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Response of Exotic Invasive Weed *Alternanthera philoxeroides* to Environmental Factors and Its Competition with Rice

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Abstracts: A greenhouse experiment was conducted to determine the influence of the environmental factors such as low-temperature, drought stress, salt-alkali and flooding on the survival rate, propagation rate, fresh biomass and viability of the *Alternanthera philoxeroides* and its competitive ability against rice by using bioassay method. A high viability of 84% was found when the stems were treated at 4℃ and then grew under normal conditions, while no viable plant was noted when the stems were treated at -20℃ and grew under normal conditions. Compared to the fresh stem with water content of 93.5%, the survival rate, number of propagated stems and fresh biomass of *A. philoxeroides* derived from the stems with water content of 30.2% were reduced by 45%, 33% and 74% respectively. The treatments of 0.1% salt-alkaline solution led to loss of viability of *A. philoxeroides.* The stems of *A. philoxeroides* could grow in wet soil with different depths of water-layer. The *A. philoxeroides* at density of 23-180 plants/m^2 reduced the rice grain yield by 43-50% at the rice plant density of 100 plants/m^2.

Key words: *Alternanthera philoxeroides*; hydrophytic type; environmental factor; rice; viability; competitive ability

The exotic invasive weed *Alternanthera philoxeroides* (Mart.) Griseb causes new problems in farming systems in China by decreasing yield and quality of rice and other upland crops greatly [1]. For example, in the rice growing region of Shanghai, the occurrence frequency of *A. philoxeroides* with harm level over grade 2 increased to 12.7%, only inferior to *Monochoria vaginalis*, and *Echinochloa crusgalli* (18.6% and 15.3%, respectively) [2]. The increase rate of occurrence frequency for *A. philoxeroides* reached 100% in the recent 20 years, while that for *M. vaginalis*, *Sagittaria pygmaea* and *Leptochloa chinensis* were 88.5%, 77.9% and 24.7%, respectively. Therefore, new techniques for *A. philoxeroides* management need to be developed to prevent its spread. How to control this invasive weed is also a critical assignment for Chinese researchers engaged in crop protection.

Asexual propagation is the main cause of the fast spread of *A. philoxeroides* except for the artificial introduction. Zhang et al [3] noted that no seed of *A. philoxeroides* were produced after flowering in Jiangsu Province, while Zhang et al. [4] found that *A. philoxeroides* had only 6.5% seed setting rate in Zhengzhou City, Henan Province [4]. Lin and Qiang [5] reported that the hydrophytic, hygrophilous and xerophytic types of *A. philoxeroides* could propagate by creeping stem and rhizome even with one node or root possessing a bud eye even 0.2 cm in length. Xiang et al [6] reported the most effective measure to prevent the propagation, spread and diffusion of *A. philoxeroides* by asexual diaspores. Shen et al [7] studied the influence of the environmental factors on the rhizome growth of terricolous type of *A. philoxeroides* and reported that the seedlings could emerge under 10-40℃ with the optimum temperature at 20-30℃, under the soil humidity ranging from 10% to 55% with the optimum soil humidity of 30%. It couldn't emerge at the soil humidity <5% and >60%, meanwhile the suitable soil depth was 0-2 cm and the optimum soil depth was 0.5 cm. The rhizome is very sensitive to drought. When the water content of rhizome was 42.4-78.2%, the emergence rate reduced to 21.7-73.4%, and when the rhizome water content

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was below 22.2%, it couldn't emerge.

The biotypes of *A. philoxeroides* differed in the response to the environmental factors. Tao et al [8] reported that water content significantly influenced the morphogenesis of hydrophytic, hygrophilous and xerophytic types of *A. philoxeroides*. However, there is few report on the pertinence of the environmental factors with the hydrophytic type of *A. philoxeroides* and therefore further study should be carried out to establish the management system.

In current study, the responses of hydrophytic type of *A. philoxeroides* to environmental factors and its competition with rice plant were investigated in order to further explain the characteristics of local distribution. The main objective of this study is to provide scientific bases for setting up an effective management method, and develop the ecological control techniques of *A. philoxeroides.*

MATERIALS AND METHODS

Plant materials

The propagation stems of hydrophytic type of *Alternanthera philoxeroides* were collected from the south river of the Fuyang Experimental Station, China National Rice Research Institute (CNRRI), and washed with tap water. The stems with same size were cut into segments with 3 cm length and stored in brown paper bag.

