Effect of ammonia on the ultrastructure of duckweed species

CHONG Yun-xiao[1,2], HU Hong-ying[2], QIAN Yi[2]
(1 College of Resources and Environment, South China Agric. Univ., Guangzhou 510642, China;
2 Environmental Simulation and Pollution Control State Key Joint Lab, Department of Environmental Science and Engineering, Tsinghua University, Beijing 10084, China)

Abstract: The changes of ultrastructure under scanning electron microscopy and transmission electron microscopy were described in two duckweed species of *Lemma aequinoctialis* and *Spirodela polyrrihza* cultivated in ammonia medium in present study, contrasted by the ones growing under nitrate medium. The result showed that outstanding cell wall in lower epiderm is became crinkly and disappeared partly in fronds under ammonia medium. The stomata on the upper epiderm is opened much wider far more than ones under nitrate medium. The chloroplast membrane limiting became blurred and disappeared partly in fronds under ammonia medium. The whole chloroplast of fronds under ammonia medium presented the trending of disassembly. And the internal membrane network including grana lamellae and stroma lamellae break off. In contrast to ones under nitrate medium, more starch grain appeared amongst stroma. Based on the results of observation, the toxicity mechanism of unionized ammonia (NH_3) and ammonium ion (NH_4^+) to duckweed were respectively discussed.

Key words: duckweeds; ammonia; ultrastructure; toxicity
CLC number: Q942.1 **Document code:** A **Article ID:** 1001 - 411X (2005) 04 - 0077 - 04

氨对紫背浮萍和稀脉浮萍超微结构的影响

种云霄[1,2], 胡洪营[2], 钱 易[2]
(1华南农业大学 资源与环境学院, 广东 广州 510642;
2清华大学 环境科学与工程系 环境模拟与污染控制国家重点实验室, 北京 100084)

摘要:选择紫背浮萍和稀脉浮萍,利用 SEM和 TEM分别对在对照液硝酸盐氮培养液和含有较高氨氮培养液中生长个体的超微结构进行了观察和分析. 结果表明,与在硝酸盐氮培养液中生长的个体相比,在氨氮培养液中生长的 2种浮萍上表皮的气孔张开程度明显地增大,下表皮细胞突起的细胞壁消失,细胞发生明显的变形;生长在含有氨氮培养液中的 2种浮萍个体的叶绿体内膜结构均受到严重的损伤,叶绿体中淀粉粒明显增多,整个叶绿体处于解体状态,而生长于硝酸盐氮培养液中浮萍的叶绿体则完好无损.

关键词:浮萍; 氨; 超微结构; 毒性

Duckweeds species, namely Lemnaceae species, are a group of free-floating aquatic plants. They have leaf-like body, called frond. The frond could take up nutrient such as nitrogen and phosphorus from water. To control eutrophication with low cost, it is a preferred choice to convert these nutrients into plant protein by duckweeds up-taking[1-5]. Duckweed species are suitable to be poultry and fish feed[6,7].

Ammonia, largely existing in domestic wastewater, is main nitrogen resource to duckweed species. However, ammonia is toxic to aquatic organism and could inhibit the growth at high concentration. In addition, due

Received date: 2005 - 02 - 25 **Biography:** CHONG Yun-xiao (1974 -), female, Ph. D.; E-mail: cyx04@scau.edu.cn
Foundation item: Major State Basic Research Development Program of China (G1999045711)

to their small size and ease of growth, duckweed species were often used as experimental material to testing the aquatic plant toxicity of ammonia in water[8-10]. But the past investigations or testing more paid more attentions to the relationship between ammonia level and visual symptom such as frond number change, color, root change etc.

Nitrate is another nitrogen resource for duckweed species and generally it has no toxicity except extremely high level. Using the fronds growing under nitrate medium as comparison, the damage of ammonia to ultrastructure of two duckweed species fronds were described here. The objective is to make clear the toxic mechanism of ammonia to duckweed species, furthermore, the mechanism to aquatic plant

1 Materials and methods

1.1 Collection and acclimation of duckweed species

Spirodela polyrrihza (Linn.) Schleid and *Lemna aequinoctialis* Welwitsch are respectively common species of two main genera of *Lemna* and *Spirodela* in Lemnaceae. And they were often used in wastewater treatment. The materials were collected from a wetland nearby Dianchi Lake (located in Kunming City, Yunnan Province, China) and acclimated to the artificial balance media suitable to culture of duckweed[11].

