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Primary Research Paper

Seed germination of three species of *Vallisneria* (Hydrocharitaceae), and the effects of freshwater microalgae

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Abstract

Two experiments were conducted to investigate seed germination under natural temperature and light regimes and to evaluate the influence of freshwater microalgae on seed germination of three *Vallisneria* species. Seeds exposed to natural seasonal temperature and light changes for 24 months germinated only in spring, perhaps indicating an annual dormancy/non-dormancy cycle. The ecological background of the natural habitat seems to play predominant roles in seed germinability. Mean cumulative seed germination percentages of *Vallisneria natans*, *V. denseserrulata*, and *V. spinulosa* of the first year were 35.2, 19.2, and 11.2%, respectively, and of the second year were 80, 72, and 32.4%, respectively. Germination rates differed significantly among the three *Vallisneria* species exposed to natural seasonal temperature and light changes in the first year, but differed only between *Vallisneria spinulosa* and the other two species in the second year. The differences of germination may influence the geographical distribution and thus be related to different survival strategies of the species. Seed germination rates in culture solution inoculated with algae were significantly higher than in solution with no algae. A strong correlation occurred between total biomass of freshwater microalgae and seed germination of the three studied species. Apparently, the extracellular products of freshwater microalgae may play an important role in seed germination. These results support the hypothesis that *Vallisneria* colonization may be mediated by algae facilitation.

Introduction

The genus *Vallisneria* (Hydrocharitaceae) includes about six to 10 species, three of which are recorded in China: *V. spinulosa* Yan, *V. denseserrulata* (Makino) Makino, and *Vallisneria natans* Hara (Sun, 1992). *Vallisneria spinulosa* is endemic to China, only occurring in middle and lower reaches of Yangtze River; *V. denseserrulata* is restricted to China and Japan; and *V. natans* is a cosmopolitan species especially in tropical and subtropical zones (Lowden, 1982; Sun, 1992). The *Vallisneria* species can be found in several kinds of freshwater bodies, such as lakes, ponds, rivers, and paddy fields (Sun, 1992). Plants of *Vallisneria* play important roles in freshwater ecosystems, providing food for waterfowl, nursery habitat for fish, substrate for invertebrates, and have a strong influence on water quality (Korschgen et al., 1997). All *Vallisneria* plants can produce both large numbers of seeds and numerous winter buds (Xiong & Li, 2002). In these species sexual reproduction may predominantly serve for long-distance dispersal and long periods of dormancy, whereas vegetative propagation is suitable for dispersal over short distances and overwintering (Bartley & Spence, 1987).

Germination is a critical stage in the life cycle of plants, and often controls population dynamics, with major practical implications (Radosevich et al., 1997). Several studies have been conducted to investigate reproduction of *Vallisneria* (Donnermeyer & Smart, 1985; You & Song, 1995;

Li & Cui, 2000; Spencer et al., 2000), all of which concerned temperature and substrate effects. Little is known about germination patterns of *Vallisneria* under natural seasonal temperature and light changes. Facilitation acts as a succession mechanism when, either directly or indirectly, one species favors the establishment of another (Connell & Slatyer, 1977; Van Andel et al., 1993; McCook, 1994). Many studies have investigated seed germination, but most focused on factors, such as seed weight, temperature, salinity, light, soil disturbance, plant extract, and oxygen concentration (e.g. Shipley & Parent, 1991; El-Sheekh & El-Saied, 1999; Khan et al., 2001; Isselstein et al., 2002). However, only a few studies have evaluated the influences of organisms on seed germination. Experiments have demonstrated that algae can maintain substrate humidity and favor seed germination in tropical dune slack species (Vázquez et al., 1998). Another experiment has demonstrated that within specific ranges in the inoculate, rhizospheric bacteria are capable of increasing the number of germinated seeds of two wheat species (Somova et al., 2001). But to our knowledge, the potential influence of freshwater microalgae on seed germination of submerged plants has not been studied although there are a large number of microalgae that coexist with them.

The objectives in the present study were to: (a) investigate germination of three *Vallisneria* species under natural seasonal temperature and light changes, (b) determine the influence of algal conditions on germination, and (c) evaluate the possibility of a facilitation process in which algae play an important role.