The germination of the propagation stem and cultivation of the seedlings of *A. philoxeroides* were carried out in a greenhouse from January to May, 2006. During the experiment the average, maximum and minimum temperatures were 24.7℃, 29.8℃ and 19.6℃, respectively from January to March, while the temperature and light were in nature in April and May.

Cold tolerance experiment of the propagation stems of *A. philoxeroides*

The propagation stems were kept in the same refrigerator (Haier, BCD-205F) at 4±1℃, -20℃ and room temperature (22±5℃) for 10 h, 24 h, 48 h and 72 h respectively, with four replications.

For each treatment, five stem segments were planted into a pot (23 cm in diameter, 18 cm in height) filled with 5 kg soil, and the water layer was kept at 2 to 3 cm over the soil surface. The numbers of seedlings and stems, fresh weight of *A. philoxeroides* plants were investigated 21 days after planting.

Drought tolerance experiment of the underground stem of *A. philoxeroides*

The stem segments with expected water contents were obtained after air-drying at 25-30℃ in room with four replications, with oven-dried treatment (100℃, 2 d, 10 h/d) as a check. The water content of the stem segment was calculated as the followed formula: Adjusted water content $= [(F \times W) - (F - D)]/F \times 100\%$, where, '*F*' represents the fresh weight of the samples, '*D*' represents the dry weight of the samples in the check and '*W*' represents the water content of the check.

The treated stem segments were planted in the pot. All the other culural conditions and investigated traits were the same as described in the cold tolerance experiment.

Salt-alkali tolerance experiment of the propagation stem of *A. philoxeroides*

The salt-alkali water solution was made by 1 NaCl : 1 KNO_3 (*W/W*) and the concentrations applied were 0%, 0.1%, 0.25%, 0.5%, 1.0%, 2.5%, 5.0%, respectively. The stem segements were soaked into 5 mL salt-alkali water solution in a Petri dish (9 cm in diameter) at room temperature for 72 h, with 5 stem segments per Petri dish, and all the treatments were replicated four times.

The treated stem segments were planted in the pot. All the other cultural conditions and investigated traits were the same as described in the cold tolerance experiment.

Influence of flooding on the germination of the propagation stem of *A. philoxeroides*

The stem segments of *A. philoxeroides* were planted in the pot with different water layers of 0 (wet soil), 1, 3, 5, 10, 20, and 30 cm over the soil surface, respectively. All the other cultural conditions and investigated traits were the same as those in the cold tolerance experiment.

Competition of *A. philoxeroides* with rice

The used rice variety Zhong 156 (*Oryza sativa* L.)

was supplied by CNRRI. The seeds were sterilized with 10% dithiocyano-methane, then the germinated seeds were sowed in the plastic pots (45 cm ×30 cm × 15 cm) filled with 10 cm soil layer, with the row spacing of 15 cm and three rows per pot at the density of 100 plants/m^2.

The stems of *A. philoxeroides* were transplanted between two rows of rice plants at the density of 23, 45, 90, 180 and 360 plants/m^2 at 30 d after seeding the rice, with the *A. philoxeroides* at the density of 45 plants/m^2 without rice plant as control and the rice at the density of 100 plants/m^2 without *A. philoxeroides* as positive control. Then the stem number of *A. philoxeroides* and the rice plant height were investigated at 45 d after rice planting. At the harvest stage, the stem number and dry weigh of *A. philoxeroides*, number of effective tillers, number of filled grains per panicle and 100-grain weight of rice were investigated. The rice grain yields (Y) were evaluated by the following formula. Y (kg/ha) $= T \times S \times W \times A/10$, where, '$T$' represents the number of tillers per pot, 'S' represents the number of filled grains per panicle, 'W' represents dry weight of 100 grains, 'A' represents the acreage of the plastic pots.

Statistical analyses

All data were analyzed with Tukey's multiple range test by DPS software (7.0) [9].

