STUDY ON MECHANISMS OF VETIVER'S TOLERANCE TO SUBMERGENCE

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Abstract: Vetiver grass (*Vetiveria zizanioides*) has been widely confirmed to have very strong tolerance to flooding and even complete submergence and, therefore, is a considerably excellent species for stabilization and revegetation of "wet" barren slopes and for wastewater treatment. However, it is poorly documented why vetiver is so tolerant to submergence. To understand the mechanisms of vetiver's tolerance to submergence, an experiment was conducted to examine ecophysiological, biochemical, and anatomical characteristics of vetiver leaves under submergence compared to other three grasses, St. Augustine (*Stenotaphrum secundatum*), carpet grass (*Axonopus compressus*), and bahia grass (*Paspalum notatum*). After stressed by submergence, the contents of photosynthetic pigments of St. Augustine and carpet grass decreased while those of vetiver and bahia grass increased from 18- to 54-day submergence, especially for vetiver, its chl(a+b) and car/chl increased by 63.9% and 72.8%, respectively. Within 36 d submergence, Fv/Fm and _PS_ of St. Augustine and carpet grass declined distinctly, but those of vetiver and bahia grass only declined slightly. This suggests that the latter two grasses were influenced very little with special reference to their photosynthetic apparatus; therefore their photosynthesis was not prohibited conspicuously by submergence. Under 36 d submergence, the activity of SOD in St. Augustine and carpet grass was inhibited by 23.5% and 32.1% respectively, and their MDA contents increased on a large scale and accordingly their membrane lipid peroxidation was obviously aggravated; on the contrary, the activity of SOD in vetiver and bahia grass almost kept stable, and their MDA contents did not acutely increase, indicating that the two species had a stronger anti-oxidation ability under the submergence condition. When the stress duration was relatively short (fewer than 60 d), vetiver and bahia assumed similar tolerant mechanisms with respect to ecophysiology and biochemical response; however, when the duration was up to 60 d, the damage of submergence to the ultrastructure of some important organelles of bahia grass, such as vascular bundle, chloroplast, mitochondrion, especially granum and stromata thylakoids of chloroplast, was much severer than the damage to that of vetiver. The ultrastructure of vetiver was not influenced or damaged distinctly unless it suffered from longer submergence like 120 d. On the whole, vetiver and bahia grass could endure submergence much longer than St. Augustine and carpet grass in terms of ecophysiological feature and biochemical response, but under long time of submergence, e.g. 60 d or more, vetiver showed the mechanisms of stronger tolerance than bahia grass in the aspect of ultrastructure.

Keywords: submergence, mechanism of tolerance to submergence, photosynthesis characteristic, chlorophyll fluorescence, ultrastructure

Abbreviations for text: chl – chlorophyll; car – carotenoid; Fv/Fm – PS_maximum photochemical efficiency; MDA – Malonyldealdehyde; q_p – photochemical quenching of variable chlorophyll fluorescence yield; SOD – superoxide dismutase; TEM – transmission electron microscope; _PS_ – quantum yield of PS_ electron transport

Abbreviations for Plates: C – cell; CH – chloroplast; CM – chloroplast membrane; CW – cell wall; FR – fret or stroma lamella; GT – granum thylakoid; M – mitochondrion; MC – mitochondrion crista; MM – mitochondrion membrane; S – starch; ST – stroma thylakoid; VL – vacuolar lipid

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1 Introduction

At present, there are increasing attentions paying for the soil erosion problem of riparian zones (Gregory *et al.*, 1991; Deng *et al.*, 2001; Xia *et al.*, 2002). Since riparian vegetation has important influences on physical and biological characteristics of riparian zones, more and more ecological measures are being applied to protect the riparian zones from erosion. That is to say, river banks and reservoir slopes can be stabilized and riparian landscapes can be modified through planting some appropriate plants used for soil and water conservation. However, few plants have been found effectively for the ecological goal to date. The main reason is that riparian zones usually suffer from seasonal inundation, while plants used for erosion control are almost all xerophytes, and they would be suffocated to death after a spell of complete submergence. Hydrophytes can endure long time of flooding or even complete submergence, but their effects for slope stabilization are generally quite poor.

Effects of flooding or submergence on plants may embody many aspects, including physiology, biochemistry, morphology, anatomy, and so on (Mielke *et al.*, 2003). For example, shoot growth is reduced because flooding affects leaf formation and expansion of leaves and internodes (Kozlowski, 1997). Furthermore, flooding also results in decrease of leaf chlorophyll contents, stomata closure, inhibition of photosynthesis, accumulation of starch and other non-structural carbohydrates (McKevlin *et al.,* 1995; Gravatt and Kirby, 1998; Mielke *et al.*, 2003; Chen *et al.*, 2005). On the whole, there have been well documented that effects of flooding on eco-physiology and biochemistry of plants, but changes of leaf ultrastructure after submergence and mechanisms of plant's tolerance to submergence remain to be documented.

Vetiver (*Vetiveria zizanioides*), a miracle grass, has a series of excellent characteristics, such as deep, extensive and penetrating root system, fast tiller-forming rate, and strong adaptability to adverse conditions, which make it become a very ideal plant for soil and water conservation and erosion control in tropics and subtropics. And especially, vetiver can bear up to 120 d of complete submergence, which has much stronger tolerance than many other grasses for soil and water conservation (Xia *et al*., 2003). It is obvious that vetiver overcomes the disadvantage that the riparian environment, especially flooding, inhibits the growth of many plant species and even suffocates them. Therefore, it is an excellent species for ecological protection of riparian zones. It is just the reason, vetiver is also regarded as an ideal species for wastewater treatment through establishing constructed wetland (Summerfelt *et al.*, 1999; Truong and Hart, 2001; Truong and Xia, 2003).