Materials and methods

Seed material

Fully ripe fruits of the three studied species were randomly collected directly from plants in October/November 2000 from the Yunihu Lake, which is located at the middle reaches of the Yangtze River Basin in Gongan County, Hubei Province, South Central China (113°32′–113°36′ E, 29°58′30°01′ N). The Yunihu Lake is shallow with abundant aquatic plants. For each species, entire fruits were collected from 20 different plants and thoroughly mixed. Fruits were transported to the laboratory at Wuhan University, dried and stored at room temperature until germination studies were initiated. All experiments were conducted with seed batches collected in 2000.

Seed germination experiments

Experiment 1: Pattern of seed germination of the three studied seeds

This experiment was conducted from 15 January 2001 to 25 December 2002 in the laboratory. Five hundred seeds in all of each species were detached from their fruits, and then thoroughly mixed to minimize effects of a single parental plant on germination.

For all experiments, seeds of each species were chosen at random from that species-mixed collection. Seeds were placed in 9-cm diameter Petri dishes containing 30 ml of fresh tap water. Five replicates of 100 seeds of each species were used for each treatment. All Petri dishes were placed in the laboratory and exposed to natural seasonal temperature and light changes. Fresh tap water was added to every Petri dish as necessary to maintain around 30 ml during experiment.

During the experiment, daily temperature was recorded twice at 8 AM and 7 PM and the number of germinated seeds of each species was recorded weekly. A seed was considered to have germinated when the radicle was 1 mm and germinated seeds were discarded after counting. Germination rate was calculated by the number of seeds germinated during a month in relation to total initial seed number (viable + non-viable) (in %).

Experiment 2: Relationship between freshwater microalgae and seed germination

This experiment was performed in the laboratory from 9 March 2002 to 15 June 2002 because a large number of freshwater microalgae were found in all Petri dishes of experiment 1. One thousand seeds of each species were detached and thoroughly mixed to minimize effects of a single parental plant on germination. For all experiments, seeds of each species were chosen at random from that species mixed collection. All seeds of the three species were surface sterilized for 20 min in 3.25% sodium hypochlorite to minimize the survival of microbial contaminants before the beginning of experiments.

One thousand seeds of each species were divided into 10 replicates of 100 seeds. Each batch of 100 seeds was placed in a 9-cm diameter Petri dish containing a 30 ml of culture solution [32 mg $Ca(NO_3)_2$; 10 mg KH_2PO_4; 12 mg $MgSO_4$; 20 mg Na_2CO_3 and trace $FeCl_3$ in 1000 ml H_2O]. Five of the 10 Petri dishes of each species were inoculated with a mixed dilution of microalgae obtained from Petri dishes of experiment 1. In each Petri dish, about 100 cells of mixed microalgae (including: *Melosira varians* Ag., 25 cells; *M. granulata* var. *angustissima* Müll., 35 cells; *Navicula simplex* Krassk. 10 cells and *Chroococcus minutus* (Kütz.) Näg. 30 cells) were used. The other five dishes of each species sever as control, only 30 ml of culture solution was added into it. All Petri dishes were placed in controlled temperature chambers at constant temperature regime (20 °C), in darkness or light with a 12/12 h photoperiod (1200 lux). Culture solution was added to every Petri dish as necessary to maintain 30 ml during the experiment.

Each week during a 14-week period, the number of germinated seeds from each species was recorded in a germ-free isolator. A seed was considered to have germinated when the radicle was 1 mm. Germinated seeds were discarded after counting. At the same time, freshwater microalgae were classified and counted under optic microscope with 0.1 ml counter chamber and the size of each microalgae was measured using a measured micrometer. The volume of each microalgae was calculated according to its shape and size ($V_{Melosira\ varians} = \pi r^2 h$; $V_{M.\ granulata\ \mathrm{var.}\ angustissima} = \pi r^2 h$; $V_{Navicula\ simplex} = lwh$; $V_{Chroococcus\ minutus} = 4\pi r^3/3$, where r, l, w, and h are the radius, length, width, and height, respectively). The biomass of each species was calculated ($G = \rho v$, where G, ρ, and v are weight and density and volume, respectively) based on the density of freshwater microalgae closely equals to water (1 mg/ml) (Zhang & Huang, 1991). The total biomass of all species in one Petri dish was summed. Seed germination percentages were calculated by the number of seeds germinated during a week in relation to total initial seed number (viable + non-viable) (in %).

