Original Research

Eco-Physiological Responses of *Scirpus planiculmis* to Different Water-Salt Conditions in Momoge Wetland

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Abstract

As a common plant in Momoge Wetland, *Scirpus planiculmis* is the major food source for Siberian Crane (*Grus leucogeranus*), which is one of the most valuable waterfowl in Momoge Wetland. Through a simulation experiment, this study investigated the effects of different water table depths (-5, 5, 10, 20, and 30 cm) and salinities (300, 1,000, 3,000, 4,000, and 5,000 mg/L) on the eco-physiological characteristics (height, leaf area per plant, chlorophyll content, and chlorophyll fluorescence) of *Scirpus planiculmis*. The results indicate that for the seedlings of *Scirpus planiculmis*, the effects of water depth on plant height, leaf area per plant, and chlorophyll content were significant, while salinity affected height increment, chlorophyll content, and chlorophyll fluorescence parameters. For the plants of *Scirpus planiculmis*, the water depth had a significant effect on height increment and chlorophyll content, while salinity had a significant effect on height increment, chlorophyll content, and chlorophyll fluorescence parameters. These results indicate a suitable ecological response thresholds of water table and salinity on the growth of *Scirpus planiculmis* seedlings and plants, which were 5-10 cm and less than 3,000 mg/L for seedlings, 30 cm and 300-4,000 mg/L for plants.

Keywords: *Scirpus planiculmis*, Momoge wetland, water-salt interaction, eco-physiological response

Introduction

Momoge Wetland, which is located in the semi-arid and concentrated saline-alkali region of western Songnen Plain in China, is an inland wetland and aquatic ecosystem reserve that provides a habitat for *Grus Leucogeranus* and other rare birds. It is also an ecological barrier to prevent the saline desertification of western Songnen Plain [1-3]. In recent decades, due to various natural and human factors, significant changes in the hydrological characteristics of this area have taken place. Drought and salinization of the wetland has posed a serious threat to cranes, other waterfowl, and wetland ecological systems in general [4-6].

Scirpus planiculmis is a typical wetland vegetation in Momoge Wetland. Being the main food source of endangered species (*Grus leucogeranus*), its growth and propagation directly affect the quality of *Grus leucogeranus* habitat [5], and it is now critical to study *Scirpus planiculmis* in the wetland. At present, the study of *Scirpus planiculmis* mainly concentrated in its biological characteristics, physical and chemical removal method [7, 8], and *Scirpus planiculmis* purification ability of heavy metals in sewage and sludge research [9]. Few studies have been undertaken to investigate *Scirpus planiculmis* growth conditions. In addition, in recent years, in order to alleviate the water shortage situation of the wetland and accommodate farmland drainage generated from large-scale farmland development, Momoge Wetland Conservation will receive a

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greater amount of farmland drainage as a result of water diversion into the wetland. Therefore, determining the appropriate amount of farmland drainage into the Momoge Wetland and revealing the effect of increased salinity by farmland drainage on wetland vegetation (*Scirpus planiculmis*) are directly relevant to regional water management and ecological restoration, which is of significant interest to local environmental protection agencies and researchers.

The objectives of this study were to analyze the physiological and ecological response characteristics (height increment, leaf area per plant, chlorophyll content, and chlorophyll fluorescence) of *Scirpus planiculmis* seedlings and plants in Momoge Wetland to different water table depths and salinity gradients through the artificial control method; subsequently, to determine suitable ecological water levels and salinity thresholds of *Scirpus planiculmis* seedlings and plants. The results of this study were to provide a scientific basis for the protection of food sources for the rare waterfowl crane, and for saltwater management and vegetation restoration.

Materials and Methods

The Plant Material

Scirpus planiculmis seedlings and wetland soil in the location of seedling growth were collected in May 2012 from Goose Head Bubble Experimental Area of Momoge Wetland, western Jilin Province, China (approximately 45º54'2"N, 123º51'25.7"E). Detailed information about the Goose Head Bubble Experimental Area of Momoge Wetland can be found in another paper [10].