However, why is vetiver so tolerant to submergence, and more tolerant than other grasses? And what are there differences regarding the changes of morphology, physiology, and anatomy among theses grasses during submergence? Undoubtedly, it will help us understand the miracle grass more fully and comprehensively, and also help us make use of vetiver more scientifically and rationally, especially for the purpose of protecting riverbanks and reservoir slopes to discover these differences and the mechanisms of vetiver's tolerance to submergence. Therefore, this present study, through a pot experiment, aims at investigating effects of complete submergence on vetiver and other three grasses, bahia grass (*Paspalum notatum*), carpet grass (*Axonopus compressus*) and St. Augustine (*Stenotaphrum secundatum*), which are all quite common in South China for soil and water conservation. The objective is to identify the mechanisms of vetiver and the other three grasses to tolerate submergence.

2 Materials and Methods

2.1 Experimental materials

Vetiver, bahia grass, carpet grass and St. Augustine were selected for the experiment.

Among them, vetiver is the sole high-stalk type and the other 3 species are the procumbent type. All tested species were collected from the grass nursery of the South China Botanical Garden; they were all mature and healthy. A former experiment has shown very different tolerance among them that vetiver was has the strongest tolerance to submergence, enduring 100~120 d of complete mergence, followed by bahia grass, up to 60~70 d and then carpet grass, up to 32~40 d; and the poorest species was St. Augustine, enduring at most 32 d (Xia *et al.*, 2003).

2.2 Research methods

2.2.1 Effects of submergence on photosynthesis and biochemical response of plants

The experiment was conducted with a method of water-cultivation. A cement tank with a volume of 2.0 m long _2.0 m wide _1.3 m high was first constructed and then pots in which the tested plants grew were put into the tank. Soil was mixed thoroughly before it was put into pots. Thereafter grass seedlings were transferred into identical pots with the mixed soil, 12 pots for each species and 2 tillers in each pot. Every grass species was divided into 2 groups, namely 6 pots for each group, one group for submergence treatment and the other for open-air control. The treatment group was transferred to the tank 30 d after plants grew in pots. In order to guarantee the 3 prostrate grasses got enough sunshine, a frame with the height of 0.8 m was installed first in the tank and then these seedling pots were put on the frame. However, vetiver pots were put on the cement ground in the tank and moreover vetiver plants were cut within 1.2 m high. Then tap water was used to fill the tank until all plants were submerged completely in water. During the period of submergence, water was kept flowing all the time through a water pipe, and the height of all vetiver plants were also controlled to avoid growing out of water surface. In the experiment, St. Augustine was submerged only for 27 d for all observed items and the other 3 grasses for 36 or 54 d.

2.2.2 Effects of submergence on leaf ultrastructure of plants

Seedlings of the four grasses were planted in pots and divided into 2 groups, one for submergence treatment and the other for control without submergence. The treatment group was submerged as described in section 2.2.1 and the control group was kept growing in the open air. After a certain spell of submergence, leaves of each submerged species were sampled to prepare for slice, and then cell ultrastructure was observed with leaf slices under an electron microscope. Concretely speaking, after St. Augustine was submerged for 9 and 18 d, carpet grass for 16 and 32 d, bahia grass for 30 and 60 d, and vetiver for 60 and 120 d, their leaves were separately collected for ultrastructure observation. In the meantime, leaves of the control group were also sampled for the same purpose.

2.3 Methods of analysis and measurements

2.3.1 Determination of photosynthetic pigment contents

After 4 grass species were submerged for 18, 27, 36, 45 and 54 d, leaf wafers were sampled and then extracted with 80% (v/v) acetone. The extraction solutions were placed for 3 d in dark and then chlorophyll a and b [chl(a+b)], and carotenoid (car) were measured with spectrophotometer (Lambdas 25, Perkin Elmer Inc., USA).

2.3.2 Measurement of chlorophyll fluorescence

Chlorophyll fluorescence kinetic emission of leaves was determined by a pulse amplitude-modulated chlorophyll fluorometer (PAM 101 Chlorophyll Fluorometer, Heinz Walz, Effeltrich, Germany). The initial fluorescence yield of leaves was recorded 30 min after adaptation in the dark. A single saturating pulse of white light (7000 _mol $m^{-2}s^{-1}$) was then administered to obtain the maximum fluorescence yield. The intensity of actinic light was 250 _mol $m^{-2}s^{-1}$ with a flash of 2 s and an interval of 30 s. On days 9, 18, 27 and 36 of submergence for every species, leaves of each plant for the 4 grasses were measured. Then, chlorophyll fluorescence parameters, Fv/Fm and _PS_ were calculated according to the

formulas provided by Rohacek and Bartak (1999).

2.3.3 Extraction and assay of leaf MDA content

For each treatment and each species, 0.500 g of fresh leaf sample was ground with 0.05 mol/L phosphate buffer (pH 7.0) and a little quartz sand in ice bath. Afterwards the mixture was centrifuged at 20,000 _ g for 30 min and the supernatant was collected, added with distilled water to a volume of 5 ml; from which 1.5 ml was sampled, mixed with 2.5 ml 0.5% thiobarbituric acid (prepared with 20% trichloroacetic acid solution) and then heated in 100°C water bath for 30 min. After cooled down with ice bath, the mixture was centrifuged again for 5 min and then the supernatant was measured the light-absorbed values by spectrophotometer at 532 and 600 nm wavelengths. Leaves were sampled on days 9, 18, 27 and 36 of submergence.

2.3.4 Measurement of SOD activity

SOD was determined by the inhibition of nitroblue tetrazolium reduction as described by Gannopolitis and Ries (1977). One unit of SOD activity is defined as 50% enzyme amount that can inhibit the photo-deoxidization of nitroblue tetrazolium.

