EFFECTS OF SEDIMENT TYPE ON STEM MECHANICAL PROPERTIES OF THE SUBMERGED MACROPHYTE *HYDRILLA VERTICILLATA* (L.F.) ROYLE

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ABSTRACT

The decline of submerged macrophytes during the eutrophication progress can be partly attributed to the adverse effects of fertile sediment on morphology and physiology of plants. However, little is known about its effects on mechanical properties, although this has been confirmed in terrestrial plants. In this study, the morphological traits, mechanical properties (tensile force, tensile stress and bending index) and cellulose content of *Hydrilla verticillata* grown on three types of sediment (sand, clay and loam) with different nutrient levels, were examined experimentally. Results showed that considerable variations in mechanical properties of plants were observed among the three sediments and along the stem, with the highest values in clay sediment and a decreased trend from the lower to upper segment, especially when grown in loam sediment, indicating that mesotrophic sediment benefits to higher mechanical resistance of stems than the other two sediments, and plants were prone to break at the other two segments, rather than the lower segment. Stem cross sectional area and internode length were significantly correlated with tensile force, and tensile stress was a linear function of stem cellulose content. Plants grown in clay sediment showed the shortest plant height and the maximum branch numbers, which are morphologically advantageous to reduce the hydraulic force. In conclusion, fertile sediment stimulated the plant growth at the expense of *H. verticillata* performance with respect to mechanical resistance. Decrease of mechanical resistance could contribute to the decline of submerged macrophytes with the enrichment of sediment nutrient during the process of eutrophication.

KEYWORDS: *Hydrilla varticillata,* sediment type, mechanical properties, segments, cellulose content, cross sectional area

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1. INTRODUCTION

Hydraulic forces resulting from waves and winds can strongly influence plant growth and distribution of submerged macrophytes [1-3]. The mechanical properties of stems including tensile and bending resistances are important features of submerged macrophytes in adapting to wave disturbance of aquatic environment [4, 5] and differ along waves and water depth gradient in field observation [4-6]. However, little is known about their response to sediment nutrient condition, which contributes to the decline of submerged macrophytes with eutrophication [7-9], supported by extensively studies on their morphology and physiology [10, 11].

The mechanical properties of terrestrial plants are significantly affected by nitrogen level: moderate nitrogen fertility benefits to strong culms but higher nitrogen application can lead to higher lodging [12-15]. High level of nitrogen fertility promotes vegetative growth, producing lanky and succulent culms, which generally leads to a higher risk of bending or/and bulking, especial of basal internodes, the target for lodging resistance [15, 16]. Additionally, the mechanical properties of aquatic plants differ along the stem [17, 18], due to the difference in the ontogenesis stages and hydraulic forces along the stem [2, 19]. Therefore, the fertile sediment during eutrophication may cause adverse effects on mechanical properties of submerged plants stems with differences along the stem.

Hydrilla verticillata (L. f.) Royle, a worldwide perennial submerged macrophyte and a pioneering species for lake restoration, can grow on different sediment types, has a vertical growth form and prefers stagnant water, usually with a long slender stem, which is easily broken by drag force [20-23]. However, the mechanical properties of this species are unknown. The objectives of this study are to: (i) investigate the effects of sediment nutrient condition on the mechanical properties of *H. verticillata* stems, (ii) the relationship between the morphology, cellulose content and the mechanical properties, and (iii) the differences in mechanical properties along the stem.

2. MATERIALS AND METHODS

The study was conducted at the Donghu Experimental Station of Lake Ecosystem (30°33 N, 114°21 E), China, from July 26 to August 15, 2008. Three types of sediment (sand, clay and loam) were used in this experiment, representing infertile, moderately fertile and fertile sediments, respectively. The sand was commercially-available washed sand (0.1cm diameter; TP, TN and organic matter content DW were undetectable), while clay (TP = 0.70 mg g^{-1} DW, TN = 3.41 mg g^{-1} DW and organic matter content = 8.13%) and loam (TP = 1.40 mg g^{-1} DW, TN = 6.32 mg g^{-1} DW and organic matter content = 11.70%) sediments were collected from the mesotrophic and eutrophic areas of Lake Donghu, respectively.