The experimental soil was the wetland soil from sample collecting sites. The salinity of soil sample was (0.512±0.0038) g/L, measured when the water-soil ratio is 5:1. The same amount (5 strains) of seedlings were planted to specifications for 25 cm×23 cm×17 cm of small experimental barrels (*Scirpus planiculmis* bulbs were mainly distributed in soil under the ground about 10 cm). The thickness of soil was 20 cm. The soil moist surface was kept through daily watering. The small experimental barrels were placed under natural conditions for cultivation, which was covered by transparent film to shield the seedlings from rain. Experiments processed after a half month, when the height of *Scirpus planiculmis* reached 18.308±2.011 cm.

Experimental Methods

Small experimental barrels were placed in larger experimental barrels (76 cm×50 cm×60 cm), and the water level gradient was controlled by using different height of support mesa. The experimental setup is shown in Table 1. Because the soil from Momoge Wetland Conservation Area was mainly soda saline soil, the salinity of this experimental treatment was prepared by NaCl and $NaHCO_3$ in a ratio of 2:1. 300 mg/L was the salinity level of Goose Head Bubble Experimental Area of Momoge Wetland; 1,000 mg/L was the salinity that the aquatic plants began to suffer from stress [11]. 4,000 mg/L is the most critical salinity of aquatic plant death [12]. During the experiment, transparent film structures were used to avoid precipitation interference. The water level and water salinity in the barrels was timing (daily) monitored, and appropriate adjustments were made to keep the water in the barrels and the salinity constant.

Table 1. Design of water table depths and salinities.

Salinity (mg/L)	Water level (cm)				
300	-5	5	10	20	30
1,000	-5	5	10	20	30
3,000	-5	5	10	20	30
4,000	-5	5	10	20	30
5,000	-5	5	10	20	30

Negative signs indicate that water is below the surface of the soil

After 3 weeks (in the early June), 25 barrels and 3 representative plants in each small barrel were selected, and the plant height, leaf area, and chlorophyll content of each plant were determined. At the same time, the number of plants in every small bucket was calculated. The rest of the plant was kept cultivating in accordance with the previous methods. A month later (in the middle of July), the height of *Scirpus planiculmis* was 55.836±3.544 cm, which represents mature plants [13]. And the remaining 25 barrels were selected to do the same experimental treatment. The height, leaf area per plant, chlorophyll content, and chlorophyll fluorescence parameters of *Scirpus planiculmis* seedlings and plants before and after treatment was monitored to analyze the effects of water levels and salinity on eco-physiological characteristics of *Scirpus planiculmis* seedlings by one-way ANOVA and multivariate ANOVA methods.

Physiological Measurements

Leaf area was measured by LI-3000 leaf area meter. Chlorophyll content was measured by a CCM-200 chlorophyll content meter (Opti-Sciences, Tyngsboro, MA, USA). Before the measurement of chlorophyll fluorescence, the leaf was kept 5 minutes dark using a black leaf clip (the same as the photosynthetic rate measurement). After the dark adaptation, a portable chlorophyll fluorescence measuring instrument (FP100, PSI, Brno, Czech Republic) was used to analyze chlorophyll fluorescence of leaf blade.

Results and Discussion

Effects of Water Levels and Salinity on Eco-Physiological Characteristics of *Scirpus planiculmis* Seedlings

From Fig. 1, results showed that the water level, salinity, and interaction of both all had significant influence on the height increment and chlorophyll content of *Scirpus planiculmis* seedlings ($p = < 0.01$). And water level had significant influence on average leaf area per plant of *Scirpus planiculmis* seedlings (p < 0.01).

The height increment of *Scirpus planiculmis* seedlings was the biggest (40.753±1.723) cm at 10 cm water level (Fig. 1a), of which the significant level was 39.637% and 122.739% higher than height increment at -5 and 30 cm water level (p<0.01), respectively. The results illustrated