Data analysis

Data were analyzed using ANOVA and regression procedures in SPSS version 11.0. Average germination percentages and 95% confidence limits were calculated for each treatment. We applied two-way ANOVA to examine the effects of species and freshwater microalgae on germination percentages. Linear regression was used to estimate relationship between total biomass of freshwater microalgae and seed germination percentages. Least-significant difference (LSD) was applied to estimate the difference among the three species of *Vallisneria.*

Results

Experiment 1: Pattern of seed germination of the three studied species

The three species studied showed similar germination patterns. The seed sample of *Vallisneria*, collected in October/November did not germinate in winter (January–March) of the first year. They began to germinate in April (spring), then entered dormancy from July (early summer) and came out of dormancy in early spring (March) of the second year. All of the three species exhibited one small peak of germination in April–May (spring) in first year and March–May in second year (spring) (Fig. 1). Mean cumulative seed germination percentages of *Vallisneria natans*, *V. denseserrulata*, and *V. spinulosa* of the first year were 35.2 ± 2.37% ($n = 5$), 19.2 ± 1.16% ($n = 5$), and 11.2 ± 1.39% ($n = 5$), respectively, and of the second year were 80 ± 2.21% ($n = 5$), 72 ± 1.25% ($n = 5$), and 32.4 ± 2.32% ($n = 5$), respectively (Fig. 1).

Seed germination rates of the three studied species exposed to natural seasonal temperature and light changes differed significantly in the first year ($p < 0.05$). However, significant difference in seed germination rates existed between *V. spinulosa* and the other two species in the second year, but no difference between *V. denseserrulata* and *V. natans* was found (Fig. 1).

Experiment 2: Relationship between freshwater microalgae and seed germination

Higher percentages of germination for the three species were observed in dishes containing a culture solution inoculated with microalgae than in those with culture solution only. Mean cumulative seed germination percentages of *Vallisneria spinulosa*,

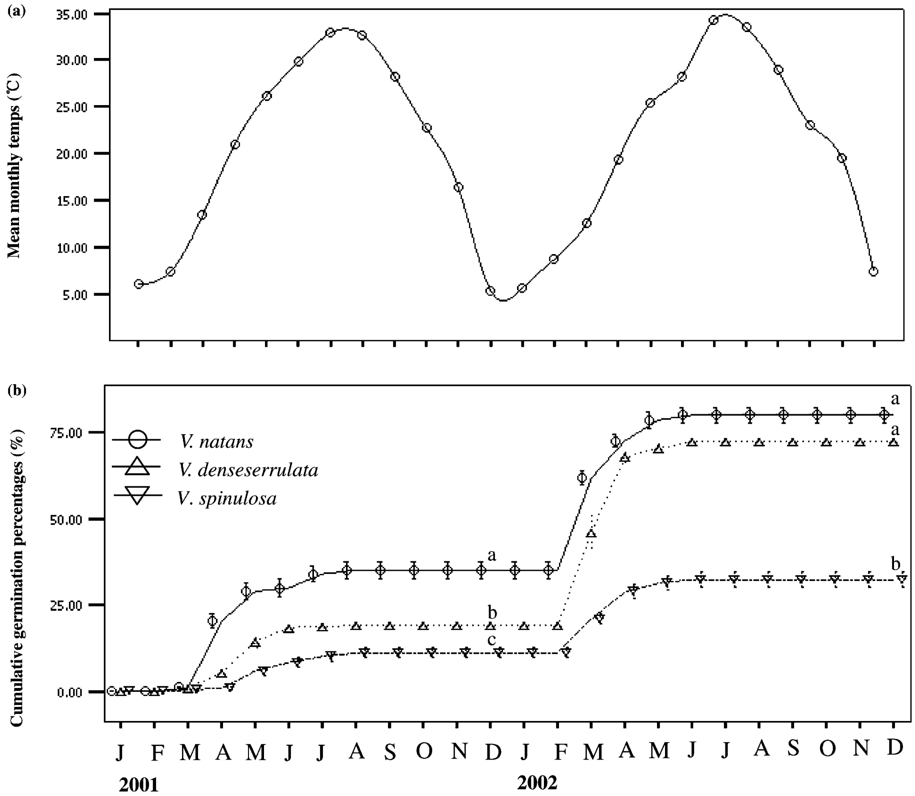

Figure 1. (a) Mean monthly air temperatures in the non-heated greenhouse in Wuhan, China, during the study period. (b) Cumulative germination percentages (Mean ± SE) of 2000-collected seeds of *Vallisneria natans*, *V. denseserrulata* and *V. spinulosa* incubated for 24 months in the greenhouse, letters indicate significant differences ($p < 0.05$).

