STUDIES ON ION BEAM APPLICATION TO IMPROVE AQUATIC MACROPHYTE REMEDIATION CAPACITY IN EUTROPHIC WATERS

LI Miao[1], WU Yue-Jin[1], ZHANG Jun[1], YU Han-Qing[2], WU Xiao-Lei[3] and YU Zeng-Liang[1]

(1. *Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, the Chinese Academy of Sciences, Hefei, China* 230031;
2. *School of Chemistry and Material Science, the University of Science and Technology of China, Hefei, China* 230026;
3. *Department of Environmental Science and Engineering, Tsinghua University, Beijing, China* 100084)

Abstract: Using ion beam biotechnology in combination with soil-less plant cultivation on artificial substratum (floating beds), the experiments were conducted with *Ipomoea aquatica* Forsk. Plants were attached to floating-beds which were placed on the surface of artificially nutrient-enriched tank water, in order to study the purification and remediation efficiency of ion beam-treated *I. aquatica* cultivars. The results show that N^+ ion beams with 25keV energy and dosages of 0, 2.6, 3.9, 5.2, 6.5, 7.8, 9.1 $\times 10^{13} N^+$(ions)/cm^2 affected *I. aquatica* dry seeds differently, with the dose of $3.9\times 10^{13} N^+$(ions)/cm^2 improving effectively the performance as expressed by various biological indices. After ion beam application, *I. aquatica* cultivars grew well in nutrient-enriched water bodies, increasing the growth of leaves and stem, number of leaves, length and area of roots, plant height, and weight more remarkably than observed in the control. The net removing rates of TN, TP were as high as 75% and 82%, respectively. Especially under the dose of $3.9\times 10^{13} N^+$(ions)/cm^2, the net removing rates of TN, TP were highest, for 77% and 85%, respectively. It was proved that ion beam application improves phytoremediation and may be used to purify nutrient rich water bodies.

Key words: Ion beam treatment; Aquatic macrophyte; *Ipomoea aquatica* Forsk; Nutrient-enriched waters; Remediation capacity
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Ion beams have been widely used in the semiconductor industry and applied for surface modification of materials since the 1970s, while nobody had discussed how these ion beams could be used in genetic modification in biological science, even few people paid attention to biological effects induced by ion beam and their possible use in genetics. In 1986, Yu et al. have discovered the genetic effects in rice mediated by low energy ions[1—5]. Since then ion beam radiation has been widely used to improve crops and the performance of microbes. By using N^+ ion implantation into rice seeds, Yu et al. firstly discovered the genetic effects on rice in M_2 generation, thereby opening a new field: ion radiation mutation[1—5]. It has been shown that the method has many advantages, such as lower damage rate, higher mutation rate, and a wider mutation spectrum. Recently developed ion beam biotechnology has resulted in seven new varieties and more than 3000 breeding materials of rice, wheat, tomato, maize, sweet-potato, rape, soybean, and tulip. The planting area of these varieties is over one million hectares, and the gain output has increased by 95 million tons. The crops exhibit their good characteristics with higher yield rate, broader disease resistance, shorter growing period and higher quality from ion beam induced mutation. Ion beam mutation has also been used for modification of micro-organisms. Nine new strains of industrial microbes have been cultivated and a new strain for producing vitamin C has resulted in high production rates. In 1993, Yu et al. also discovered that ion beam etching could form micro-holes on the walls of cells. Based on this discovery, foreign genes have been successfully delivered into mature embryo cells of rice, wheat, and tobacco etc. Using this method, DNA from one kind of crop has

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Corresponding author: Yu Zeng-Liang

been transferred into other plants[1—5]. With the development of low energy ion beam application, more studies have been performed in plants and microbes breeding, gene modification, risk assessment of environmental ion exposure, and the role of low energy ions in the chemical origin and evolution of life.