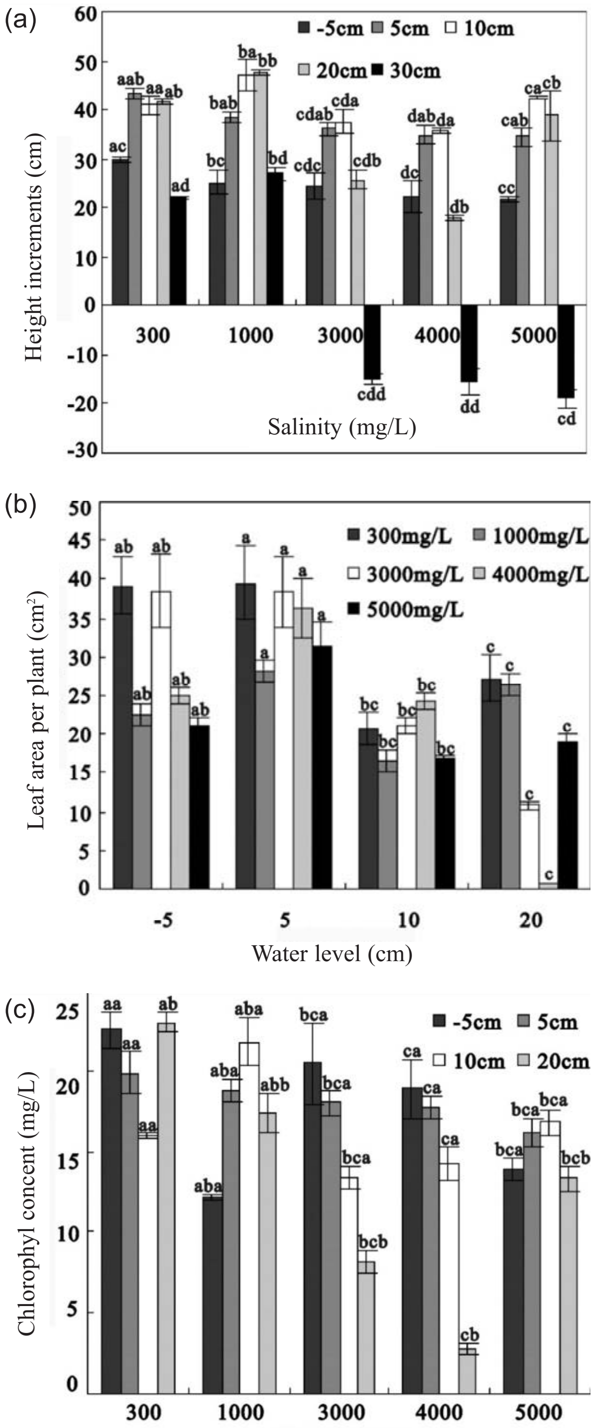

Fig. 1. The variation in eco-physiological characteristics of *Scirpus planiculmis* seedlings under different water-salt conditions: (a) height increment, (b) leaf area per plant, (c) chlorophyll content.
Different letters indicate that the differences between different treatment reach p<0.05 level of significance (the same below).

that the suitable water level for the growth of *Scirpus planiculmis* seedlings was 10 cm. And the mortality rates of *Scirpus planiculmis* seedlings were as high as 60% at 30cm water level, which indicated that 30 cm was the critical water level for seedling growth, so 30 cm water level was not considered in the subsequent analysis for seedlings. It may be that the early stage of plant growth (May), low temperature, high water level, water temperature picking up slowly result in relatively slow plant growth rate [14, 15]. The height increments of *Scirpus planiculmis* seedlings decreased with the increase of salinity. The height increment of seedlings at 300 mg/L salinity was significantly higher than those at other salinities, which indicated that the increase of salinity inhibited the height increments of *Scirpus planiculmis* seedlings. Under the water level of 30 cm, in addition to the salinity treatment of 300 and 1000 mg/L, the *Scirpus planiculmis* seedlings in the other salinity treatment group all died. The results indicated that the increase of salinity would limit the tolerance range of *Scirpus planiculmis* seedlings to high water levels. Salter et al. also had similar results [16].