1.2 Cultivation of duckweed species

The fronds of *S. polyrrihza* and *L. aequinoctialis* were cultivated firstly to adapt the conditions with nitrate or ammonia as only nitrogen resource. So the nitrogen in the artificial balance medium was adjusted to two conditions: $\rho(NH_4^+-N) = 20$ mg/L and $\rho(NO_3^--N) = 20$ mg/L by replacing NH_4NO_3 with NH_4Cl and $NaNO_3$ respectively. The pH of medium was adjusted to 7.0 ± 0.3. Twenty healthy fronds of each species were placed into the media. The cultivation was performed in plastic container with 200 mL medium. In order to make frond adapt the growth condition fully, the cultivation lasted 24 days. At the end of the cultivation, 5 fronds of in each condition were taken out randomly for the observation of ultrastructure. The experiments were conducted in laboratory condition with fluorescent lamps of light intensity of 2 000 - 3 000 lx (16 h-light, 8 h-dark). The temperature was 26 - 30 ℃.

1.3 Treatment of samples for SEM & TEM

The fronds were fixed in 4% (*w*) glutaraldehyde and postfixed in 1% (*w*) osmium tetroxide in 0.1 mol/L phosphate buffer with pH 7.1. Dehydration was accomplished in a graded acetone series lastly.

Some samples were taken out for the observation of chloroplast of parenchymatic tissue by transmission electron microscopy (TEM). The samples were infiltrated and embedded in Epon (Spurr). Thin sections were cut with a diamond knife, stained with Reynold's lead citrate and examined with a Hitachi-600 TEM at 80 kV at last. Other samples were taken out for the observation of the epidermis of upper and lower sides by scanning electron microscopy (SEM). The whole frond was cut into segments firstly, fixed in glutaraldehyde, dehydrated in acetone, critical-point dried, sputtered with gold and examined with a Hitachi-505 SEM at last.

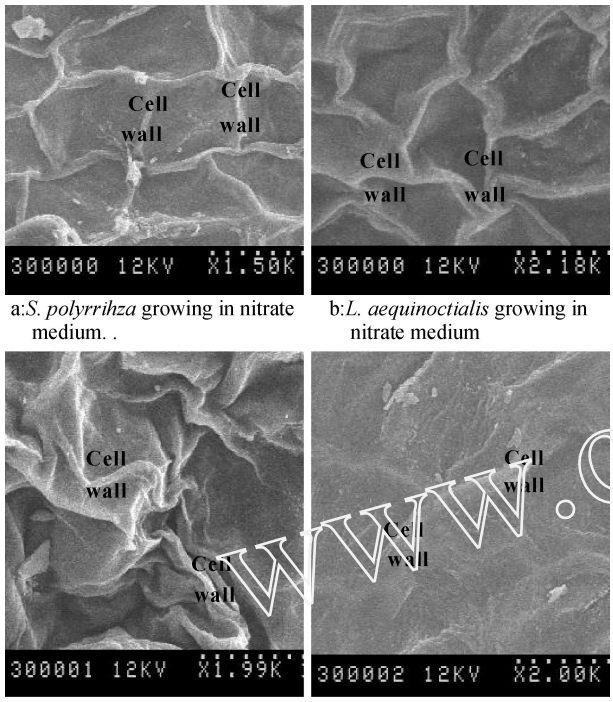
2 Results

2.1 Changes of ultrastructure in lower epidermis of frond

The lower epidermis of fronds growing under ammonia medium looked obviously different from ones under nitrate medium. For fronds under ammonia medium, the outstanding wall between cells became very crinkly in *S. polyrrihza* (Fig. 1c) and even disappeared partly in *L. aequinoctialis* (Fig. 1d), which made the cell take on blurred array. The wall between cells stand out on the lower epidermis of frond under nitrate medium, which separated the cells and made the cell arrange orderly (Fig. 1a and Fig. 1b). It seemed that ammonia in the medium could directly cause the damage to the wall of lower epidermis cell.

2.2 Changes of ultrastructure in upper epidermis of frond

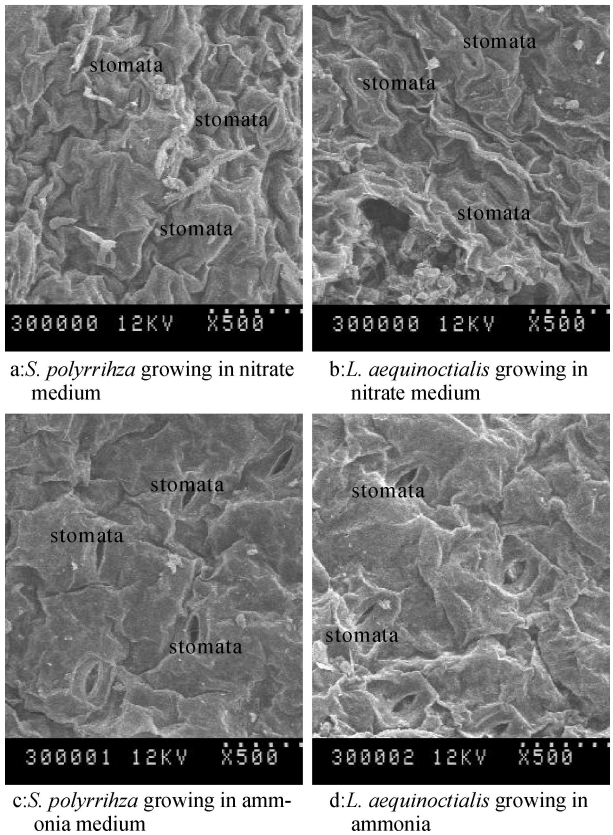
Like the conditions of lower epidermis, the upper epidermis of fronds under ammonia medium looked obviously different from those under nitrate medium. The most change was the behavior of stomata. They kept open slightly in frond under nitrate medium (Fig. 2a and Fig. 2b), but opened much bigger under ammonia medium (Fig. 2c and Fig. 2d). The epidermis cells surrounding stomata also were a little different. They were more expanding under ammonia medium, not like one under nitrate medium. It seemed that ammonia could affect the mobility of stomata of duckweed species fronds.