Lake Chaohu, with an average depth of about 3m, is one of the five largest freshwater lakes in China. Before the 1950s, it was well-known for its scenic beauty and for its richness in aquatic species. The serious eutrophication has caused negative ecological effects and impaired human health while also affecting the social and economic utility of the lake. Over the last decades, with the population growth and the economic development in the drainage area, nutrient-rich effluents have drained increasingly into the lake. This has resulted in contamination and eutrophication, disrupting the lake ecosystem structure. The deterioration of water quality has caused unpleasant taste of fish and fish kills are now quite regularly reported during summer seasons[6, 7]. So controlling eutrophication and restoring the ecosystem of the Lake Chaohu have strongly been suggested and attracted the attention of scientists, local and central governments as well as the general public. As typical nation-wide case studies on lake eutrophication, comprehensive research on Lake Chaohu has been carried out for more than 20 years, including the investigation and assessment of pollution sources and water quality, the eutrophication mechanism, the strategies for ecological restoration and for environmental management. Some environmental and ecological engineering measures have been implemented, and acquire a certain effects[6, 7]. However, it is not easy to control eutrophication ultimately, since the lake ecosystem is a very complex system with many interacting natural and man-made factors.

Aquatic plants have shown tremendous capacity to reduce the level of nutrients (N and P), biological oxygen demand (BOD), absorb toxic metals, and foster settlement of total suspended solids from eutrophic waters. Nitrogen and phosphorus nutrient removal via cultivated aquatic plants is necessary to reduce eutrophication in such as lakes, reservoirs, ponds, and marshes. The nitrogen and phosphorus nutrient uptake by aquatic plants was very widely researched and practically used in many water bodies[8, 9], in particular in artificial wetlands. However, the use of aquatic plants in purifying eutrophic water is still mainly at experimental stage and is currently a developing area in water environmental management. It largely depends on the life stage and genetics potential of the macrophytes, their purification ability is limited in nature. In eutrophic water bodies purification often requires that grown-up biomass of aquatic plants must be removed from the water bodies and disposed or recycled. However, removal of surplus biomass of aquatic plants from water is rather difficult to handle. *I. aquatica* is a floating plant, growing rapidly, with a high biomass yield and relatively easy to handle. As a pollution tolerant species it is growing widely in China and it is supposed to purify nutrient-polluted water. To improve its purification efficiency, we have used ion beam irradiation technology to induce modifications to the performance of *I. aquatica*, and it is postulated that the effect of ions beam contributes to the purification ability of the plant.

1 Materials and methods

1.1 Plant material The experiments were performed with *I. aquatica* Forsk. obtained from "the Fengle seeds corporation", with the "Thailand narrow leaf" variety being selected as test plant. The conditions for ion beam treatment on *I. aquatica* seeds were established at the Department of IBBE, ASIPP, CAS, China (unpublished data). The untreated plants were used to provide the experimental control. The seeds of *I. aquatica* by ion beam treatment were sown. Then, the plantlets were grown up to their height of about 5—10cm at the beginning of the experiments.

1.2 Low energy ions implantation Ion beam treatment using N^+ ions with low energy of 25keV and current of 20mA were chosen as the sources which were produced by the ASIPP's ion beam bio-engineering instrument. For each ion beam treatment, the specimens were fixed onto a petri dish arranged in two groups, one to be exposed to the beam and another as a vacuum treated control. The pulse treatment technique was used; the pulse time was 10s and the interval between two pulse was 50s. In each pulse, the application dose was $D_0 = 2.6 \times 10^{13}$ ions/cm^2. The dosages used were counted as accumulating data (from 1000 to 3500) multiplied by D_0. Pulsed beam modes were adopted using periodical beam sweeping across the exposure holes of the sample holder with each pulse bombarding the target to a dose of 0, 2.6, 3.9, 5.2, 6.5, 7.8 and 9.1 $\times 10^{16} N^{+}$ (ions)/cm^2, marked symbol were B_0, B_1, B_2, B_3, B_4, B_5, B_6 respectively. The operating pressure in the target chamber was kept at approximate 10^{-3} Pa which caused the ambient temperature to be approximate 0℃ or lower. During application the specimens were normally exposed to this environment for 1.5—2h. Four replicates were used for individual species in each experiment. The experiment on each species was repeated at least three times.

1.3 Planted floats The planted floats (33cm × 32cm × 3cm), made from polyethylene foam, were used to support floating-beds for higher plants growth near or at the water surface. On the bottom of the float, there was a 2cm layer of sand. The sand was washed to minimize the availability of nutrients from this substrate. The bottom of the float had three rows of holes (diameter 1cm and four holes per row), through which the roots of plants could elongate into the water. To prevent loss of sand, the holes were filled with sponge. The floats were then placed in plastic trays (46cm × 33cm × 24cm) filled with artificial nutrient rich water similar to those from known water bodies, and planted with young seedlings grown from ion beam-treated seeds of *I. aquatica*. After measuring the initial fresh weights, the 12 plants were transplanted evenly in per float per treatment and per replicate, and then covered by a sand layer of 1.0cm, total 7 treatments add 1 control, and replicated three times. There were total 288 plants used in the whole experiment. The plastic trays without treated plants were used as controls. The plastic trays were weekly and exclusively irrigated with distilled water because of evaporation loss.