H. verticillata were pre-incubated in tap water at a 1.0-m depth for 2-months after collection from Liangzi Lake, Hubei Province, China (30°65 N, 114°31 E) on May 26, 2008. At the beginning of the experiment, apical shoots of *H. verticillata* (15.00±0.52 cm in length with similar morphology, 0.64±0.05g, fresh weight) were uniformly planted in 72 sediment-filled plastic pots (14 cm diameter, 10 cm depth, one shoot per pot) with the lower 5 cm buried in the sediment. Each sediment treatment included 24 pots, which were uniformly incubated in three aquaria (60 cm × 25 cm × 25 cm, eight pots per aquarium) containing 50 cm of tap water (NO_3-N: 1.06 mg L^{-1}, NH_4^+-N: undetectable, PO_4^{3-}-P: 0.04 mg L^{-1}). Each aquarium was regarded as an independent replicate of the treatments. The plants were cultured outdoors under a transparent polycarbonate roof and the water in each aquarium was renewed every 3 days. Twenty days later, all the plants were harvested for measurements of the morphological and mechanical properties as well as the cellulose content.

The morphological properties of *H. verticillata* include plant weight, height, branch number, and the weight, length, cross sectional area and internode numbers of 3 individual segments closest to and farthest away from the sediment, and the middle parts between them from an intact stem (the lower, middle and upper segments, in equal length) [17]. Length and diameter of *H. verticillata* stem were determined by tape and caliper gauge, respectively. According to the definition of the specific root length [24], specific internode length of segments was calculated as the ratio of internode length to internode weight. Stem cross sectional area was calculated by the diameter regarding that the stem cross section as approximate a cycle.

Mechanical properties, including the tensile force, tensile stress [2, 17, 18, 25], and bending index of the segments, were measured in a relatively straightforward manner [26, 27]. Bending index was defined as log (Φ), where Φ represents the bending angle of the segment measured by a protractor, according to the definition of lodging score of Spring Wheat (*Triticum aestivum* L) [28].

Each segment was carefully fixed near its base to the gauge by a clamp. Then an increasing parallel tensile force was applied slowly to the upper clamp until mechanical failure, the distance between the two clamps was 0.03 m. The maximal tensile force before shoot broken was defined as tensile force (N). Tensile stress (MN m^{-2}) for each segment was calculated by dividing the tensile force (N) by cross sectional area of the broken end (mm^2) [25].

The plant collected from each aquarium was oven dried at 80 °C for 3 days, and then ground into fine powder. Approximately 0.02 g of powdered sample was extracted with acetic-nitric reagent (30 min, 100 °C) and centrifuged (2000 × g). After removed the supernatant, the sample was extracted again by distilled water and acetone, respectively. Finally, the cellulose contents of the residue were measured by a modified acetic-nitric method [29].

Statistical analysis was carried out with the software SPSS (version 16.0). The mechanical variables were tested for normality (One-Sample Kolmogorov-Smirnov Test) and homogeneity (Levene's test), and data were square root, sin or/and cos transformed when equality of variance was not satisfied between treatments. A two-way ANOVA with sediment nutrient level and segment was performed on mechanical data, and a one-way ANOVA with sediment nutrient level was performed on the morphological and cellulose data of the plants. Differences between individual means were determined with Duncan's test at the 0.05 significance level, while, the correlation of tensile force with cross sectional area and tensile stress with cellulose contents were tested using linear regression.

3. RESULTS

The results showed that *H. verticillata* grown in the clay sediment had the highest mechanical strength indicated by most mechanical property indices (including tensile stress, 2.01 ± 0.90 MN m^{-2}, bending index, 2.02 ± 0.05, average value), except for the tensile force of the upper segment, which was significantly lower than that in the loam sediment (Fig. 1). The tensile force (1.56 ± 0.43 N), tensile stress (1.70 ± 0.60 MN m^{-2}) and bending index (1.89 ± 0.12) were all lowest for plants grown in the sand sediment (Fig. 1b, c). These results showed that sediment nutrient condition had significant effects on the tensile force and bending index, but no significant effects on the tensile stress (Table 1).

Mechanical properties of *H. verticillata* showed significant differences along shoot segments (Fig. 1). Although the trend of variations were different among sediments and locations of stems, mechanical properties of plants decreased from the lower to upper regions of the stems, especially when grown in the loam sediment (Fig. 1).

The plant height, weight and the stem cross sectional area of *H. verticillata* were all highest in the loam and lowest in the sand sediment, respectively (Table 2; Fig. 2d). The highest length and weight values of internode were observed at the loam treatment, while their lowest were at the clay treatment (Fig. 2a, b). However, the highest specific internode length, the branch number and cellulose content of the plant (Fig. 2c; Table 2) were all found at the clay treatment. Whereas, the lowest specific internode length was at the loam treatment (Fig. 2c), and both the lowest branch number and cellulose content were observed at the sand treatment (Table 2). The cross sectional area and internode length decreased gradually upward along the shoots at all treatments (Fig. 2d; Fig. 2a)

TABLE 1 - The effects of sediment nutrient level and segment on mechanical properties of *H. verticillata* stems. Values are the SS of two-way ANOVA. Statistical significance is indicated by asterisk(s): ^{ns} > 0.05, * < 0.05, ** < 0.01, *** < 0.001.