V. denseserrulata, *V. natans* in dishes without microalgae were 11 ± 3.16% ($n = 5$), 30.2 ± 5.97% ($n = 5$), and 40.6 ± 6.11% ($n = 5$), respectively, and 20.2 ± 4.15% ($n = 5$), 49.2 ± 8.90% ($n = 5$), and 59.2 ± 7.33% ($n = 5$), respectively, in dishes inoculated with microalgae (Fig. 2).

Seed germination rates of three studied species differed significantly in culture solution ($p < 0.05$). However, a significant difference in seed germination rates existed between *V. spinulosa* and other two species in solution with microalgae, but no difference occurred between *V. denseserrulata* and *V. natans* (Fig. 2). Although significant differences in germination percentages occurred among the three species and between the treatments, germination patterns with respect to microalgal presence was similar among the three species (Table 1). Strong correlations existed between seed germination percentages of *V. natans, V. spinulosa*, *V. denseserrulata*, and total biomass of freshwater microalgae ($p < 0.001$) (Fig. 3).

Discussion

Seeds exposed to natural seasonal temperature and light changes for 24 months suggested an annual dormancy/non-dormancy cycle. That is, seeds came out of dormancy and could germinate to high percentages from March to May (in spring) and were dormant during the remainder of the year (Fig. 1). Seasonal dormancy cycles are common among plants (Van Assche & Vanlerberghe, 1989; Schütz, 1997). Dormancy patterns can vary depending on life-history traits and the habitat of a species (Schütz, 1997). The ability to remain dormant for a long period is associated with seeds of species from unpredictable environments, such as those that grow in flood-prone areas. Seeds of various species and especially those growing in aquatic habitats enter dormancy while they are flooded because deep-water germination of such seeds would lead to a failure in establishment of seedlings (Farmer & Spence, 1987; Honek &

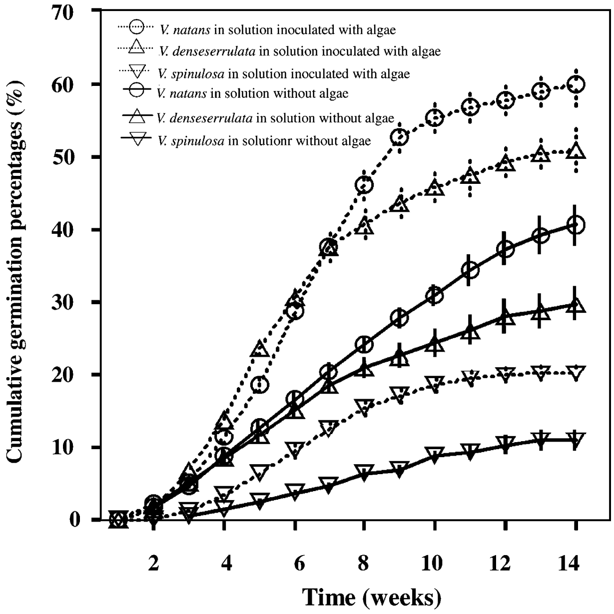

Figure 2. Cumulative seed germination percentages (mean ± SE) of 2000-collected seeds of *Vallisneria natans*, *V. denseserrulata* and *V. spinulosa* under two treatments during 14 weeks.