1.4 Artificially nutrient-enriched water According to the eutrophication standard of Lake Chaohu declared in 1990, its high eutrophication level is based on average nutrient concentrations of 4.60mg/L, 0.55mg/L for TN, TP. These levels were referred to for preparing the nutrient enriched test water for the growth chamber experiments. Distilled water was used while nutrients were added in different concentrations using NH_4Cl, $Ca(NO_3)_2 \cdot 4H_2O$, NaH_2PO_4, thereinto 1/4 was ammonia-nitrogen, 3/4 were nitrate-nitrogen, while the phosphorus was added as soluble inorganic P consisting of mono-ammonium phosphate. Desired amounts of hydroponic culture solution were dissolved in tap water and diluted to the designed TP and TN concentrations. The solution was replaced fortnightly. Only one initial nutrients levels (TN concentration: 4.60mg/L and TP concentration: 0.55 mg/L) was considered for treatment. The treatments were undertaken in a two-factorial design producing seven treatments of different dosages. The all selected dosage and energy levels were similar to those in the biological effects of nitrogen ion beam application on *I. aquatica* M_1 generation (unpublished data).

1.5 Growth chamber hydroponic culture system set-up A growth chamber hydroponic culture system was used in this study to investigate the suitability of I. *aquatica* to remove TP and TN from nutrient-enriched Lake Chaohu water. The growth chamber hydroponic culture system was set-up in a green house to simulate the Lake Chaohu eutrophication status. *I. aquatica* young plants were transferred to the growth chamber of the hydroponic culture system, at the start of the experiment. The untreated plants were used as controls. There were 12 plants in each float, two replicates for each treatment, and 12 plants in each replicate, total 7 treatments plus 1 control. Total 288 plants were used in the experiment. After 56 growing days the experiment was terminated and the growing state of creeping-stem plants was recorded.

1.6 Sample collection and chemical analysis At the end of the growth trials, plants were carefully removed from the floats, gently washed with tap water, blotted with absorbing paper and the fresh weights were measured. After drying at 70℃ for 48h, the dry weights and water contents were recorded. During the course of the experiments, water samples were collected every seven days from each system. The concentrations of total phosphorus and total nitrogen in the culture solutions were measured by using the standard methods[10—13]. The N content of plant material was determined by the semi-micro Kjeldahl method and the P content was according to the Ames' method. For comparison, plant samples collected from the cultivating seedlings of land sites were also analyzed.

2 Results

2.1 Effect of N^{+} ion at different doses on germination of *I. aquatica* seeds

The influence of ion beam irradiation to seeds on the germination rate induced in *I. aquatica* is shown in Tab. 1. Different dosages had different influences on the germination rate. In the case of nitrogen ion irradiation to the "Thailand narrow leaf" variety, the germination rate increased when the dosage increased to levels employed in B_1 to B_3, and especially the treated dose $B_2 = 3.9 \times 10^{16} N^+$ (ions)/cm^2 was the most effective one and had obvious a long-term activated effect. However, the germination rate decreased when the dosage increased to levels higher than B_3. This showed that ion irradiation possesses the advantage of causing lower damage to *I. aquatica* seeds than other methods, and remediate fast after application. The appropriate dose condition could stimulate growth, nevertheless growth was blocked and restrained by exorbitant dosages.