RESULTS

Response of the stem segments of *A. philoxeroides* to the coldness

The number of viable plants and fresh biomass of *A. philoxeroides* significantly declined with the decrease in the treatment temperature (Table 1). After being treated at 4℃, the stem still germinated and grew. However, it could not survive after being treated at -20℃ (Table 1). The treatment time ranging from 10 h to 72 h had no significant influence on the germination and growth of the propagation stem of *A. philoxeroides* (data not shown).

Table 2. Effects of different water contents of propagation stems on the viability of *A. philoxeroides*.

Water content (%)	Rate of viable plant (%)	No. of stems per pot	Fresh biomass of plant (g/pot)
93.5	100 a	6.75 a	5.24 a
76.0	100 a	6.75 a	5.00 ab
57.0	100 a	6.25 ab	4.12 b
40.8	85 a	5.25 b	1.33 c
30.2	55 b	4.50 b	1.39 c
11.4	35 c	2.00 c	0.46 c

Values within each column followed by the same letters are not significantly different at the 5% level by Tukey'multiple range test.

Influence of the water content of propagation stem on the viability of *A. philoxeroides*

The water content of fresh stem of *A. philoxeroides* was high, even reaching 93.5%. With the reduction in the water content of propagation stems, the rate of viable plants, the propagated stem number and fresh biomass decreased significantly (Table 2). When the water content declined to 30.2%, the rate of viable plants, propagated stem number and fresh biomass of *A. philoxeroides* were decreased by 45 percent points, 33% and 74%, respectively.

Influence of the salt-alkali solution on the viability of propagation stem of *A. philoxeroides*

The propagation stem of *A. philoxeroides* was sensitive to salt-alkali. As shown in Table 3, after being treated with 0.1% salt-alkali solution, the rate of viable plant, propagated stem number and fresh biomass of the *A. philoxeroides* were significantly lower than

Table 1. Effects of low temperature on the viability of the propagation stem of *A. philoxeroides*.

Temperature treated (℃)	Rate of viable plants (%)	No. of stems per pot	Fresh biomass (g/pot)
22± 5 (Room temperature)	100 ± 0 a	16.7± 1.53 a	20.1±0.98 a
4± 1	84± 0 b	14.5 ± 1.04 a	13.6±1.12 b
-20	0 c	0 b	0 c

Values within each column followed by the same letters are not significantly different at the 5% level by Tukey'multiple range test.

Table 3. Effects of the saline-alkali solution on the viability of propagation stem of *A. philoxeroides*.

Saline-alkaline concentration (%)	Rate of viable plant (%)	No. of stems per pot	Fresh biomass (g/pot)
0.00	100 a	9.8 a	9.6 a
0.10	5 b	0.5 b	0.3 b
0.25	5 b	0.8 b	0.5 b
0.50	5 b	1.0 b	0.8 b
1.00	5 b	0.5 b	0.3 b
2.50	0 b	0.0 b	0.0 b
5.00	0 b	0.0 b	0.0 b

Values within each column followed by the same letters are not significantly different at the 5% level by Tukey'multiple range test.

those of untreated control. The addition of 2.5% salt-alkali solution resulted in the failure of germination of the propagating stems.

Influence of flooding on the viability of propagation stem of *A. philoxeroides*

The propagation stem of *A. philoxeroides* could survive flooding with different water depths. The plant height of *A. philoxeroides* significantly increased but the stem number significantly decreased as the water depth increased. The maximum plant height was observed in the treatment of 20-cm water layer, whereas the maximum stem number was in the untreated check. However, in the treatment of 30 cm water layer, the plant height of the *A. philoxeroides*, stem number and fresh biomass decreased significantly, indicating the conditions with less of oxygen caused by deep water was not suitable for the growth of *A. philoxeroides* (Table 4).

Competition of *A. philoxeroides* against rice

As shown in Table 5, the rice plant height decreased significantly with the increasing density of *A. philoxeroides* at similar rice plant density. There is a significantly difference in rice plant height between the control and the *A. philoxeroides* treatment at 360 plants/m^2. But the plant height of *A. philoxeroides* significantly increased with the increasing density, due to the decreased transverse space speeded up the vertical growth of *A. philoxeroides* plants (Table 5).

At rice maturity, the stem number and above-ground dry weight of *A. philoxeroides* increased significantly with the increase in the density of *A. philoxeroides* (Table 5). Compared with the control rice plants, the plant height of the rice in different treatments decreased significantly with no significant difference between different treatments (Table 5).