a:*S. polyrrihza* growing in nitrate medium. .　b:*L. aequinoctialis* growing in nitrate medium

c:*S. polyrrihza* growing in ammonia medium.　d:*L. aequinoctialis* growing in ammonia medium

Fig 1　The SEM photograph of the lower epidermis



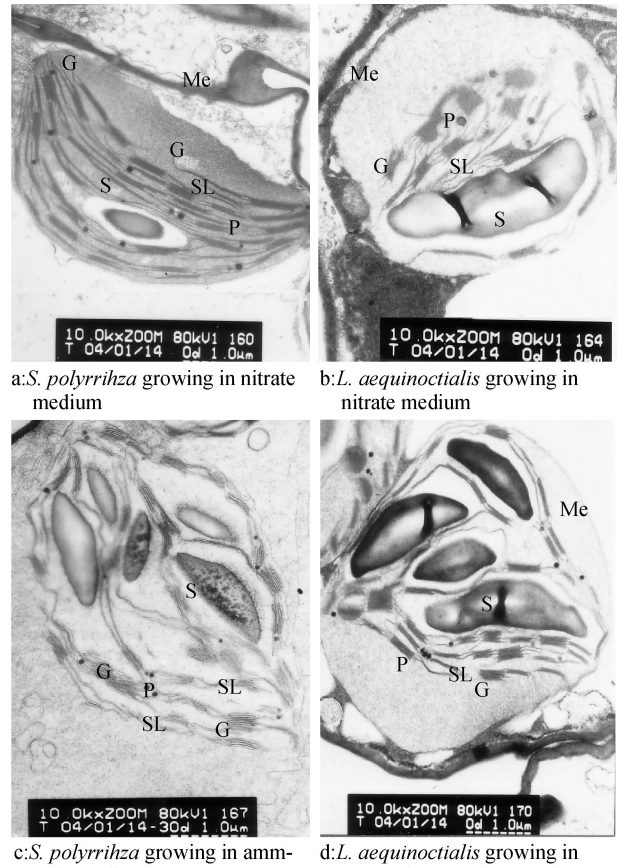
a:*S. polyrrihza* growing in nitrate medium　b:*L. aequinoctialis* growing in nitrate medium

c:*S. polyrrihza* growing in ammonia medium　d:*L. aequinoctialis* growing in ammonia

Fig 2　The SEM photograph of the upper epidermis

2.3　Changes of ultrastructure of the chloroplast

The chloroplasts of fronds under nitrate medium kept complete structure (Fig 3a and Fig 3b). The membrane of the chloroplast was very clear. The lamellae forming grana piled up densely together. The stroma lamellae connecting the grana kept continuous and formed intact stroma lamellae network. The dense stroma contained a starch grain and several globuli. On the contrary, the chloroplast of fronds under ammonia was destroyed evidently (Fig 3c and Fig 3d). The membrane became blurred and disappeared partly, even disappeared totally in *S. polyrrihza* (Fig 3c). The grana lamellae was not be adpressed densely any more, but arrayed loose. The stroma lamellae connecting grana was cut off, which made stroma lamellae network break off. More starch grain appeared amongst stroma. The chloroplast of fronds under ammonia tended to be disassembly. All these changes presented more obvious for *S. polyrrihza* (Fig 3c).



a:*S. polyrrihza* growing in nitrate medium　b:*L. aequinoctialis* growing in nitrate medium

c:*S. polyrrihza* growing in ammonia medium　d:*L. aequinoctialis* growing in ammonia

P: plastoglobuli S: starch grain SL: stroma lamellae G: grana Me: membrane of chloroplast

Fig 3　The SEM photograph of the chloroplast of the fronds

3　Discussion

It was observed that the size of fronds under ammonia medium was smaller than ones under nitrate medium. This should have relationship with the damage of ammonia to the cell wall, which possibly caused by the NH_4^+. Britto et al[12] has proposed that NH_4^+ could re-

duce the content of some essential cations such as the calcium, magnesium in plant issue by replacement. These cations, especially calcium had very important role to keep the strength of plant cell wall. Possibly it is NH_4^+ that resulted in the changes of lower epiderm is cell wall under ammonia medium.