	Tensile force ^{A}	Tensile stress ^{B}	Bending index ^{C}
Sediment type	0.011 *	0.197 ^{ns}	0.017 *
Segment	0.060 ***	2.610 ***	0.016 *

^{A} After sqrt-transformation. ^{B} After sqr-ln-square-transformation. ^{C} After sin-transformation.

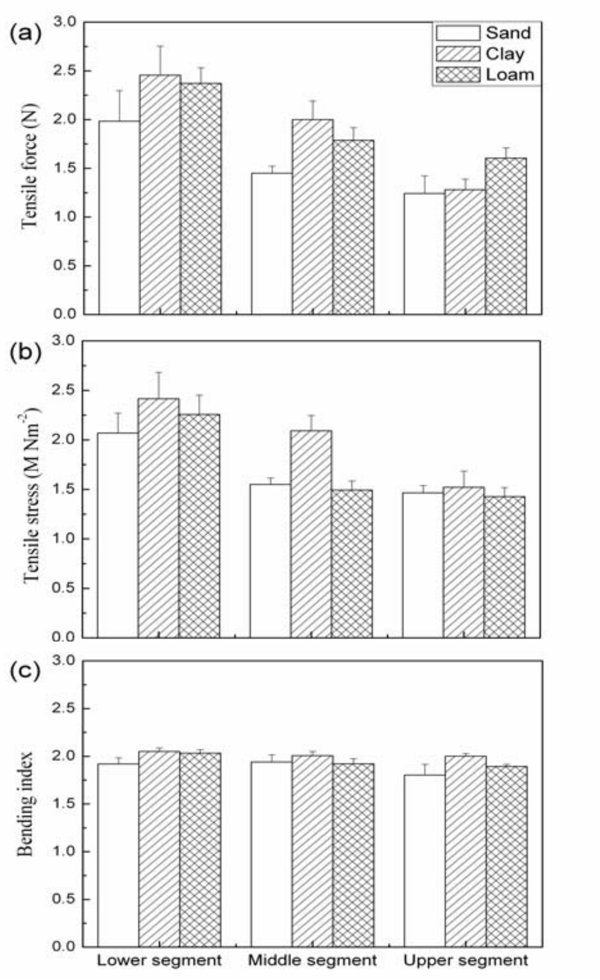

FIGURE 1 - Variation in mechanical parameters of *H. verticillata* grown in three sediments. Mean and standard error are displayed.

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TABLE 2 - The morphological parameters and the cellulose contents (Mean ± standard error, *N* = 5, 24) of the whole plants.

	Sand	Clay	Loam
Shoot weight (g) ^{A}	4.28±0.28 a	4.88±0.56 a	5.39±0.55 a
Plant height (m)	0.44±0.06 a	0.43±0.02 a	0.62±0.03 b
Branch number	3.00±0.00 a	5.17±0.45 b	4.5±0.28 b
Cellulose content (% DW)	15.17 ± 3.02 a	22.48 ± 4.12 b	18.35 ± 2.44 a

The same letter indicates no significant difference between treatments (Duncan's test at the 0.05 significance level). ^{A} After ln-sqrt-transformation.