2.3.5 Observation of cell ultrastructure

Leaf samples (1 mm _ 6 mm for each) from the middle section of the fresh leaves were fixed with 4% glutaraldehyde, and rinsed with 0.1 M sodium dimethylarsenate buffer. Afterwards, samples were fixed with 1% osmium tetroxide in the same buffer, and then washed with the same buffer again. Specimens were then dehydrated in a graded ethanol series and epoxy propane, and embedded in Epson 812 resin. Samples were sliced with Leica S-typed ultramicrotome. Ultrathin sections were double-stained with uranyl acetate and lead citrate. Electron micrographs were obtained with a JEM-1010 TEM.

3 Results and Discussion

3.1 Effects of submergence on photosynthetic characteristics of plants

3.1.1 Change of contents of photosynthesis pigments after submergence

Plants carry out photosynthesis relying mainly on photosynthetic pigments, including chlorophyll a and b and carotenoid in them. As shown in Fig. 1, the total contents of chl(a+b) and ratios of car/chl for carpet grass and St. Augustine declined along with the increase of submergence duration. It indicated that the photosynthetic pigments of the two species were destroyed by submergence. On the contrary, chl(a+b) and car/chl of vetiver and bahia grasses increased as submergence prolonged, which was especially obvious for vetiver grass. From 18 d to 54 d, chl(a+b) of vetiver increased by 63.9%, and its car/chl increased by 72.8%. It seemed that a period of submergence stimulated the photosynthetic pigments of vetiver grass and bahia grass other than destroyed them, indicating that the two species may maintain high light absorbing quantum by increasing photosynthetic pigments as a response to decrease of light intensity under water. Therefore, it is probably one of the main reasons why vetiver and bahia grass have strong tolerance to submergence.

3.1.2 Responses of chlorophyll fluorescence parameters to submergence

Measurement of chlorophyll fluorescence parameters can give insights into the ability of plant photosynthetic apparatus to tolerate environmental stress and its damaged extent caused by stress (Maxwell and Johnson, 2000). Fv/Fm generally declines when plants are stressed or damaged (Bjorkman and Demmig 1987; Rohacek, 2002). Before submergence, the 4 grasses represented similar Fv/Fm values, with 0.650, 0.676, 0.683 and 0.625 for vetiver, bahia grass, carpet grass and St. Augustine, respectively (Fig. 2-A). After 9-day submergence, Fv/Fm of the 4 grasses decreased obviously; as submergence continued, carpet grass and St. Augustine continued to decline, while vetiver and bahia grass changed slightly and even assumed a rising tendency. In the end, the two intolerant species, St. Augustine and carpet grass declined conspicuously, up to 26.4% and 30.3%, respectively,

while the two tolerant species, vetiver and bahia grass fell down only by 6.9% and 9.9%, respectively (Fig. 2-A) when submergence was over on day 27 or 36. Mieke *et al.* (2003) also found that there was no effect of soil flooding on Fv/Fm of *Genipa Americana*, indicating that there was no damage to the photosynthetic apparatus of the species.

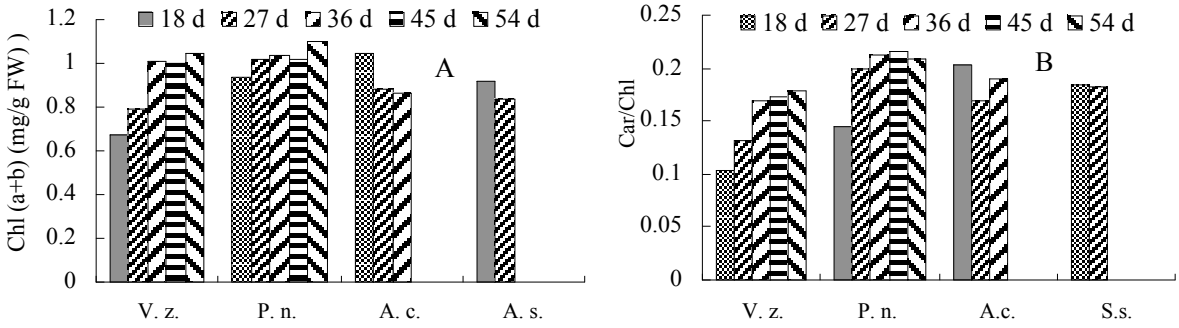

Fig. 1 Changes of leaf chlorophyll contents (A) and car/chl (B) of four grasses with submergence time

V. z., P. n., A. c., and S. s. represent *V. zizanioides*, *P. notatum*, *A. compressus*, and *S. secundatum*, respectively

As to _PS_, the 4 grasses also had similar _PS_values before submergence; then all declined as submergence occurred. However, with submergence further continuing, _PS_ of carpet grass and St. Augustine continued a rapid declining trend, whereas that of vetiver and bahia grass declined very slowly, especially for vetiver grass showing quite stable under submergence. _PS_ values of vetiver, bahia grass and carpet grass fell down by 5.5%, 13.1% and 46.2%, respectively, 36 d after submerging, while St. Augustine down by 38.6% after submergence for 27 d (Fig. 2-B).