For the higher plant, the behavior of stomata is mainly controlled by the osmotic pressure of stomatal guard cell. Many factors could affect the osmotic pressure of stomatal guard cell such as potassium, abscisic acide etc. But there is seldom report that ammonia could cause the change of the osmotic pressure. Furthermore, Severi et al[13] founded that the stomata in full grown fronds of *Lemna* species always kept open and the guard cell had lost the function, but for *Spirodela* species this function still exists. From the observation result of this study, both stomata of *S. polyrrihza* and *L. aequinoctialis* opened wider under ammonia medium than nitrate medium. We didn't consider that the stomata behavior was caused by the effect of ammonia to the osmotic pressure. We tend to conclude that the crimpy shape of other epiderm is cells, especially those of lower epiderm is draw out the stomata indirectly, which was caused by the toxicity of NH_4^+ indirectly.

Whole chloroplast of plant is wraped by double layer membranes. The inside one is stroma lamellae and grana lamellae, which is composed of folding single layer membrane. Based on the result of this study, the membrane structure of two duckweed species under ammonia medium was damaged evidently. The unionzed ammonia (NH_3) could disturb cell membrane when it traverses largely. Obviously, unionzed ammonia (NH_3) resulted in the damage of the chloroplast because it was existent in ammonia medium of this study. The level of NH_3 was very low, about 0.15 mg/L according the equilibrium between un-ionized ammonia (NH_3) and ammonium ion (NH_4^+)[9]. But it resulted in great damage to the chloroplast of duckweeds. This confirmed that the toxic mechanism of ammonia to duckweed should be more attributed to NH_3 instead of NH_4^+.

References:

[1] BERGMAN B A, CHENG J, CLASSEN J, et al. Nutrient removal from swine lagoon effluent by duckweed [J]. Transaction of ASAE, 2000, 43(2): 263 - 269.

[2] GIJZEN H J. Anerobes, aerobes and phototrophs: A winning team for wastewater management[J]. Water Science and Technology, 2001, 44 (8): 123 - 132.

[3] RANA N, AGAMI B M, ORON G. A pilot study of constructed wetlands using duckweed (*Lemna gibba* L) for treatment of domestic primary effluent in Israel[J]. Water Research, 2004, 38: 2 240 - 2 247.

[4] REED S C, CRITES R W, MIDDLEBROOKS E. Natural system for waste management and treatment[M]. 2nd ed. New York: McGraw-Hill, Inc, 1995. 158.

[5] ZIMMOA O R. van DER STEEN N P, GIJZEN H J. Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilization ponds treating domestic wastewater[J]. Water Research, 2003, 37: 4 587 - 4 594.

[6] BERGMAN B A, CHENG J, CLASSEN J J, et al. *In vitro* selection of duckweed geographical isolates for potential use in swine lagoon effluent renovation [J]. Bioresource Technology, 2000, 73 (1): 13 - 20.

[7] RUSOFF L L, BLAKENEY E W, CULLY D D. Duckweeds (Lemnaceae family): A potential source of protein and amino acids [J]. Journal of Agricultural Food Chemistry, 1980, 28: 848 - 850.

[8] CLEMENT B, BOUVET Y. Assessment of landfill leachate toxicity using duckweed *Lemna minor*[J]. The Science of Total Environment, 1993, 41(suppl): 1 179 - 1 190.

[9] KORNER S, DAS S K, VEENSTRA S, et al. The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*[J]. Aquatic Botany, 2001, 71: 71 - 78.

[10] WANG W. Ammonia toxicity to macrophyte (common duckweed and rice) using static and renewal method [J]. Environment Toxicity and Chemistry, 1991, 10: 1 173 - 1 177.

[11] ZHAO J J, WANG L H. The culture of *Lemna aeguinoctiolis* 6746 for experiment study of photoperiod [A]. TANG Z C. Experimental Guide of Modern Plant Physiology[C]. Beijing: Science Press, 1999. 76.

[12] BRITTO D T, KRONZUCKER H J. NH_4^+ toxicity in higher plants: a critical review [J]. Journal of Plant Physiology, 2002, 159: 567 - 584.

[13] LANDOLT E. Biosystematic investigation on the family of duckweed: The family of Lemnaceae——a monography study[M]. Zurichbergstrasse: Geobotanischen Insistute ETH, Stiftung Rubel, 1987. 38.

【责任编辑 周志红】