Tab. 1 Effect of N^+ ion treatment with different dosages (B_0 to B_6: see material and methods) at the stage of seed germination of *Ipomoea aquatia* Forsk on the rate of germination and overall growth assessment. Two replicates for each treatment, a total of 7200 seeds were used in the experiment

Dose	Tested seed numbers	Germination number	Germination rate%	Growth state
CK	300	210±10.5	70±3.5	Normal
B_0	300	210±10.5	70±3.5	Normal
B_1	300	225±11.3	75±3.8	High
B_2	300	243±12.2	81±4.1	Higher
B_3	300	231±11.6	77±3.9	High
B_4	300	195±9.6	65±3.3	Common
B_5	300	144±7.2	48±2.4	Bad
B_6	300	84±4.2	38±1.9	Worse

2.2 Effect of N^+ ion application at different doses on seedling characteristics of *I. aquatica*

There had been a higher influence on growth and development low energy N^+ ions application was undertaken during the seedling phase of *I. aquatica*. The leaves appeared curly which was abnormal and the plant shape changed. In the case of irradiation with nitrogen ion beams, the induced mutation rate was 1.2% induced in plants in the field, moreover the mutation increased when the dose increased. The majority of the treatments with various dosages of N^+ ions beam radiation had little influence on seedling and young plant rates in the field. The seedling rate and young plant rate was higher than control and other treatments in the field when the dose was at level $B_2 = 3.9 \times 10^{16} N^+$ (ions)/cm^2, and at $B_3 = 5.2 \times 10^{16} N^+$ (ions)/cm^2. But these rates decreased when the dosage increased to the levels of B_5 and B_6. The experiments indicated the importance of appropriate dose treatment to achieve accelerated *I. aquatica* outgrow of seedling successfully. Different doses N^+ ion beam have definitely initiated an activated growth effect as expressed by some of the studied biological characteristics (such as seedling rate, young plant rate, plant height, etc.) of *I. aquatica* seeds when treated in seedling phase with low energy ions irradiation. From the total mutation range of every biological character, there was the trend of surge changes among dosages.

Tab. 2 Effect of N^+ ion treatment applied at different dosages on selected performance characteristics of *I. aquatica*. CK= controls B_0 to B_6= explanations see material and methods. Two replicates for each treatment, a total of 5000 individual young plants were investigated in the experiment

Characters	CK	B_0	B_1	B_2	B_3	B_4	B_5	B_6
Seedling rate (%)	62.6±3.1	62.5±3.1	63.9±3.2	68.8±3.4	67.8±3.4	60.4±3.0	42.2±2.1	35.7±1.8
Young plant rate (%)	61.3±3.1	61.3±3.1	62.6±3.1	64.0±3.2	63.2±3.2	58.1±2.9	39.8±2.0	31.9±1.6
Plant height (cm)	4.1±0.2	4.0±0.2	4.5±0.2	5.4±0.3	5.1±0.3	4.9±0.3	4.6±0.2	4.4±0.2
Fresh weight (g)	0.30±0.02	0.30±0.02	0.38±0.02	0.49±0.03	0.47±0.02	0.41±0.02	0.39±0.02	0.31±0.02
Leaves number	4±0.2	4±0.2	4±0.2	6±0.3	6±0.3	4±0.2	4±0.2	4±0.2
Leaf length (cm)	3.4±0.17	3.5±0.18	3.6±0.18	3.9±0.20	3.9±0.20	3.7±0.19	3.7±0.19	3.7±0.19
Stem thickness (cm)	0.16±0.008	0.16±0.008	0.18±0.009	0.26±0.013	0.25±0.013	0.23±0.012	0.22±0.011	0.19±0.010
Root length (cm)	5.2±0.26	5.1±0.26	5.6±0.28	6.8±0.34	6.5±0.33	6.0±0.30	5.7±0.29	5.4±0.27
Root range (cm)	2.8±0.14	2.8±0.14	3.4±0.17	4.2±0.21	4.2±0.21	4.0±0.2	3.8±0.19	3.5±0.18
Side root number	12±0.60	11±0.55	15±0.75	20±1.00	18±0.90	18±0.90	14±0.70	13±0.65

2.3 Effect of N^+ ion implantation different doses on the growth of *I. aquatica* in eutrophic water bodies

Low energy ions application on *I. aquatica* showed normal and positive growth effects in media representing nutrient levels known from nutrient-enriched waters and this was shown under most treatment conditions when compared with the control. The leaves appeared fresh green; the root, stem, and leaf were full-grown. The grow-out characteristics were improved further. There was more obvious increase than in controls for leaf length, leaf number, stem thickness, and other factors. After four days, plants in every dose treatment grew new roots three days earlier than in the control. It was most obvious for the treatments $B_2 = 3.9 \times 10^{16} N^+$ (ions)/cm^2 and $B_3 = 5.2 \times 10^{16} N^+$ (ions)/cm^2 (Tab. 3). At higher dose levels, however, the grow-out characters were worse compared to controls, therefore only appropriate dose treatment could help improve *I. aquatica* performance. The growth characteristics were in the order of $B_2 > B_3 > B_4 > B_1 > B_5 > CK > B_0 > B_6$.

