | | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
| 10 | 10 | 0.0 | 16.2 | 30.1 | 64.9 | 75.9 |
| | 20 | 0.0 | 26.3 | 37.3 | 72.5 | 83.5 |
| | 30 | 0.0 | 30.9 | 47.5 | 62.0 | 71.9 |
| | 40 | 0.0 | 8.6 | 26.6 | 44.2 | 58.7 |
| | 50 | 0.0 | 1.8 | 18.9 | 32.6 | 42.8 |
| 15 | 10 | 0.0 | 100.0 | — | — | — |
| | 20 | 0.0 | 100.0 | — | — | — |
| | 30 | 0.0 | 54.2 | 87.0 | 92.5 | 100.0 |
| | 40 | 0.0 | 40.0 | 79.3 | 88.5 | 100.0 |
| | 50 | 0.0 | 26.2 | 48.4 | 49.6 | 71.2 |
| 20 | 10 | 0.0 | 100.0 | — | — | — |
| | 20 | 0.0 | 100.0 | — | — | — |
| | 30 | 0.0 | 38.7 | 53.2 | 74.0 | 85.8 |
| | 40 | 0.0 | 24.2 | 42.9 | 58.7 | 62.8 |
| | 50 | 0.0 | 18.3 | 31.0 | 45.5 | 58.4 |
| 25 | 10 | 0.0 | 72.8 | 86.9 | 100.0 | — |
| | 20 | 0.0 | 64.3 | 79.3 | 89.6 | 100.0 |
| | 30 | 0.0 | 34.4 | 43.5 | 63.5 | 84.9 |
| | 40 | 0.0 | 16.5 | 23.7 | 46.5 | 60.2 |
| | 50 | 0.0 | 10.8 | 22.0 | 40.8 | 58.1 |
| 30 | 10 | 0.0 | 18.2 | 34.6 | 44.0 | 78.0 |
| | 20 | 0.0 | 29.8 | 40.5 | 63.8 | 87.9 |
| | 30 | 0.0 | 24.8 | 36.5 | 49.4 | 69.4 |
| | 40 | 0.0 | 11.6 | 20.6 | 32.6 | 54.8 |
| | 50 | 0.0 | 10.4 | 18.7 | 31.1 | 49.4 |

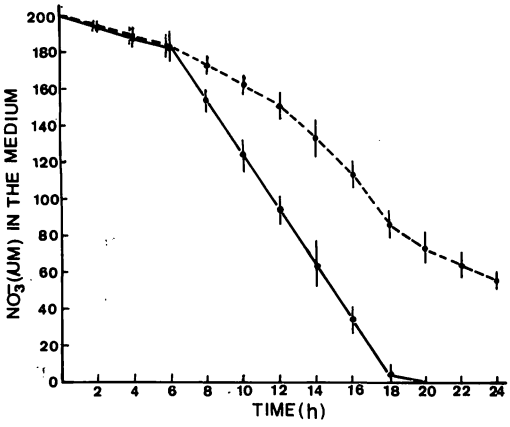


Fig. 1. Decrease of nitrate-nitrogen in the medium due to the uptake by nitrogen-starved *Gracilaria tenuistipitata* var. *liui* in the light (—) and in the dark (---).

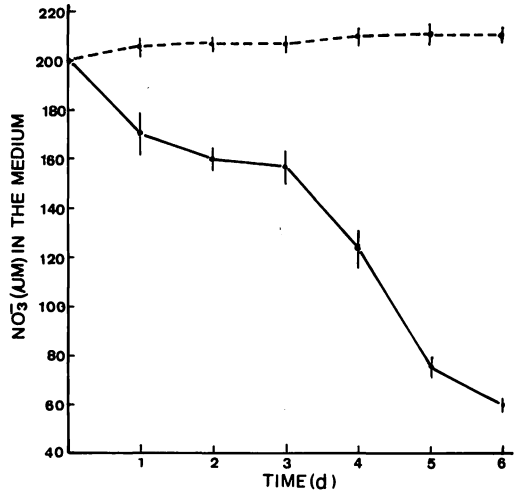


Fig. 2. Changes of nitrate-nitrogen in the medium due to the uptake (or release) by *Gracilaria tenuistipitata*. var. *liui* in the light (—) and in the dark (---) after the materials experienced the same conditions as shown in Fig. 1.

medium, whereas the "dark" plant had assimilated only 65%. In addition, Fig. 2 shows that plants in the light continued to assimilate $NO_3^-$ from the fresh medium while those in the dark actually lost some of the previously assimilated $NO_3^-$ to the medium.