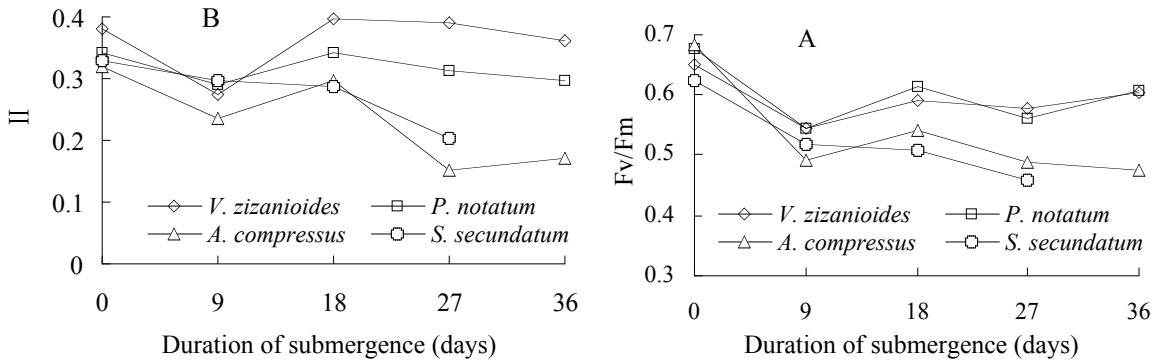

Fig. 2 Changes of Fv/Fm (A) and _PS_(B) of four grasses during submergence

In addition, different from the other 3 grasses, vetiver's q_p was higher after submergence than prior to submergence (data not shown here). This suggests that submergence may stimulate the PSII reaction centre of vetiver grass more open and improve its photochemical electron transfer efficiency, thus maintaining a high photosynthesis rate. All the above results showed that photosynthetic apparatus and photosynthesis capacity of vetiver and bahia grass were not affected obviously by submergence; on the contrary those of carpet grass and St. Augustine were affected seriously.

3.2 Effects of submergence on biochemical response of grasses

3.2.1 Changes of peroxidation of membrane lipids after submergence

MDA is the end product of membrane lipid peroxidation and therefore its accumulation is an important mark that membrane's structure and function are damaged

and destructed (Halliwell, 1981). Thereby, the extent of lipid peroxidation is expressed usually by leaf MDA content (Lin *et al.*, 1984; Ghanati *et al.*, 2005). Before submergence, MDA contents of the 4 grasses in the normal growing state were close each other, between 52~58 nmol g^{-1}, and then immediately ascended after being submerged. On day 27, MDA content of St. Augustine was up to 112.8 nmol g^{-1}, increased by 98.2%; on day 36, MDA content of carpet grass increased by 1.26 times than that prior to submerging, while vetiver and bahia grass increased only by 84.1% and 66.7%, respectively (Fig. 3). That was to say, vetiver and bahia grass could resist excessive lipid peroxidation more effectively than St. Augustine and carpet grass under submergence, thus protecting their membrane system farthest from destruction. This was probably the result that carotenoid contents of the latter two grasses increased during submergence (Fig.1-B). Carotenoid has been documented to play the most important role in quenching singlet oxygen ($^1O_2$) produced in plants, which can prevent $^1O_2$ induced peroxidation of unsaturated fatty acids and, therefore, protect the various membrane systems, especially the photosynthetic membranes of chloroplast from destruction (Telfer *et al*., 1994).

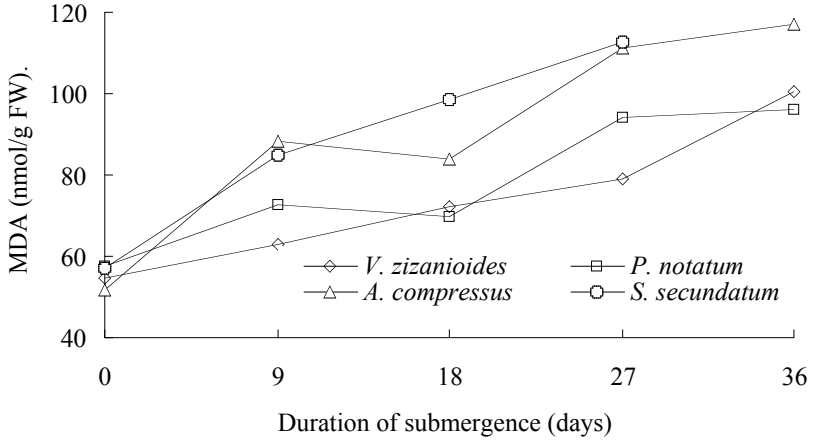

Fig. 3 Leaf MDA contents of four grasses with submergence time

3.2.2 Changes of the SOD activity after submergence

Plants active oxygen clearing capacity and their antioxidation ability are marked by activity of enzyme-catalyzed defensive system (like SOD) and content of non-enzyme-catalyzed antioxidant (like carotenoid) in them. The damage of environmental stress to plants and their resistance to stress are related closely to the activity of SOD in them (Gannopolitis and Ries, 1977; Lin *et al.*, 1984). SOD activity of St. Augustine and carpet grass went down obviously from day 9 to 27 or to 36 after submergence, by 23.5% and 32.1%, respectively, whereas that of vetiver and bahia grass stayed almost stable or even went up for vetiver during the same period; furthermore, the SOD activity of the latter two species were significantly higher than that of the former two (Table 1).

Table 1 Leaf SOD activity (unit/g FW) of four grasses with submergence duration

Species	9 days	18 days	27 days	36 days
A. compressus	316.3	252.5	230.4	214.9
S. secundatum	282.2	251.4	215.9	--
V. zizanioides	372.4	440.5	299.2	389.5
P. notatum	404.6	312.3	266.5	395.2

3.3 Effects of submergence on leaf ultrastructure

The ultrastructure of plant leaves, a sensitive index reflecting environmental situations, has been used more and more to study the effects of such adverse conditions as

toxicity of heavy metals, drought, and low temperature on plants in recent years (Xu *et al.*, 2003; Lin *et al.*, 2005). However, the change of ultrastructure under submergence stress has been poorly documented.