The average leaf area per plant of *Scirpus planiculmis* seedlings was the biggest under the water level of 5 cm (Fig. 1b), and the water level of -5 cm came secondary, which were significantly bigger than that under other water levels. The results indicated that low water level conditions were much more favorable to leaf area expansion of seedlings, and when the water level was higher than 20 cm, it can significantly inhibit the leaf area expansion of seedlings. Wang et al. found that the leaf area of two species Sagittaria decreased with the increase of water level [17]. Ye et al. also found total leaf area of *Bruguiera gymnorrhiza* seedlings were significantly greater in the low water level than the high water level [18]. This may be because environmental hypoxia caused by flooding led to plant leaf loss and smaller leaf area [19]. Although average leaf area per plant of *Scirpus planiculmis* seedlings between different salinity had no significant difference, it all showed an inhibitory effect compared to 3,000 mg/L.

The chlorophyll content first increased and then descended with the increasing water level (Fig. 1c). The chlorophyll content was the biggest at 5 cm water level, and the lowest at 20 cm. The chlorophyll content between water levels of 5, 10, and -5 cm had no significant difference, but all significantly greater than 20 cm water level treatment, which indicated that the inhibitory of salinity to chlorophyll content of seedlings was mainly high water level inhibitory. The chlorophyll content of seedlings under the salinity of 3,000, 4,000 and, 5,000 mg/L were significantly less than that under 300 mg/L (p<0.01). The difference of chlorophyll content under salinity of 300 and 1,000 mg/L was not significant. Bu et al. found that the chlorophyll content of *Calamagrostis angustifolia* leaves increased with the increase of water level in significant waterlogging (10-30 cm) conditions, which were different from our experimental results [20]. This might result from the increase of salinity reducing the adaptive capacity of seedlings to water level, showing that there is a synergistic inhibition function between high water level and high salinity.

Table 2. Correlation analysis among chlorophyll fluorescence parameters of seedlings, water levels, and salinities.

	Ψo	φEo	φDo	PIabs	ABS/RC	Tro/RC	Eto/RC	Dio/RC	Fm/Fo	Fv/Fo	Fv/Fm
Salinity	0.02	-0.488*	0.795**	-0.692**	0.286	-0.193	-0.163	0.769**	-0.858**	-0.858**	-0.795**
Water level	0.346	0.386	-0.17	0.289	0.008	0.113	0.301	-0.143	0.141	0.141	0.17
Ψo		0.777**	0.091	0.514*	-0.044	-0.085	0.555*	0.039	-0.078	-0.078	-0.091
φEo			-0.556*	0.875**	-0.131	0.206	0.663**	-0.513*	0.559*	0.559*	0.556*
φDo				-0.707**	0.164	-0.425	-0.304	0.873**	-0.985**	-0.985**	-1**
PIabs					-0.499*	-0.052	0.285	-0.805**	0.751**	0.751**	0.707**
ABS/RC						0.823**	0.654**	0.621**	-0.216	-0.216	-0.164
Tro/RC							0.78**	0.066	0.365	0.365	0.425
Eto/RC								0.073	0.264	0.264	0.304
Dio/RC									-0.883**	-0.883**	-0.873**
Fm/Fo										1**	0.985**
Fv/Fo											0.985**

* and ** represent significant difference on 5% and 1% respectively

The results of correlation analysis of chlorophyll fluorescence parameters and water level, salinity are shown in Table 2. The fluorescence parameters of seedlings had a little correlation with water level, but closely associated with salinity. The quantum yield of dissipation of energy (φDo), performance index (PIabs), dissipation energy flux per active reaction center (Dio/RC), the ratio of maximum fluorescence to non-variable fluorescence (Fm/Fo), potential activity of PSII(Fv/Fo), and the quantum yield or efficiency of photochemistry in PSII(Fv/Fm) [21] were significantly correlated with salinity ($P<0.01$), and in addition to the φDo and Dio/RC parameters, the other parameters were negatively related with salinity.