Martinkova, 1992). One possible explanation of why germination in these three species does not take place in the summer and autumn is an adaptation to the floodwater. In the location where the three species grow, there is an annual water-level cycle with high water levels in summer and autumn. Flood water may prevent seeds from germinating, thus they re-entered dormancy by summer and could not germinate the remainder of the year. Apparently, their life cycles are regulated by favorable (e.g. low water level) and unfavourable (e.g. high water level) conditions for germination in its habitat in spring and summer–autumn, respectively. If it is the case, germination at the beginning of spring, after the cold period, would maximize the chances of successful growth for these plants and their long-term maintenance in Yunihu Lake. Such dormancy patterns restrict germination to limited periods and prevent germination in seasons unfavorable for seedling establishment (Karssen, 1982; Baskin & Baskin, 1985). From our results, we conclude that an endogenous biorhythm could prevent seed germination in seasons unfavorable for seedling establishment and seeds are programmed to ‘wait’ until the following spring before germination. Therefore, germination of *Vallisneria* seemed to be regulated by some internal clock. Alternatively, some ecological factors such as the temperature, whose changes were conspicuous along seasons, may be an external ‘trigger’ for germination.

Our study revealed there was a significant difference in seed germination percentages of the three species of *Vallisneria* exposed to natural

Table 1. A two-way ANOVA on the relationship between freshwater microalgae and species on the seed germination of three *Vallisneria* species

Source of variation	d.f.	Mean square	*F*	*p*
Species	2	3131.23	95.32	0.000 ***
Freshwater microalgae	1	1984.53	60.41	0.000 ***
Species × Freshwater microalgae	2	97.23	2.96	0.071 ^{ns}
Error	24	32.85		

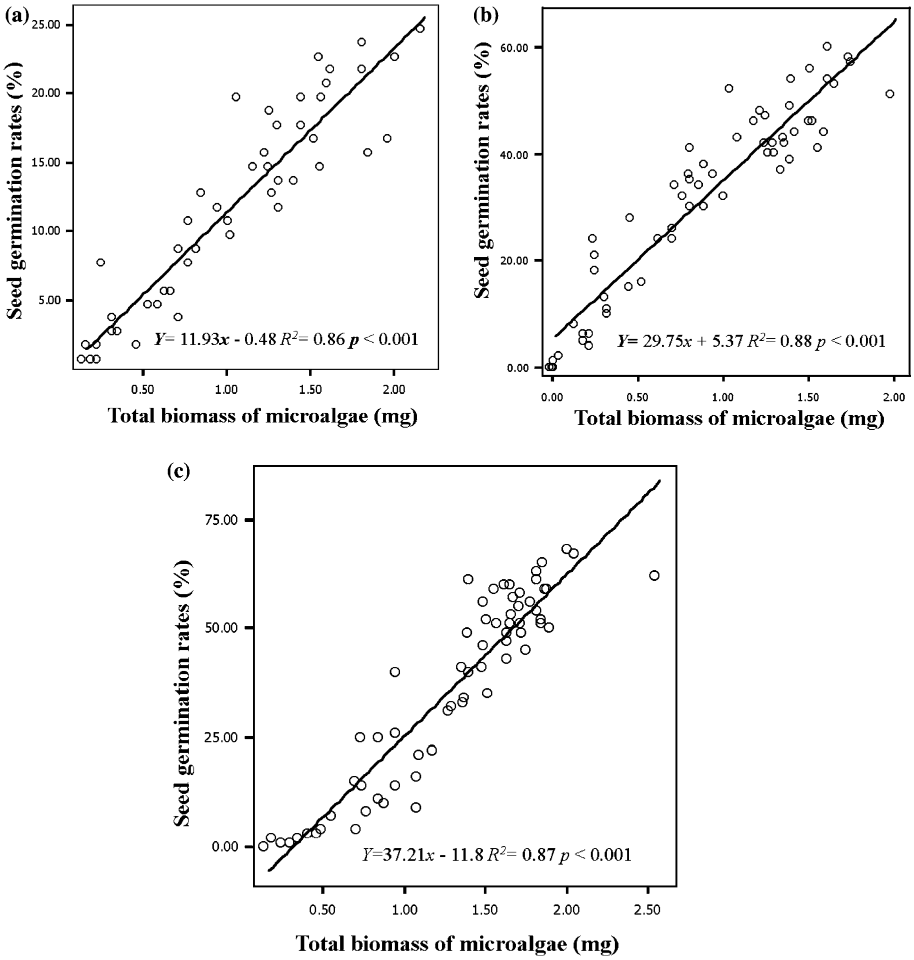

Figure 3. Regression between total biomass of freshwater microalgae in each Petri dish and seed germination rate of the three species of *Vallisneria* (a: *V. spinulosa*; b: *V. denseserrulata*; c: *V. natans*) in everyweek during the study period.