*Experiment III*

The results of this experiment shown in Fig. 3 indicate that segments of the basal portion assimilated about 80% of $NO_3^-$ in the

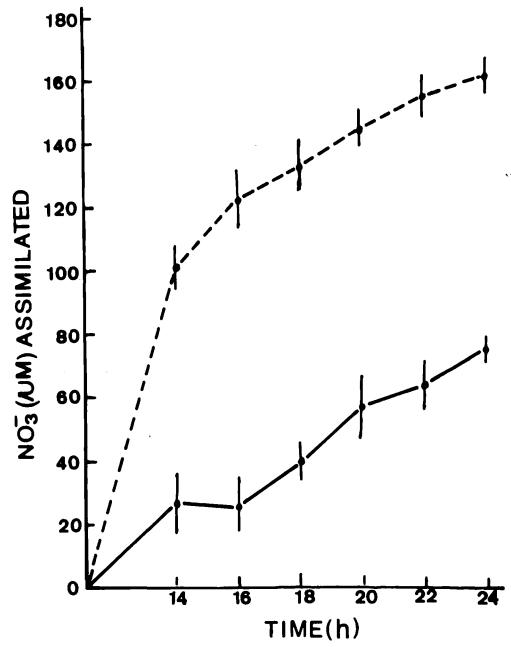


Fig. 3. Uptake of nitrate-nitrogen by the apical (—) and basal (---) segments of nitrogen-starved *Gracilaria tenuistipitata* var. *liui*.

medium within 24 hr, whereas those of the apical one assimilated less than 40% of the original concentration. However, segments of the apical portion increased their wet weight and produced more new branches than the basal segments after 7-day cultivation (unpublished data).

## Discussion

Earlier studies (LAPOINTE and RYTHER 1979, RYTHER and HANISAK 1981) on the culture of *Gracilaria* have shown that healthy plants can lose their dark reddish-brown color and become pale-yellow when they are held under very low $NO_3^-$ concentrations. The change in color is indicative of nitrogen deficiency, with C/N values changing from about 6 to nearly 30, after stocking in the tank with unenriched running seawater. Plants used in this study indicated a change in color from dark reddish-brown to pale-yellow, while C/N values increased from about 5 to 15, indicating nitrogen deficiency.

Rapid N-assimilation by N-starved

*Gracilaria* depends upon carbohydrate reserves (RYTHER *et al.* 1981), which in turn are affected by temperature and salinity. EHRKE (in GESSNER 1970) found that photosynthetic rates of *Fucus serratus, Plocamium cocconeum* and *Enteromorpha compressa* were higher than respiration at lower temperatures (<20°C). In this experiment, the N-starved *Gracilaria* growing at 15 and 20°C assimilated $NO_3^-$ more quickly than those growing at 30°C. This could be due to the rate of photosynthesis of these plants exceeding that of respiration, thereby increasing their carbohydrate reserves during the preconditioning period. Therefore they may have been able to assimilate $NO_3^-$ more rapidly at the expense of carbohydrate reserves. On the other hand, as temperature increases beyond the optimum range for growth of the plants, the rate of respiration exceeds that of photosynthesis (EHRKE in GESSNER 1970), thereby reducing the carbohydrate reserves. This may explain the slower assimilation of $NO_3^-$ at 25 and 30°C than at 15 and 20°C. The low uptake rate of $NO_3^-$ at 10°C might be due to the slow metabolic rate of the plants at this temperature.

HUANG (1980) found that *G. tenuistipitata* var. *liui* showed the highest photosynthetic rate at 25°C in salinities of 10–20‰ and that the rate decreased as the salinity increased from 20 to 50‰. These findings agree with our present results that plants can assimilate more $NO_3^-$ in lower salinities (10–30‰) than in higher ones (40–50‰). These results support the observation of GESSENER and SCHRAMM (1971) that although both photosynthesis and respiration decrease with increased salinity, salinity affects more photosynthesis than respiration.