Correlation analysis also indicated that the key parameters that were closely related to salinity were selected and the variation of parameters with salinity under different water levels was shown in Fig. 2. It can be seen that φEo, PIabs, Fm/Fo, Fv/Fo, and Fv/Fm showed a decreasing trend

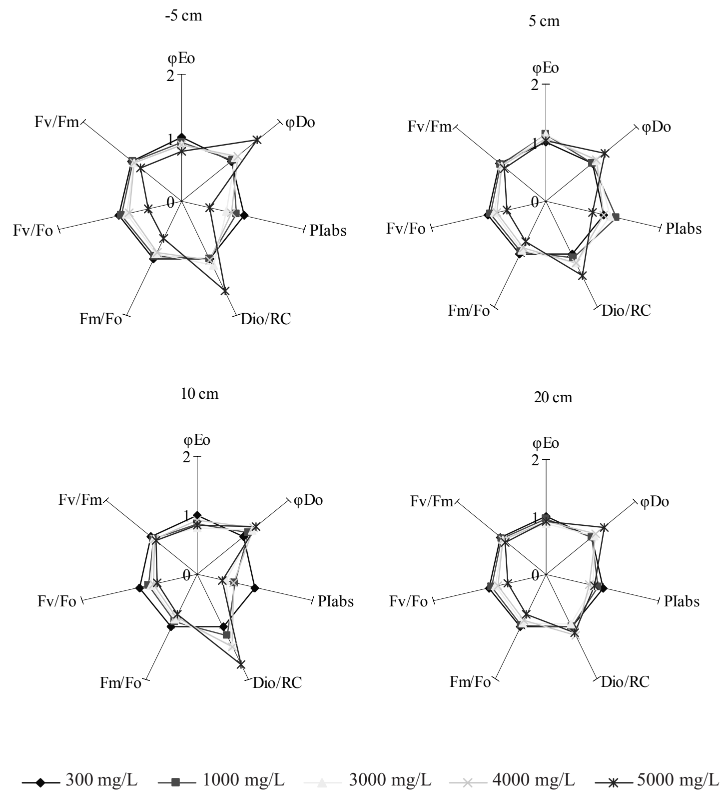

Fig. 2. Response characteristics of the chlorophyll fluorescence parameters of *Scirpus planiculmis* seedlings on salinities under different water levels (all parameters were normalized to the values of treatment of 300 mg/L).

with the increase of salinity under -5 cm water level. While φDo and Dio/RC increased with the increase of salinity, which indicated that seedlings indeed suffer salinity stress, and the increase of the heat dissipation may be a protective mechanism. When the salinity was less than 4,000 mg/L, the variations of the above parameters were small, and when salinity was 5,000 mg/L, the PIabs and Fv/Fm reduced sharply, and Dio/RC increased by 53%. Under the water level of 5 cm, the variation trend of Fm/Fo, Fv/Fo, Fv/Fm, φDo, and Dio/RC with salinity was similar to that in the -5 cm water level treatment. While φEo and PIabs were first increased then decreased with the increase of salinity, and maximum values were obtained in 1,000 mg/L salinity treatment. Under 10 and 20 cm water level treatments the change trend of the parameters with salinity was the same to -5 cm water level treatment but more smooth, while Dio/RC and PIabs had more obvious changes in 10 cm compared to 5 cm water treatment results under the same salinity. The change trend of parameters with salinity under the water level of -5, 10, and 20 cm was consistent with -5 cm water level treatment, and was more smoothly compared to other water treatments, which indicated that the rise of water level produced relief to salinity stress.

Effects of Water Levels and Salinity on Eco-Physiological Characteristics of *Scirpus planiculmis* Plants

Fig. 3 showed that the height increment and chlorophyll content of *Scirpus planiculmis* plants was significantly influenced by water level and salinity ($p<0.05$) (Figs. 3a and c). The results of significance test of leaf area per plant showed that the average leaf area per plant showed no significant difference at different water levels and salinities (Fig. 3b).

Plant height increments resulting from the changes of water levels were (Fig. 3a): -5, 5, 10, 20, and 30 cm, i.e. the height increment intensified with rising water levels. The height increment at 30 cm water level treatment was significantly greater than that at 5 cm and -5 cm water level treatment, and it showed no significant difference between 10cm and other water level treatments, which indicated that the increase of water level promoted the growth of plant height. Luan et al. found that with the advancement of *Carex pseudocuraica* growth period, its maximum plant height is constantly to near deep water levels, which is consistent with the results of this study [22]. The variation characteristics of height increments of different water level treatment with the change of salinity were: 3,000, 4,000, 1,000, 300, and 5,000 mg/L, demonstrating that the height increment initially intensified and then subsided with the increase of salinity (Fig. 3a). The height increment at 5,000 mg/L salinity treatment was significantly less than that at 3,000, 4,000, and 1,000 mg/L salinity treatment, and it showed no significant difference between 300 mg/L and other salinity treatments, which indicated that the increase of salinity promoted the growth of plant height, but when the salinity exceeded 4,000 mg/L, the height increments of *Scirpus planiculmis* plants would be restrained.