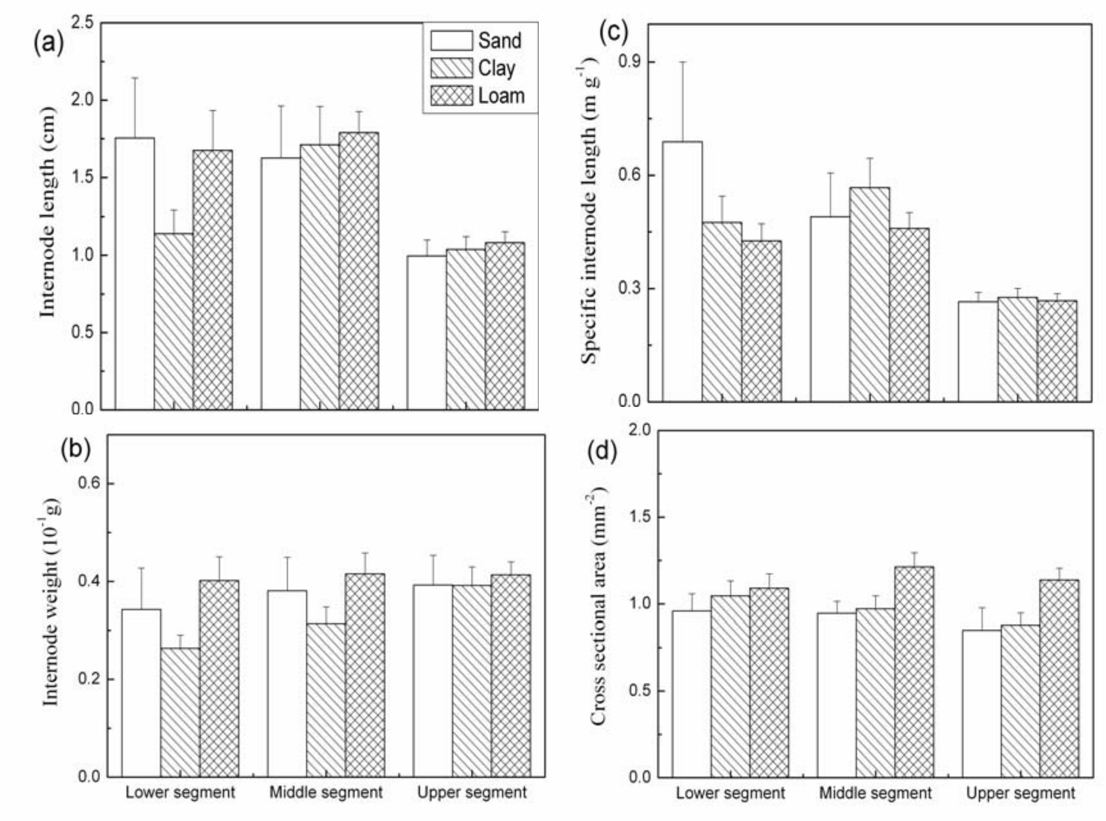

FIGURE 2 - Variation in morphological parameters of *H. verticillata* grown in three sediments. Mean and standard error are displayed.

All the mechanical parameters were positively correlated with each other (Table 3). The tensile force was positively correlated with the internode length and the cross sectional area (Table 3; Fig. 3a). The tensile stress had negative correlations with the internode weight and stem cross sectional area, and a positive correlation with specific internode length and cellulose contents (Table 3; Fig. 3b). There were not relationship between morphological parameters and the bending index (Table 3).

TABLE 3 - Correlation coefficient (r) between the cellulose content, morphological and mechanical parameters.

	Tensile force	Tensile stress	Bending index
Tensile force[A]	1	0.718 **	0.222 *
Tensile stress	0.718 **	1	0.285 **
Bending index	0.222 *	0.285 **	1
Internode length	0.220 *	0.134	0.096
Internode weight	0.042	-0.309 **	-0.085
Specific internode length	0.109	0.340 **	0.206
Cross sectional area	0.458 **	-0.247 *	-0.05
Plant weight	0.207	-0.064	-0.235
Plant height	0.028	-0.03	-0.153
Branch number	0.01	0.008	-0.123
Cellulose content	0.270	0.821 *	0.250

* and ** significant at $P < 0.05$ and 0.01, respectively.
[A] Parameters in bold print were mechanical parameters.

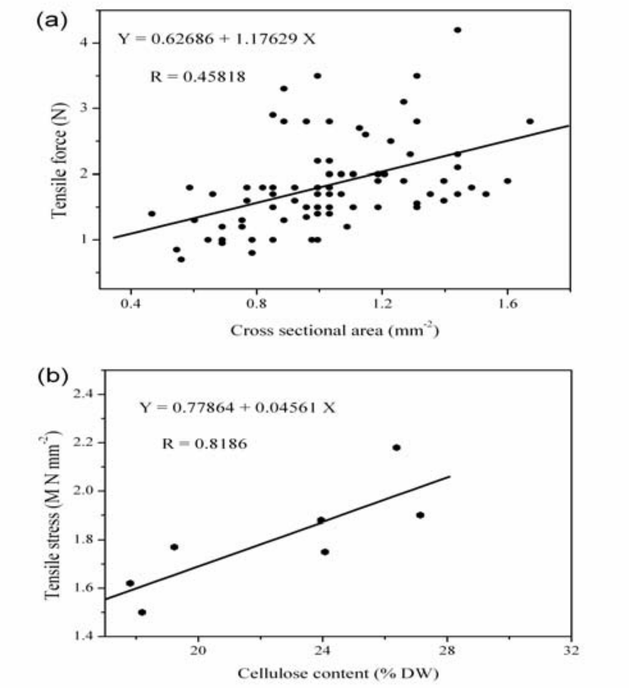

FIGURE 3 - The relation between tensile force and stem cross-sectional area, tensile stress and cellulose content for *H. verticillata* grown in three sediments.