Tab. 3 Effect of N^+ ion treatment with different dosages on the growth of *I. aquatica* in nutrient-enriched waters (simulating eutrophic conditions). Values are mean values of the respective numbers indicated. Two replicates for each treatment, a total of 288 individual plants were used in the experiment

Dose	Plant height (cm)	Fresh weight (g)	Root weight (g)	Root length (cm)	Root number	Root range (cm)	Stem thickness (cm)	Leaf number	Leaf length (cm)
CK	12.4±0.62	2.46±0.12	0.59±0.030	11.1±0.56	30±1.5	5.4±0.27	0.52±0.026	8±0.4	4.2±0.21
B_0	12.1±0.61	2.45±0.12	0.56±0.028	10.7±0.54	25±1.3	5.0±0.25	0.48±0.024	8±0.4	4.0±0.20
B_1	13.8±0.69	2.69±0.14	0.88±0.044	12.4±0.62	36±1.8	6.2±0.31	0.59±0.030	10±0.5	4.3±0.22
B_2	16.6±0.83	3.48±0.17	1.66±0.083	15.3±0.77	48±2.4	7.6±0.38	0.74±0.037	16±0.8	5.4±0.27
B_3	15.9±0.80	3.28±0.16	1.60±0.080	14.6±0.73	44±2.2	7.1±0.36	0.70±0.035	14±0.7	4.9±0.25
B_4	14.8±0.74	3.04±0.15	1.29±0.065	13.3±0.67	39±2.0	6.6±0.33	0.66±0.033	12±0.6	4.7±0.24
B_5	13.5±0.68	2.69±0.14	0.85±0.043	12.1±0.61	35±1.8	5.9±0.30	0.55±0.028	10±0.5	4.3±0.22
B_6	11.2±0.56	2.15±0.11	0.38±0.019	10.8±0.54	24±1.2	4.7±0.24	0.43±0.022	8±0.4	3.8±0.19

2.4 The effects of N^+ ion treatment at different doses on *I. aquatica* removal efficiency for TN in nitrogen-enriched water bodies

Tab. 4 shows the remediation effects of N uptake by *I. aquatica* plants that have been treated by N^+ ion beam irradiation. The ability for TN uptake was improved in principle. This obviously can increase the remediation effect in nutrient-enriched waters when using this plant. It is clear that TN was removed in each treatment better than in the control. The removal rate and net removal rate was 85% and 75% respectively. The remediation effect was dose dependent from B_1—B_6 but was higher than in the control B_0. Treatments $B_2 = 3.9 \times 10^{16} N^+$ (ions)/cm^2 performed best. The TN removal rate and net removal rate was 87% and 77%, respectively. The results indicated that it is feasible to use the technique of ion beam irradiation as an performance improving biotechnology, while combining the technique with floating-beds (soilless cultivation technique) where plants are attached to artificial substrates, thereby improving the ability of nitrogen uptake by *I. aquatica* to nutrient-enriched waters.

Tab. 4 The removal effectiveness of *I. aquatica* after N^+ ion beam treatment at different dosages based on an initial TN level as found in nutrient-enriched waters. CK = control; B_0 to B_6 = treatment dosages (see material and methods). Data represent mean values. Two replicates for each treatment, a total of 288 individual plants were used in the experiment

Treatment	CK	B_0	B_1	B_2	B_3	B_4	B_5	B_6
Pre-experiment (mg/L)					4.60			
Post-experiment (mg/L)	4.14±0.21	0.86±0.04	0.78±0.04	0.60±0.03	0.64±0.03	0.69±0.04	0.72±0.04	0.74±0.04
Removal rate (%)	10±0.5	81.30±4.07	83.04±4.15	86.96±4.35	86.08±4.30	85.00±4.25	84.35±4.22	83.91±4.20
Net removal rate (%)	10±0.5	71.30±3.57	73.04±3.65	76.96±3.85	76.08±3.80	75.00±3.75	74.35±3.72	73.91±3.70