The variation characteristic of chlorophyll content of plants along with the water level was: 5, 20, 30, 10, and -5 cm, i.e. changing in accordance with bimodal peak curve as water depths increased. The Duncan difference examination results showed that the chlorophyll content under -5 cm water level treatment was significantly lower than other treatments, and the chlorophyll content between other treatments had no significant difference, which indicated that the chlorophyll formation was mainly restricted by low water levels. The chlorophyll content of *Scirpus planiculmis* plants continuously decreased with the increase of salinity (Fig. 3c). The chlorophyll content at 5,000 mg/L salinity treatment was significantly lower than that at 300 and 1,000 mg/L salinity treatments ($p<0.05$), and it showed no significant difference between 300, 1,000, 3,000, and

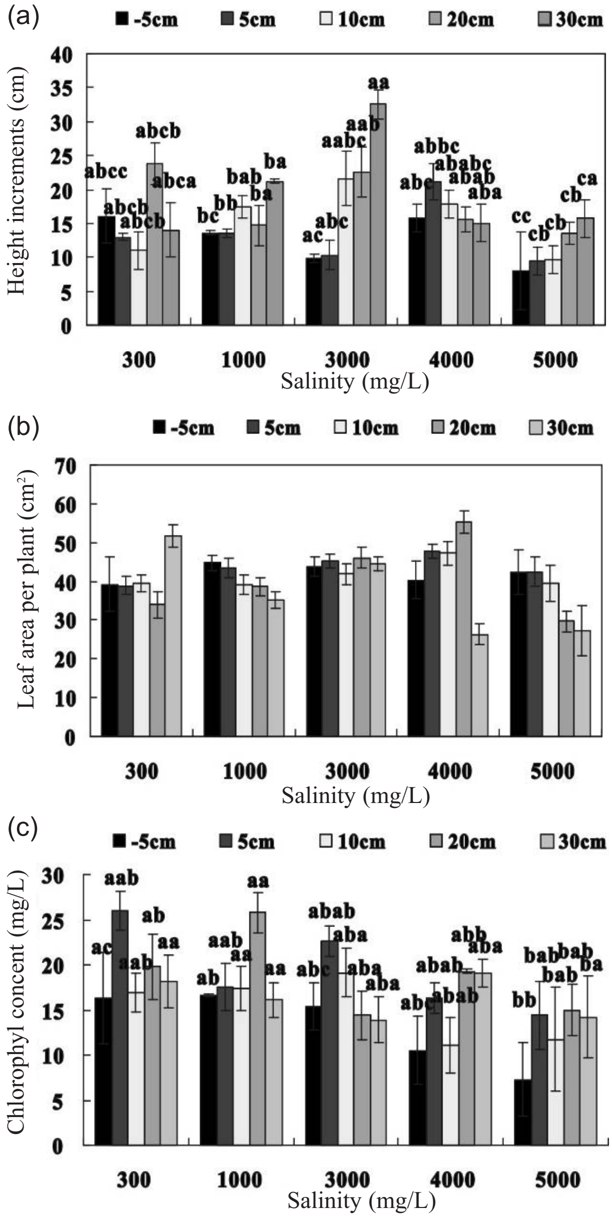

Fig. 3. The variation in eco-physiological characteristics of *Scirpus planiculmis* plants under different water-salt conditions. (a) height increment, (b) leaf area per plant, (c) chlorophyll content.

Table 3. Correlation analysis among chlorophyll fluorescence parameters of plants, water levels, and salinities.

