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Original Research

Physiological Responses of *Scirpus validus* to Nitrate Stress

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Abstract

Physiological responses of *Scirpus validus* to nitrate stress were investigated. The experiment was conducted in an artificial greenhouse over a period of 35 days. The inhibitory effects of nitrate stress on *S. validus* growth were greater at concentrations higher than 10 mmol·L^{-1}. Greater than 10 mmol·L^{-1} nitrate inhibited the growth of *S. validus*; specifically, the fresh weight, new stem height, Δroot length, surface area, and root average diameter and volume were reduced. The level of ammonium in the plants was constant, whereas total nitrogen and nitrate nitrogen levels were reduced. Under stress, nitrate damaged the photosynthetic system and strongly reduced the net photosynthetic rate, transpiration rate, quantum yield at *LCP*, *LSP*, and P_{nmax}. Furthermore, nitrate increased stomatal limitation and conductance and influenced spectral parameters, e.g., reduced both PRI and SDr/SDb. The inhibitory effect of nitrate was most pronounced at 20 mmol·L^{-1}, primarily due to penetration and non-stomatal limitation. This study identified the physiological responses of *S. validus* to nitrate stress. The observed changes in physiological indices for *S. validus*, including photosynthetic parameters and spectral indicators, suggest that nitrate can inhibit root growth, differentiation and photosynthesis in plants, leading to an overall reduction in growth.

Keywords: Nitrate, *Scirpus validus*, Spectra, Photosynthesis, Root

Introduction

Eutrophication is a phenomenon of excess nutrition that results from the natural or artificial enrichment of inorganic nutrients such that the system cannot regulate the circulation of nutrients [1]. Among 118 lakes in China, the proportion of mesotrophic lakes was 21.4% and that of eutrophication was 78.6% in 2016 [2]. It is clear that China faces serious water eutrophication, which will affect the ecological landscape and the safety of livestock drinking water [3]. Many factors lead to eutrophication and predominantly involve the addition of nitrogen and phosphorus [4]. In particular, nitrogen is an important cause of eutrophication [5], especially ammonium nitrogen and nitrate nitrogen contents. Large amounts of nitrate-enriched water pose a serious threat to human life and production. The World Health Organization [6] found that drinking high-nitrate water can endanger human health and safety [7]. As a result,

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WHO recommends that the nitrogen content of drinking water be less than 10 mg·L^{-1} [8].

At present, there are many methods used to treat eutrophication, which consist primarily of physical, chemical and biological methods [9-11]. Biological methods typically entail the use of aquatic plants to purify sewage, which has the advantages of simple operation and large economic and technical implementations. Biological treatment has become an important new technology for the remediation of polluted water [12]. The tolerance of plants to different pollutants and their removal abilities and mechanisms along with the survival and growth ability of plants under polluted environments are the theoretical and technical bases of phytoremediation. In the study of plant physiological ecology, pigment content, water content, nutrition status, photosynthetic rate and other parameters are important indicators of the physiological status of plants [13]. The root system of the plant can optimize the absorption and use of nutrients through its physiological and ecological adaptability [14]. The "red edge" of the blade reflection spectrum is sensitive to vegetation growth, which is defined as the largest slope in the reflectance spectrum of plants and would be beneficial for modelling nutrient fluxes [15].

Scirpus validus is an aquatic plant widely used in sewage treatment [16] that presents good removal rates of nutrient salt [17], heavy metals [18] and organic compounds [19]. For example, *S. validus* is effective at removing ammonia nitrogen and nitrate and at effectively promoting the transformation of nitrogen and significantly reduce the residence time of inorganic nitrogen in wetlands [20]. Li studied the purification effect and physiological characteristics of *S. validus* in wastewater treatment [13]. The results showed that *S. validus* could effectively remove ammonia nitrogen, and its net photosynthetic rate was higher in wastewater than in the controls. Fu showed that the water-purifying effect of *S. validus* was 74.41% and that the plant had a strong purifying effect on reclaimed water with a high salt content [21]. Therefore, studies on plant photosynthetic physiology and spectral characteristics that show the tolerant response of plants to different nitrate environments can provide a theoretical basis for developing methods to remediate nitrate pollution in waters using *S. validus*.