RYTHER *et al.* (1981) found that N-starved *Gracilaria* assimilated more ammonium-nitrogen following exposure to daylight than did plants held in the dark, and showed that N-starved macroscopic algae can assimilate nitrate in the darkness, as can microalgae (cf. SYRETT 1962). The same phenomenon is also shown in our study on the assimilation of nitrate-nitrogen by N-starved *Gracilaria* (Fig. 2). The plants in the "light" and in the "dark" showed no substantial difference in the uptake of nitrate-nitrogen during the first six hours of the experiment. This observation suggests that the carbohydrate reserves in both plants were almost equal at first. However, the "light" plants were able to maintain or increase their carbohydrate content due to photosynthesis and hence were able to continue $NO_3^-$ assimilation under light condition. On the other hand, the "dark" plants continued to deplete their carbohydrate reserves and ultimately began to lose the ability to assimilate $NO_3^-$. Rapid N-assimilation by starved algae depends upon carbohydrate reserves and ceases when those reserves are depleted (SYRETT 1962). In their study on two red macroalgae, D'ELIA and DEBOER (1978) found that decreases in nitrogen uptake rates occur in response to nitrogen satiation of the seaweeds. This was also true for *G. tenuistipitata* var. *liui* when the medium of both the "light" and the "dark" was replaced with fresh one after 24 hr. The plants in the "light" continued to assimilate $NO_3^-$ but at a slower rate, and only 72% of $NO_3^-$ added was assimilated after 6 days in contrast to 100% in the first 24 hr. Plants in the "dark" did not assimilate any $NO_3^-$, but actually lost some.

The color of the basal portions of the plants used in this study was generally slightly darker than that of the apical ones, suggesting that the basal region had more pigment available for photosynthesis than the apical ones. This appeared to be the case as the photosynthetic rate of the basal portion was almost 1.5 times higher than that of the apical one (unplished data). ROSENBERG and RAMUS (1982) found that N-starved plants use pigment-protein as a nitrogen source for cell division, and since cell divisions are usually more active in the apical part than in the basal part of a plant, the apical parts would tend to lose their pigments more quickly than the basal ones. This was confirmed when the apical segments produced more branches than the basal ones after 7-day cultivation.

In conclusion, our findings on the condi-

tions for nitrogen uptake by nitrogen starved *Gracilaria* could be helpful in choosing time for adding fertilizers to *Gracilaria* ponds.

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## 江 永 棉・林 俊 亮：窒素欠乏条件下に置かれた紅藻 *Gracilaria tenuistipitata* var. *liui* の硝酸塩吸収

紅藻 *Gracilaria tenuistipitata* var. *liui*（従来 *Gracilaria verrucosa* と呼ばれたもの）を窒素欠乏海水中に置いた後，温度5段階(10, 15, 20, 25, 30°C)と塩分5段階(10, 20, 30, 40, 50‰)を組合わせた25の条件下で硝酸態窒素の吸収を調べた。15°C 及び 20°C と10‰及び20‰の組合わせでは24時間以内に培地中の $NO_3^-$ (200 $\mu$M) は全て吸収されたが，低温 (10°C) 高塩分 (40及び50‰) 下では吸収は遅かった。硝酸態窒素の吸収は初めの6時間は明条件下でも暗条件下でも変りはなかったが，その後は，明条件下の藻体の方が暗条件下の藻体より急速な吸収を示した。明条件下の藻体は20時間以内に培地中の $NO_3^-$ (200 $\mu$M) を全て吸収し，新しい培地に移すとさらに吸収を続けた。これに対し，暗条件下の藻体は初めの24時間は $NO_3^-$ をゆっくり吸収し，新しい培地に移すと吸収を停止した。窒素欠乏藻体の基部片は培地中の $NO_3^-$ (200 $\mu$M) の80%以上を24時間以内に吸収したが，先端部片はわずか40%を吸収しただけであった。（台湾台北市　国立台湾大学海洋研究所）