	Ψo	φEo	φDo	PIabs	ABS/RC	Tro/RC	Eto/RC	Dio/RC	Fm/Fo	Fv/Fo	Fv/Fm
Salinity	-0.328	-0.459*	0.944**	-0.434*	0.125	-0.094	-0.460*	0.565**	-0.943**	-0.943**	-0.944**
Water level	0.005	0.036	-0.22	0.013	-0.016	0.037	0.033	-0.124	0.215	0.215	0.22
Ψo		0.989**	-0.385	0.943**	-0.559**	-0.475*	0.669**	-0.631**	0.377	0.377	0.385
φEo			-0.519**	0.955**	-0.548**	-0.433*	0.698**	-0.688**	0.512**	0.512**	0.519**
φDo				-0.498*	0.196	-0.036	-0.474*	0.650**	-0.998**	-0.998**	-1.000**
PIabs					-0.720**	-0.616**	0.485*	-0.806**	0.494*	0.494*	0.498*
ABS/RC						0.973**	0.21	0.872**	-0.192	-0.192	-0.196
Tro/RC							0.33	0.734**	0.039	0.039	0.036
Eto/RC								-0.082	0.469*	0.469*	0.474*
Dio/RC									-0.644**	-0.644**	-0.650**
Fm/Fo										1.000**	0.998**
Fv/Fo											0.998**

* and ** represent significant difference on 5% and 1% respectively.

4,000 mg/L, which indicated that when the salinity was no more than 4,000 mg/L, the chlorophyll content of *Scirpus planiculmis* plants would not be largely affected. Mild salt stress would promotes ion exchange absorption, which makes the plant chlorophyll content increase, and with the increase of salt stress, the ion toxic function makes plant water use efficiency greatly reduced and chlorophyll content lower [23].

As shown in Table 3, the fluorescence parameters had little correlation to water level. Some of the fluorescence parameters such asφDo, Dio/RC, Fm/Fo , Fv/Fo , Fv/Fm (p<0.01), and φEo, PIabs, and Eto/RC (p<0.05) showed