Under the TEM, there was a circuit of closed vascular bundle sheath cells forming a garland in the leaf of control samples of St. Augustine (Plate I-1). The sheath cells had many big chloroplasts whose shape was like a spindle due to being full of starch grains (Plate I-4, I-7). There were well-developed granum thylakoids (GT) and stroma lamella (FR) in chloroplasts (Plate I-10). The double-layered mitochondrion membranes (MM) were complete and mitochondrion cristae (MC) were very abundant (Plate I-13). After suffering from the 9-day submergence stress, the garland in the vascular bundle began to change its shape (Plate I-2); the starch grains in chloroplast disappeared, and chloroplasts (CH) were swelled to elliptical (Plate I-5, I-8); both GT and ST began to break into sparse strips (Plate I-11), the number of MC also greatly decreased (Plate I-14). Plant's photosynthetic systems (PS_and PS_) are just distributed on the membranes of GT and ST (Xie *et al.*, 2000); hence, the photosynthesis would be severely influenced if these thylakoids are destructed. 18 d after submergence, the structure of vascular bundle was still similar to that of the 9-day treatment (Plate I-13), but the number of chloroplasts decreased more, and they swelled more distinctly, almost forming a rotund shape (Plate I-6, I-9); GT and ST broke more acutely and their structure became unclear in the TEM (Plate I-12); however, the double-layered MM still kept somewhat clear with the except of MC breaking acutely (Plate I-15). In addition, vacuolar lipids (VL) increased with submergence time (Plate I-7, I-8, I-9).

As to carpet grass, the leaf ultrastructure feature of the control was very similar to that of St. Augustine (Plate_-1,_-4,_-7,_-10,_-13). 16 d after submergence, the vascular bundle began to change its shape (Plate_-2); the chloroplasts swelled and the starch grains disappeared (Plate_-5,_-8); both GT and ST began to break slightly (Plate_-11), but mitochondrion kept almost intact (Plate_-14). 32 d later, chloroplasts (CH) further swelled into round shape (Plate_-3,_-6); ST and FR became disorganization, and their number clearly decreased and became sparse, but GT basically kept complete (Plate_-9,_-12); furthermore the mitochondrion number rose, their interior became empty except that outer membranes still kept relatively intact (Plate_-15). An increase of mitochondrion number can make the respiration become intense, which would excessively consume materials and energy in cells and, therefore, accelerate death of leaves (Xie *et al.*, 2000).

Regarding bahia grass, the leaf ultrastructure, including vascular bundle, chloroplast and mitochondrion, of the control also assumed a similar feature to that of St. Augustine and carpet grass (Plate _-1, _-4, _-13). Compared to them both, however, there was a big difference that almost no starch grains could be seen in chloroplasts of bahia grass (Plate _-7). 30 d after submergence, the starch grains in chloroplasts increased (Plate _-8); on day 60, they continued to increased, and furthermore their volume became big (Plate _-9). There have been documented that flooding results in an increase of starch concentration in plant leaves (Wample and Davis, 1983; Chen *et al.*, 2005). At the middle time of submergence (30 d), ST and FR began to be disorganized, and their number became fewer (Plate _-11); however its mitochondrion were damaged slightly and MC were still abundant (Plate _-14). At the end of submergence (60 d), ST were further disorganized until they almost vanished and, furthermore, MC also began to break and outer MM burst (Plate _-15).

The leaf ultrastructure of vetiver was also observed in the TEM. The control samples assumed the almost same ultrastructure as the above 3 grasses (Plate _-1,_-4,_-7,_-

10). On day 60 of submergence, its vascular bundle structure did not change shape yet (Plate _-2); neither GT nor ST produced clear disorganization (Plate _-8); the outer MM also kept intact and its MC only broke slightly (Plate _-11); but chloroplasts (CH) swelled into round and starch grains vanished, compared with the control (Plate _-5). On day 120, its vascular structure and chloroplast's shape still did not produce a very big change (Plate _-3,_-6), but ST and GT broke, and their number became sparse (Plate_-9); moreover, the outer MM burst and its MC further disorganized (Plate _-12).

4 Conclusion

From the above results and discussion, it could be concluded that the mechanisms on the strong tolerance of vetiver to submergence contain at least three respects, ecophysiological characteristic, biochemical response, and cell optical ultrastructure.

Firstly, in the same submergence condition, the photosynthetic apparatus of vetiver and bahia grass were adversely affected relatively slight compared to the intolerant species, St. Augustine and carpet grass. As a result, their photochemical electron transfer efficiency was enhanced and, therefore, their photosynthesis was not prohibited very severely.

Secondly, MDA contents of the two tolerant species, vetiver and bahia grass increased only a little, and their SOD activity kept almost stable after submergence; on the contrary, MDA contents of St. Augustine and carpet grass increased conspicuously, and their SOD activity decreased markedly. In addition, carotenoid contents of vetiver and bahia grass were enhanced under submergence stress. This indicated that vetiver and bahia grass assumed stronger antioxidation ability to lighten the damage of membrane lipid peroxidation to them than St. Augustine and carpet grass.

Lastly, when the submergence duration was up to 60 d, vetiver was damaged by submergence stress much slighter than bahia grass with special reference to the cell ultrastructure of such important organelles as vascular bundle, chloroplast and mitochondrion; the damaged symptom of vetiver's ultrastructure was not revealed distinctly until it was submerged up to 120 d.

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A Brief Introduction to the First Author

Dr. Hanping Xia, a restoration ecologist, is working at the South China Botanical Garden, Chinese Academy of Sciences. Since 1991, he has been engaged in a wide range of R&D on the Vetiver System for the purpose of erosion control and polluted environment mitigation. He creatively initiated “the Vetiver Eco-engineering” from his working experience of many years and has advocated over 10 enterprises to run vetiver market in China. So far he has one monograph and over 40 academic papers in this aspect published. He won the first “Vetiver Champion” and “The Kind of Thailand Vetiver Award” at ICV-3. He also won two second prizes awarded by The Vetiver Network in 1998.