Compared with control rice plant, the numbers of effective tillers and filled grains per panicle of rice decreased significantly, but no significant difference was found between the treatments differed in density of *A. philoxeroides*. The increase in the density of *A. philoxeroides* led to the significant decrease in 100-grain weight of rice(Table 5). The rice grain yields in all the treatments were obviously lower than that in the control, which was consistent with the changes in plant height, number of effective tillers and number of filled grains per panicle. The grain yields of rice in the three treatments (rice 100 plants/m^2 + *A. philoxeroides* 23 plants/m^2, rice 100 plants/m^2 + *A. philoxeroides* 45 plants/m^2, rice 100 plants/m^2 + *A. philoxeroides* 90 plants/m^2) showed no significant difference. But when the density of *A. philoxeroides* increased to 180 plants/m^2 and 360 plants/m^2, the grain yields of rice decreased significantly by 50% and 53%, respectively (Table 5).

Table 4. Effects of flooding on viability of propagation stem of *A. philoxeroides*.

Water-layer depth (cm)	Plant height (cm)	Rate of viable plant (%)	No. of stems per pot	Fresh biomass (g/pot)
0	15.5 cd	100 a	10.8 a	4.3 c
1	20.5 bc	100 a	10.3 a	8.5 abc
3	24.2 b	100 a	9.3 ab	9.5 ab
5	20.8 bc	95 ab	6.5 cd	5.6 bc
10	22.4 b	85 b	5.0 d	6.4 abc
20	34.6 a	100 a	7.0 bcd	10.5 a
30	25.5 b	100 a	6.5 cd	5.0 bc

Values within each column followed by the same letters are not significantly different at the 5% level by Tukey'multiple range test.

Table 5. Competition between *A. philoxeroides* and rice.

Parameter	Treatment (Plants/m^2)[a]						
	R100+A0	R100+A23	R100+A45	R100+A90	R100+A180	R100+A360	R0+A45
45 d after rice sowing							
Plant height of rice (cm)	65.7 a	63.8 ab	64.1 ab	63.4 ab	63.4 ab	61.5 bc	—
Plant height of *A. philoxeroides* (cm)	—	18.3 bc	27.5 b	28.9 b	40.0 ab	40.0 ab	120.9 a
Rice maturity stage							
No. of *A. philoxeroides* stem	—	26.0 d	37.7 cd	89.0 bc	76.0 bcd	99.0 b	246.0 a
Dry weight of *A. philoxeroides* shoot (g/pot)	—	4.02 c	6.32 c	15.44 b	13.69 b	16.61 b	48.81 a
Plant height of rice (cm)	64.6 a	60.2 b	62.2 ab	59.0 b	60.2 b	62.2 ab	
No. of effective stems per pot for rice	31.65 a	20.40 b	20.40 b	22.80 b	19.95 b	18.75 b	
No. of grains per panicle for rice	25.4 a	22.8 ab	22.5 ab	21.5 b	21.3 b	21.3 b	
100-grain weight of rice (g)	2.99 a	2.97 ab	2.91 abc	2.87 bc	2.86 c	2.85 c	
Grain yield of rice (kg/ha)[b]	1782 a	1023 ab (−43%)	990 ab (−44%)	1042 ab (−42%)	900 b (−50%)	843 b (−53%)	

In a row, data followed by the common letters indicated no significant difference at 0.05 levels by the Turkey's test.

[a] Treatment: R100 means planting density of rice at 100 plants/m^2; R0 means no rice planting; A0, A23, A45, A90, A180, and A360 mean planting density of *A. philoxeroides* at 0, 23, 45, 90, 180, 360 plants/m^2, respectively.

[b] Data in the parentheses showed the decreasing rate of rice grain yield.

DISCUSSION

In this experiment, it was found that the propagation stems of *A. philoxeroides* survived at 4℃, indicating hydrophytic type of *A. philoxeroides* is tolerant to coldness. In East, Nouth and South-West of China, the stems and leaves of *A. philoxeroides* above water surface withered to death in winter, but the propagation stems in water could survive cold winter. In these regions, the water temperature was higher than the air temperature and relatively stable though the weather temperature was occasionally below zero, which helped the propagation stems of *A. philoxeroides* in water survive cold winter. However, when the temperature decreased to -20℃, none of the propagation stems could survive. Therefore, the stems of *A. philoxeroides* could not survive cold winter in frigid regions. Tan et al [10] reported that there wasn't *A. philoxeroides* in the areas of Tibet, Qinghai, Ningxia, Xinjiang, Inner Mongolia and Heilongjiang of China. According to this study, it could be attributed to that the stems of *A. philoxeroides* could not survive cold winter in these areas.