2.5 The effect of N^+ ion irradiation at different doses on the removal efficiency of *I. aquatica* for TP

Tab.5 shows the effects on P uptake when *I. aquatica* was treated by N^+ ion beam irradiation. The ability of TP uptake was improved. This obviously can increase the remediation effect when such treated plants are placed in nutrient-enriched waters. It is clear that TP was removed in each treatment better than in the control. The removal rate and net removal rate was 86% and 83%, respectively, which was higher than in the control B_0. Especially one may expect the best remediation effect at the dose $B_2 = 3.9 \times 10^{16} N^+$ (ions)/cm^2, where the TN removal rate and net removal rate was 89% and 85%, respectively.

Tab. 5 The removal effectiveness for total phosphorus (TP) of *I. aquatica* after N^+ ion beam treatment at different dosages based on an initial TP level as found in the eutrophic state of the investigated lake. Data represent mean values. Two replicates for each treatment, a total of 288 individual plants were used in the experiment

Treatment	CK	B_0	B_1	B_2	B_3	B_4	B_5	B_6
Pre-experiment (mg/L)	0.550							
Post-experiment (mg/L)	0.528±0.026	0.094±0.005	0.091±0.005	0.063±0.003	0.068±0.003	0.075±0.004	0.082±0.004	0.092±0.005
Removal rate (%)	4.00±0.2	82.91±4.15	83.45±4.17	88.55±4.43	87.64±4.38	86.37±4.32	85.09±4.25	83.27±4.16
Net removal rate (%)	4.00±0.2	78.91±3.95	79.45±3.97	84.55±4.23	83.64±4.18	82.37±4.12	81.09±4.05	79.27±3.96

3 Discussion

Although this study demonstrated the potential for N and P removal by *I. aquatica* after ion irradiation and subsequent culture in a controlled environment, some critical questions need to be answered before application of this methodology can be recommended for the field. Firstly, the effects of water movement on plant growth of ion irradiated plants. The water movement caused by wind, wave and row in field circumstances has a two-sided effect on plant growth of any type, whether ion-irradiated or not, while wind-created currents brings more nutrients and other elements to the rhizomes, such movement (particularly strong winds) may also cause physical damage to plant roots that have received ion-irradiation because of potential structural modifications. The actual effects of ion irradiation treatment on plant growth may depend on the intensity of water movement. Usually rapid water exchange is beneficial as it brings more nutrients to the roots, avoiding a depletion of the gradient for nutrient uptake at the root surface.

Secondly, in any natural or engineered ecosystems for wastewater treatment, the consequences of ion beam irradiation treatment on plant physiology (especially bioaccumulation of pollutants content in eutrophic waters) is poorly understood. The uptake of nutrients such as the overall N and P removal is only a fraction of the eutrophication problem. The N and P removal at system level, which involves both water plants and microorganisms and their interaction should be investigated. Thirdly, the potential of rapid spread of plants that had been modified by ion-irradiation treatment has to be tested. For the plants used in our study, *I. aquatica* one can at least state that it is a native plant. However, ion irradiation treatment on *I. aquatica* is considered to modify the plant to become an "exotic" species. The ecological and economic consequences of such modern modifications of *I. aquatica* need further research. Appropriate dosage condition could stimulate better growth. Growth was restrained by exorbitant dosages. The investigation results primarily proved that the specific ion beam application employed here improved phytoremediation capacity and this could perhaps be used to better assist purification of eutrophic water bodies. This new approach and science initiative should receive further attention for developing and using ion irradiation in order to improve aquatic phytoremediation in eutrophic water areas, thereby contributing to the environmental protection for a sustainable ecological aquatic system.

4 Conclusions

The study demonstrated that ion beam treatment applied to *I. aquatica* has the potential to enhance the performance of the plant thereby removing significant larger amounts of N and P from wastewater than the control plants. The technology was very effective when used in combination with the floating-plant systems. The method described overcomes some of the intrinsic disadvantage of floating plant system and keeps their advantages such as reliability and high efficiency as well as the option for confinement and control. Future research work is to investigate the molecular mechanism of ion irradiation in order to become able to acquire the objective mutant, and to study the transmissibility of current morphologic and physiological effects by ion beam irradiation, and to assess this methodology on *I. aquatica* health and the potential ecological risks before the technique can be applied in the open environment.

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