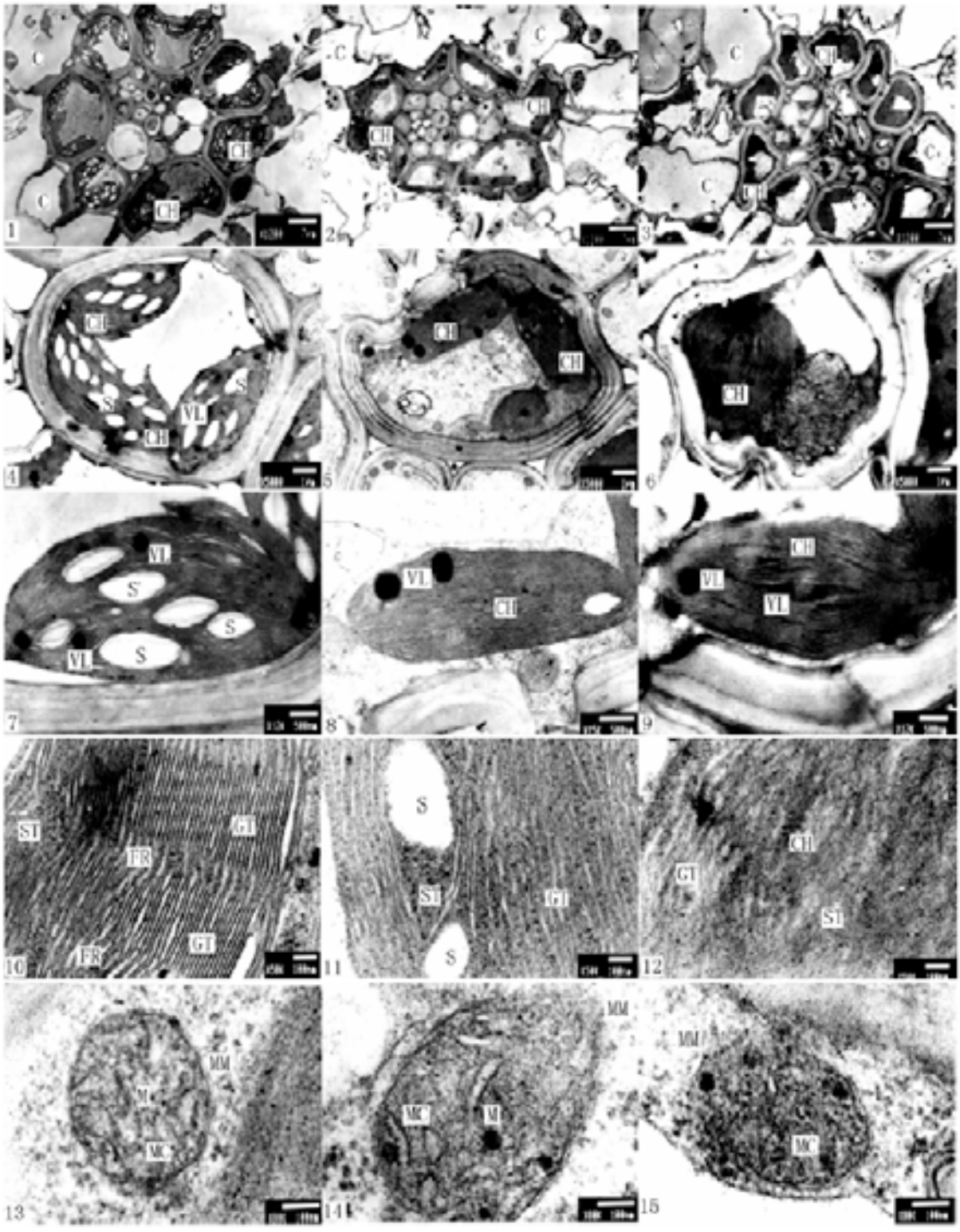

Plate I Leaf ultrastructure of St. Augustine

1~3: Vascular bundler in control, 9 d and 18 d submergence, respectively. (Bar = 7 μm for 3 plates)
4~6: Single cell and chloroplasts in control, 9 d and 18 d submergence. (Bar = 1 μm for 3 plates)
7~9: Chloroplast in control, 9 d and 18 d submergence. (Bar = 500 nm for 3 plates)
10~12: Thylakoids of chloroplast in control, 9 d and 18 d submergence. (Bar = 100 nm for 3 plates)
13~15: Mitochondrion in control, 9 d and 18 d submergence. (Bar = 100 nm for 3 plates)