The viability of *A. philoxeroides* had a close correlation with its water content. The viability decreased when the water content decreased. Shen et al [7] reported that for terricolous type of *A. philoxeroides*, the germination rate of underground stem was 21.7% when the water content was 42.4%. In this study we noted that the hydrophytic type had a high viable rate of 55% when the water content was 30.2%, indicating it had stronger tolerance to drought than the terricolous one. But we need further evidences to support the conclusions. The response of plant to drought included the phosphorylation of the proteins and the changes in gene expression [11]. The drought stress was the main factor that influences the growth and development of the plants [12]. It has been noted that when the weather was dry for a long time, *A. philoxeroides* could not grow and spread normally. *A. philoxeroides* could not grow in the drought areas such as Tibet, Qinghai and Ningxia may be not only attributed to the cold winter, but to dry weather and less rainfall.

The propagation stems of *A. philoxeroides* were very sensitive to salt-alkali (NaCl+KNO_3), which lost the viability after treated with 0.1% salt-alkali solution. And its salt-alkali tolerance was significant lower than the rice variety Chunjiang 11 which could survive 0.5% salt-alkali solution [13]. Alkaline resistant plants could grow normally in the regions of high salt-alkali, such as *Echinochloa crusgalli* [14], *Pragmites australis* [15] and *Artemisia capillaries* [16]. According to the results from this study, it could be deduced that *A. philoxeroides* might not grow and spread in salt-alkali soil.

The hydrophytic type of *A. philoxeroides* had a strong flooding tolerance, which could grow and proliferate in water-saturated soil or in deep water. Therefore, the ecological weed-controlling method of deep-water irrigation had no significant effect on the *A. philoxeroides* and it was difficult to be controlled in agricultural production system. But the traditional manual weeding and tillage were effective for the *A. philoxeroides* control [17]. The modern agriculture is dependent on the utilization of herbicides for weed control. The weed control is not an easy task for the farmers due to the lack of effective herbicides, high price and the difficulties to determine the suitable application time of herbicides. Thus, for the successful management the traditional manual weeding methods should be applied at lower population density, while chemical control with the herbicide of Fluroxypyr should be done when the density was high. The control efficiency of Fluroxypyr was 95% against *A. philoxeroides* in ditches and over 85% in rice fields, which was much better than that of Glyphosate and Bentazone [18].

Due to the competition of *A. philoxeroides*, the plant height, number of effective tillers, number of filled grains per panicle and grain yield of rice decreased significantly. Interplant of *A. philoxeroides* at the density of 23-60 plants per m^2 led to the reduction of rice yield by 42-53%. Zhang et al [19] reported that in the paddy field, *A. philoxeroides* at a density of 2-7 plants/m^2 reduced the rice yield of Wuyujing 3 by 9-28%, due to the significant reduction in the number of filled grains per panicle, whereas there was not significant decrease in the number of effective tillers and 100-grain weight, which was not in accordance with the results of this study for the low density designed.

Weeds had much stronger influence on the yield of directly seeding rice than on that of transplanted rice. *Heptachlor chinensis* at the density of 20-50 plants/m^2 reduced the number of effective tillers, number of filled grains per panicle and 1000-grain weight of hybrid rice Shanyou 63 by 11-69%, 24-28% and 18-37%, respectively, while the yield was reduced by 62%, 68% and 89% when the density were at 20, 30 and 50 plants/m^2 [20]. In this study we noted that *A. philoxeroides* at the simulated densities in transplanting paddy field had lower competitive ability against rice than that of *Leptochloa chinensis* in the direct-seeding paddy field. But this competitive ability would increase in the conditions of paddy field of directly seeding. Thus it is concluded that the *A. philoxeroides* will lead to loss of rice yield and become a noxious weed with strong competitive ability in paddy field of direct seeding method.

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