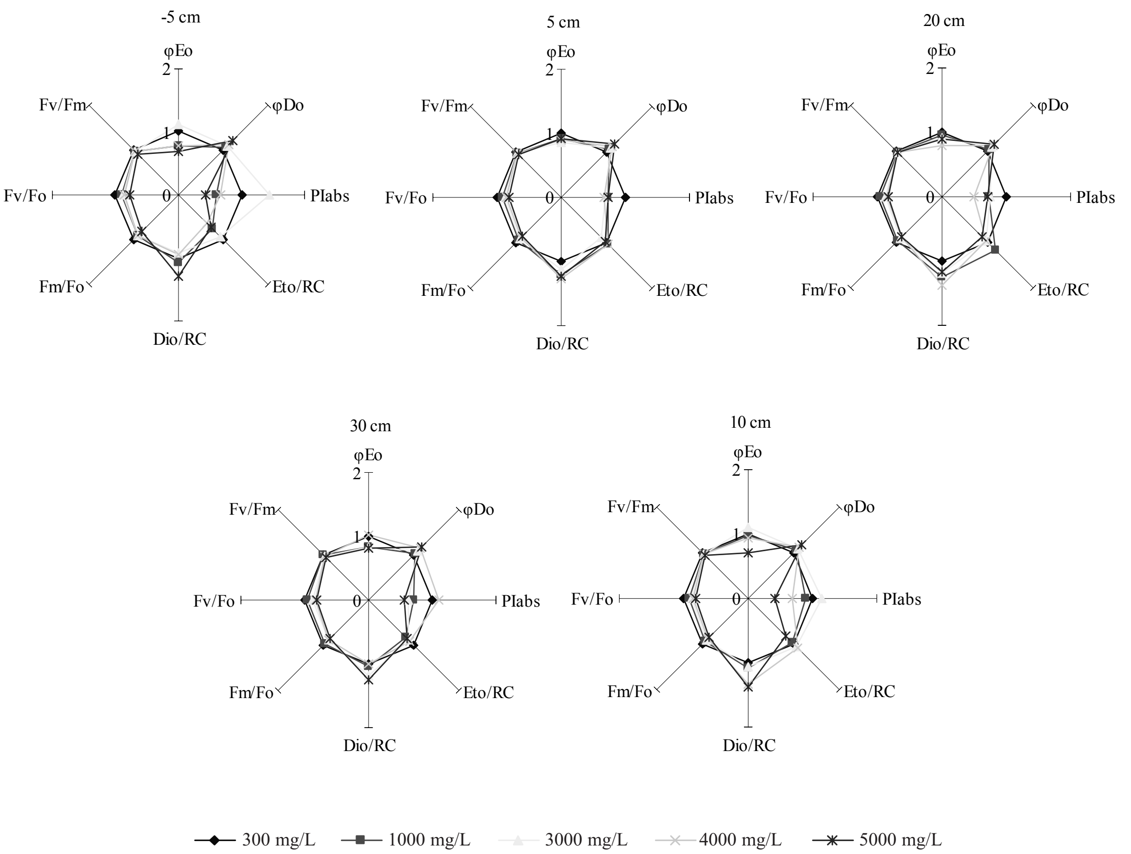

Fig. 4. Response characteristics of the chlorophyll fluorescence parameters of *Scirpus planiculmis* plants on salinities under different water levels (all parameters were normalized to the values of treatment of 300 mg/L).

significant correlation to salinity, and among these parameters, except that φDo and Dio/RC were positively correlated to salinity, other parameters were all negatively correlated with salinity. So the results indicated that *Scirpus planiculmis* plants were little affected by the water level, but significantly affected by salinity.

From Fig. 4, under the -5 cm water level, with the increase of salinity, φDo continuously increased, Fm/Fo, Fv/Fo, Fv/Fm constantly decreased, and φEo, PIabs, Eto/RC showed fluctuations decrease trend. And Dio/RC showed no significant difference between salinity that was less than 4,000 mg/L treatment and 300 mg/L, but it sharply increased at 5,000 mg/L. Under the 5cm water level, with the increase of salinity, φDo, Fm/Fo, Fv/Fo, Fv/Fm had the constantly consistent change with -5 cm, and PIabs continuously decreased with the increase of salinity, and it showed a sharp decrease in 1000 mg/L and then a little with the increase of salinity. Under the 10cm water level, with the increase of salinity, φDo, Dio/RC, PIabs, Fm/Fo, Fv/Fo, and Fv/Fm had the constantly consistent change with -5 cm, and φEo, Eto/RC showing first an increase and then a decrease trend. Under the 20 cm water level, except that Eto/RC first increased then decreased with the increase of salinity, other parameters had the constantly consistent change with -5 cm. Under the 30cm water level, all parameters had the constantly consistent change with -5 cm, but more gradual than -5 cm. This may be because under salt stress, the PSII reaction center is inactivated (Fv/Fo, Fm/Fo reduced), electron transfer rate decline (Eto/RC decreased), the photosynthetic mechanism of the plant is damaged, which affects the blade capturing light energy into chemical energy.

Conclusions

(1) Water level had a significant effect on height increments and leaf area per plant of *Scirpus planiculmis* seedlings; suitable water level found to be 5-10 cm. When salinity exceeded 3,000 mg/L, the salt started to have a significant inhibitory effect on the height, chlorophyll content, and chlorophyll fluorescence parameters (PIabs, Fv/Fm and Dio/RC); the ecological threshold of salinity for the growth of *Scirpus planiculmis* seedlings was in the range of 300-3,000 mg/L.

(2) Within the range of -5 cm to 30 cm, the increase of water level was found to promote the growth of *Scirpus planiculmis*, but it had no significant effect on leaf area per plant and the chlorophyll content of the plant. Thus the suitable ecological water level for the growth of *Scirpus planiculmis* plants was 30 cm. Within the range of salinity from 300 mg/L to 5,000 mg/L, the chlorophyll content of *Scirpus planiculmis* plants was significantly affected by salinity, but the leaf area per plant had no significant change within the range of salinity. When salinity exceeded 4,000 mg/L, the height increments of *Scirpus planiculmis* plants would be restrained, and chlorophyll fluorescence parameters were significantly influenced. So the suitable ecological threshold of salinity for the growth of *Scirpus planiculmis* plants was 300-4,000 mg/L.

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