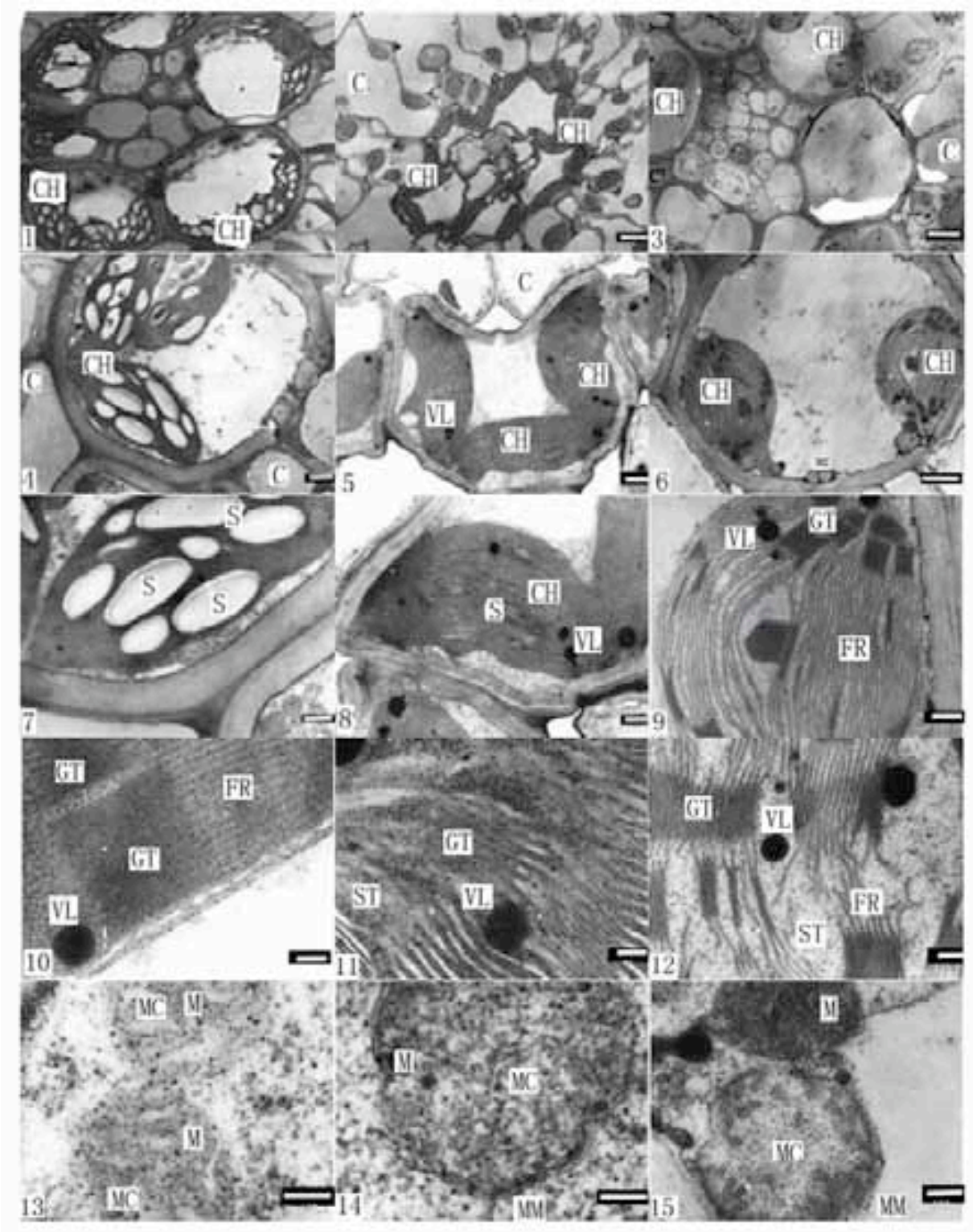

Plate _ Leaf ultrastructure of carpet grass

1~3: Vascular bundler in control, 16 d and 32 d submergence, respectively. (Bar = 7 μm for 3 plates)

4~6: Single cell and chloroplasts in control, 16 d and 32 d submergence. (Bar = 1 μm for 3 plates)

7~9: Chloroplast in control, 16 d and 32 d submergence. (Bar = 500 nm for 3 plates)

10~12: Thylakoids of chloroplast in control, 16 d and 32 d submergence. (Bar = 100 nm for 3 plates)

13~15: Mitochondrion in control, 16 d and 32 d submergence. (Bar = 100 nm for Plate 13 and 14, and 200 nm for Plate 15)

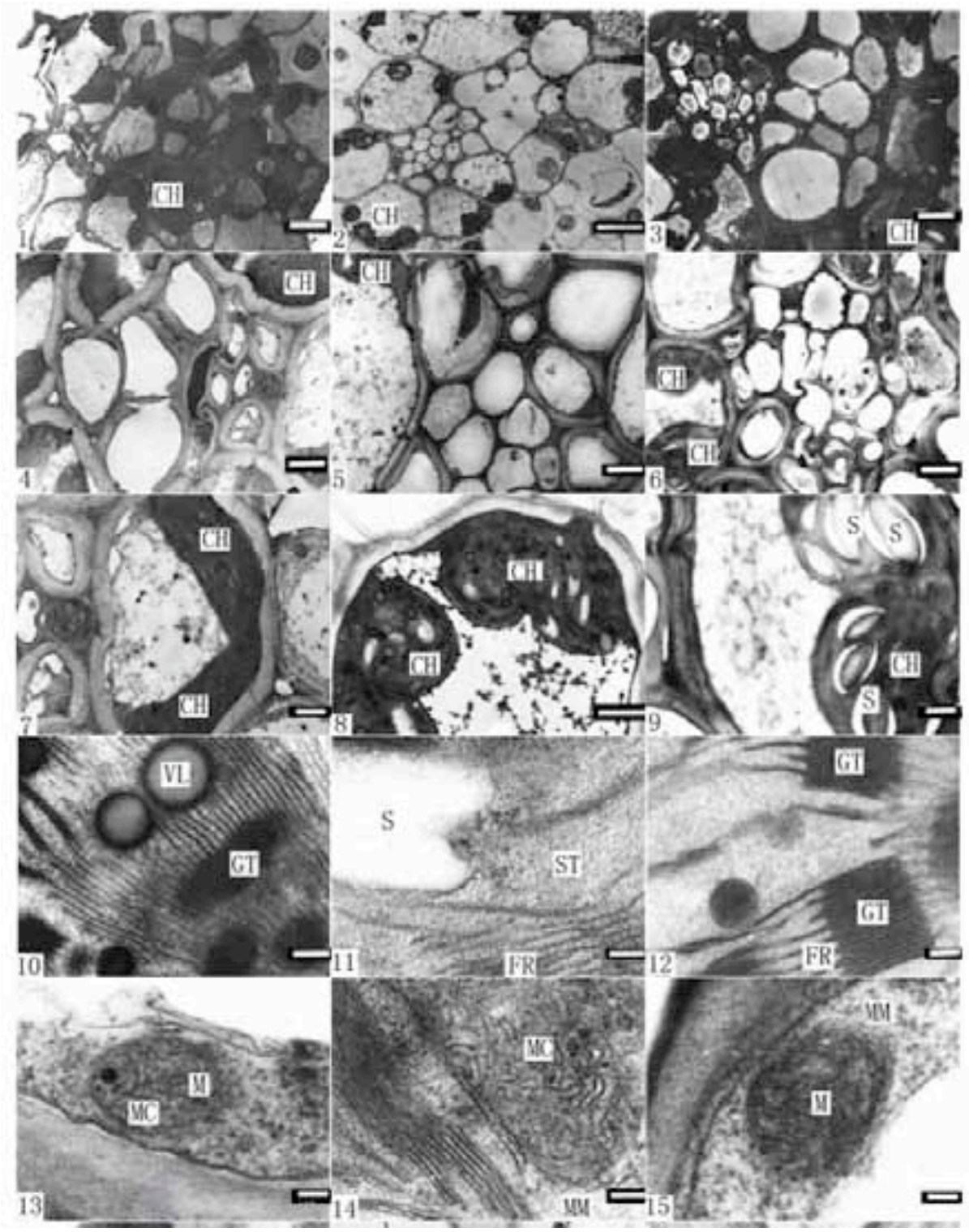

Plate _ Leaf ultrastructure of bahia grass

1~3: Vascular bundler in control, 30 d and 60 d submergence, respectively. (Bar = 7 μm for 3 plates)