seasonal temperature and light changes in the first year ($p < 0.05$) and in seed germination rates between *V. spinulosa* and the other two species in the second year (Fig. 1). The same results were observed in experiment 2 (Fig. 2). Seed germination is a critical phase in the reproductive cycle that is of great importance for species fitness (Navarro & Guitiàn, 2003). Variations in germination percentages are often interpreted as reflecting adaptations to specific ecological conditions (Venable & Lawlor, 1980; Grime et al., 1981; Martin et al., 1995). The adaptation to the environment has been accompanied by evolutionary differentiation, which has led to reproductive strategy alternation. The genotypic and ecological backgrounds of natural habitats seem to play predominant roles in the germination mechanism (Kamenetsky & Gutterman, 2000). Thus, different germination rates were observed in three *Vallisneria* species. The differences of germination may be responses to the different distributions of the three species and thus be related to different survival strategies of them. Seed traits, including seed size, germination requirements, and germination rate often vary in parallel and are thought to comprise co-evolved complexes or life-history strategies for many species (Harper, 1977; Foster, 1986; Rees & Westoby, 1997). *Vallisneria spinulosa* is an endemic species, and *V. denseserrulata* is a regional species, but *V. natans* is a eurychoric plant. High seed germination rates may be an important adaptive feature for dispersal. Thus, high germination is necessary for *V. natans* to be widely distributed. Although in this regard, the present study is preliminary, our results revealed significant differences in seed germination rates that could suggest factors that for instance are important in limiting the distribution of *V. spinulosa*.

Facilitation is an important factor in the structure and dynamics of community diversity (Vázquez et al., 1998). The different responses of seeds to the two treatments in the present study (Table 1) suggested the existence of a facilitation process associated with conditions of inoculation with microalgae. The present study provided evidence that seed germination can be greatly increased with total biomass of freshwater microalgae (Fig. 3). Apparently the presence of freshwater microalgae can be very important for the maintenance of unpredictable conditions which facilitate the germination process. The influences of algae on seed germination are very complex, perhaps due to extracellular products of algae. All algae can liberate organic substances into the surrounding medium (Jackson, 1964). Experiments have demonstrated that the extracellular products liberated from algae can amount up to 1.5% of total carbon fixed (Fogg, 1958). The production of extracellular substances by algae that can produce a variety of ecological effects can sometimes be quantitatively considerable (Lewin, 1962). Experiments have demonstrated that all aqueous and ether extracts of an axenic culture of *Nostoc muscorum* Ag. could accelerate seed germination of *Panicum miliaceum* L. significantly (de Caire et al., 1976). Freshwater microalgae apparently can accelerate seed germination (Table 1). Immediate germination favors seedling establishment. The prospects for survival and reproduction during later stages of the life-story may be strongly affected by the speed of germination (Grime et al., 1981). A positive relationship between immediate germination and seedling establishment has also been observed in other species of phanerogams (Grime et al., 1981). Success in seedling establishment almost invariably depends upon rapid exploitation of temporarily favourable conditions (Vázquez et al., 1998). So, the presence of freshwater microalgae can be very important for the maintenance of adequate conditions which sustain the germination process, especially during the short period of time in which low water level is available for seedling establishment.

To summarize, seeds of the three *Vallisneria* species germinated in spring which might correspond to an annual dormancy/non-dormancy cycle. A significant positive correlation was observed between seed germination rates and total biomass of freshwater microalgae, suggesting freshwater microalgae may facilitate seed germination of *Vallisneria*. Now, due to eutrophication and excessive human disturbance, aquatic vegetation in many water bodies in China has been seriously damaged, leading to degradation water quality and malfunction of freshwater ecosystems (Jian et al., 2003). Submerged aquatic vegetation is known to be a key structural component and regulator in ecosystems. In general, plants of *Vallisneria* are used as pioneer species during freshwater ecosystem restoration due to their strong influences on water quality (Korschgen et al., 1997) and large numbers of seeds (Xiong & Li, 2002). Thus, further research is needed to investigate in additional detail the germination pattern and to identify factors responsible for the observed effects of freshwater microalgae on seed germination.

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