4. DISCUSSION

In the present study, the results first demonstrated that sediment nutrient condition significantly affected the mechanical properties of aquatic plants (*H. verticillata*), exhibiting higher mechanical properties in sediment of medium nutrient, and lower ones in both fertile and infertile sediments. This is consistent with previous studies on terrestrial plants (Miscanthus*,* wheat and rice) [12-16] where the application of nitrogen had increased stem breaking strength but further increase resulted in a decreased trend. It should be noted that in this study, besides high level of nitrogen fertilization, loam sediment also had higher total phosphorus and organic matter than the other two sediments. The three parameters all have significant effects on the plant growth and distribution of submersed macrophytes [10, 11, 30]. However, for crops the fertile soil is the high levels of nitrogen fertilization since the 'green Revolution' began, rather than total phosphorus or organic matter [31, 32]. Therefore, in the present study, the effects of sediment type on the mechanical properties of *H. verticillata* were by their combination (TN, TP and organic matter) rather than the TN alone. Hence, how and why the three parameters in sediment alone affect the mechanical properties of aquatic plants stems deserves further detailed investigation.

In the present study, the mechanical properties of *H. verticillata* decreased considerably from the lower to the upper segment at all treatments, especially when grown in fertile sediment, in addition, the highest tensile force of upper segments were also observed at fertile sediment. These suggest that plants grown in fertile sediment are prone to break at middle part, which will inhibit the growth and survival of parent plant growth, whereas plants in the moderately fertile sediment, prefer to break at upper part, without fatal damage for parent plants. These results together demonstrate that the mechanical properties of middle to upper stem are more important to submerged macrophytes, which do not agree with the reports on terrestrial plants [15, 16] that the pushing resistance of the lower stem is regarded as main target for lodging resistance introducing by nitrogen fertilization. The different responses of submerged and terrestrial plants to sediment nutrient level may be explained by the differences between aquatic and terrestrial habitats.

In this study, vegetative growth (plant height and weight, here) increased with the increase in sediment nutrient condition, whereas the change of cellulose content to sediment nutrient condition was consistent with the response of mechanical properties. This suggests that fertile sediment improves plant growth by succulent stems and lower mechanical tissue, resulting in taller stem but with a higher mechanical fragmentation. Plant height, internode length and stem cross sectional area can determine the mechanical properties of emergent and terrestrial plants [6, 31]: stem cross sectional area is strongly correlated with the tensile force, and small plant height makes stronger emergent plant stems [4, 6, 25], the basal internode length of terrestrial plants is positively related with its mechanical properties (e.g., wheat, barley and oats) [33, 34]. In the present study, except for the relationship between the plant height or weight and mechanical parameters, there were relationships between the internode length and tensile force for the middle and upper segments, and between the cross sectional area and tensile force, which are consistent with the basal segment of crops. These indicate that the plant height may determines the bending resistance of emergent and terrestrial plants [6, 31], but not for submerged macrophyte, which are supported through buoyancy, whereas the mechanical properties of key intermodal segment both in submerged and terrestrial plants are determined by their morphology and cellulose contents.

Generally, in plant community, stems may support each other and divert critical forces to the entire stand, so as to form ‘framework-like’ protection [6, 35, 36], which suggest that higher branch numbers increase the resistance of macrophytes to mechanical stress. On the other hand, small-sized plants have advantage in reducing mechanical damage [37, 38]. In the present study, *H. verticillata* grown in sediment of medium nutrient, had higher branch numbers and lower shoot height than the plants of other treatments, indicating that moderate nutrient sediment maintained smaller mechanical stress of the plants than other nutrition conditions in similar wave disturbance of aquatic environment.

5. CONCLUSIONS

In the present study, the mechanical properties of stems in submerged macrophytes (*H. verticillata*) were affected by sediment nutrient condition. Plants grown in sediment of medium nutrient had higher resistance (i.e. higher stem mechanical properties, smaller shoot size and higher branch number) to similar wave disturbance of aquatic environment than those in both infertile and fertile sediments. Besides the sediment enrichment, the increase of ammonium (NH_4^+) concentrations and reduction in light availability in water column are also prominent stresses leading to the decline of submerged macrophytes during the eutrophication (e.g. physiological stress and reduction in photosynthesis) [24, 39-41]. Therefore, it is essential to further examine the contribution of mechanical properties to the decline of submerged macrophytes by conducting studies on the effects of high NH_4^+ concentration and reduction in light availability in water column from a mechanical perspective.

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