4~6: Enlarged vascular bundler in control, 30 d and 60 d submergence. (Bar = 2 μm for 3 plates)

7~9: Chloroplasts in control, 30 d and 60 d submergence. (Bar = 2 μm for Plate 7, and 1 μm for Plate 8 and 9)

10~12: Thylakoids of chloroplast in control, 30 d and 60 d submergence. (Bar = 200 nm for Plate 10, and 100 nm for Plate 11 and 12)

13~15: Mitochondrion in control, 30 d and 60 d submergence. (Bar = 100 nm for 3 plates)

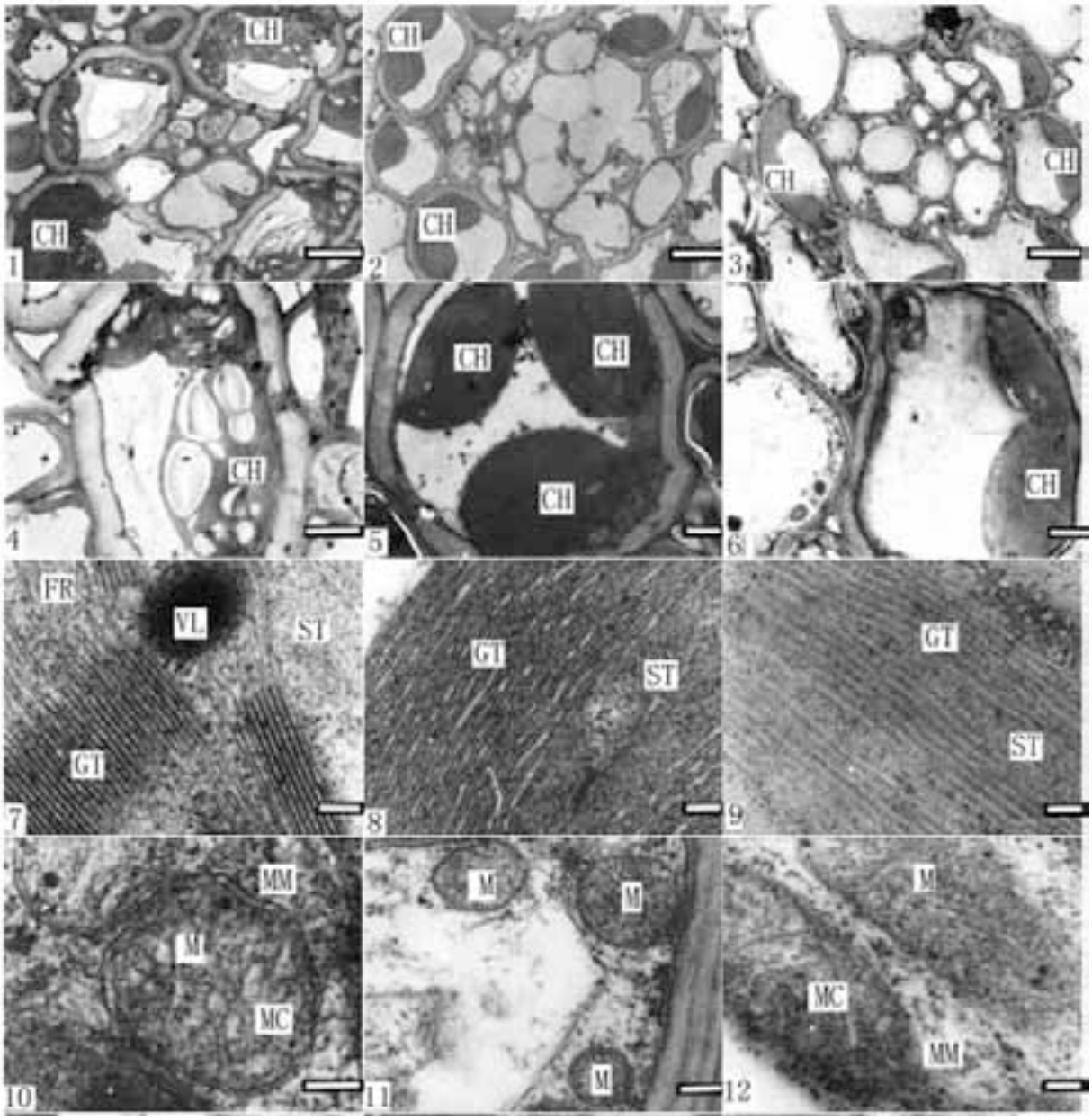

Plate _ Leaf ultrastructure of vetiver grass

1~3: Vascular bundler in control, 60 d and 120 d submergence, respectively. (Bar = 5 μm for 3 plates)

4~6: Chloroplasts in control, 60 d and 120 d submergence. (Bar = 2 μm for Plate 4, and 1 μm for Plate 5 and 6)

7~9: Thylakoids of chloroplast in control, 60 d and 120 d submergence. (Bar = 100 nm for 3 plates)

10~12: A mitochondrion in control, 60 d and 120 d submergence. (Bar = 100 nm for Plate 10 and 12, and 200 nm for Plate 11)