Materials and Methods

Experimental Set-up

In mid-March 2015, 150 robust, healthy *S. validus* plants were collected from South Lake Park for use in the experiment in Tai'an, Shandong Province, China. In the lab, sediment, plankton, withered and yellow leaves were removed from the root surfaces with tap water. Then the plants were transferred to 20-L buckets containing 15 L of tap water. The plants underwent two weeks of cultivation and domestication in the artificial glasshouse of Shandong Agricultural University to become adapted to the greenhouse environment. After domestication, the plants were fit for the simulation test. The plants were monitored biweekly to observe the treatment levels and correct any deviations from the desired treatment levels.

Nitrate and Water Level Treatments

The culture solution was prepared from Hoagland culture solution with only KNO_3 added. Seven nitrate concentrations were established: 0 mmol·L^{-1}, 0.5 mmol·L^{-1}, 1.0 mmol·L^{-1}, 2.5 mmol·L^{-1}, 5 mmol·L^{-1}, 10 mmol·L^{-1} and 20 mmol·L^{-1}, as based on a previous study [22]. The 20-L, blue plastic buckets were used for culture and each contained 15 L of nutrient solution. For each bucket, the plants (3 plants per bucket) were fixed to the top of the bucket with a foam board and covered with a black plastic bag to maintain the roots in a dark state. Each treatment included three replications (7 treatments×3 replications). The experiment lasted for 35 days. At the end of the experiment, photosynthetic indexes and spectral parameters were measured, after which the plants were harvested and washed three times with ultra-pure water. Their biomass (Fresh Weight, FW) and the nitrogen content were determined using chemical methods [23]. The length, volume, and diameter of the roots were simultaneously determined using the plant root analysis machine (WinRHIZO, Regent Instruments Company, Quebec, Canada), which can measure root length, volume, diameter, surface area, and projected area for washed roots.

Determining Photosynthetic Indexes

Determining Photosynthetic Gas-Exchange Parameters

Photosynthetic gas exchange parameters were measured at 08:00-11:30 each day. From the middle part of each test plant, 3 leaves showing ideal development were selected, and light response was measured using a CIRAS-2 portable photosynthesis system (Systems PP, USA). We applied photosynthetic active radiation (PAR) by using a light-emitting diode (LED) (range 0-2000 μmol (photon) m^{-2}·s^{-1}; peak emission wavelength: red light (90%): 620-630 nm, and white light (10%): 425-625 nm) at the following levels: 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 60, 30, and 0 μmol·m^{-2}·s^{-1}. Each PAR measurement was a duration of 120 s, with three repeats. The air temperature of the leaf chamber was maintained at approximately 26±1.5ºC, the relative humidity was maintained at 60±5%, and the CO_2 concentration was 370±6 μmol·mol^{-2}. The following physiological and environmental parameters were automatically recorded: PAR (μmol·m^{-2}·s^{-1}), net photosynthetic rate (P_n, μmol·m^{-2}·s^{-1}), transpiration rate (E, mmol·m^{-2}·s^{-1}), stomatal conductance (G_s, mmol·m^{-2}·s^{-1}), intercellular CO$_2$ concentration (C_i, µmol·mol^{-1}), and air CO$_2$ concentration (C_a, µmol·mol^{-1}). Water-use efficiency (WUE, µmol·mmol^{-1}) and stomatal limitation (L_s) were calculated as follows: WUE = P_n / E, $L_s = 1 - C_i / C_a$ [24].

Determination of Spectral Parameters

Spectral Parameter Data Acquisition

A Unispec-SC (single channel) portable spectrometer produced by PP Systems (USA) was used to measure spectral reflectance in the different treatments; the parameters of band, sampling interval and resolving power were 310-1130 nm and 1 nm, respectively. For each treatment, three samples were selected, and three leaves from each sample were measured. Corrections were made using a standard whiteboard. The average measurement value was recorded as the leaf spectral reflectance. The spectral reflectance data were input into Excel 2013.

Data Processing

After obtaining the average spectral reflectance, we calculated the differential spectral reflectance according to the following equation (1), which eliminates the spectral baseline translation to obtain the "trilateral parameter":

$$D_{\lambda_i} = \frac{\left| R_{\lambda(i+1)} - R_{\lambda\ (\mathrm{i}-1)} \right|}{2\Delta\lambda}$$

$$\mathrm{CHINDI} = \frac{R_{750} - R_{705}}{R_{750} + R_{705}}$$

$$\mathrm{PRI} = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}$$

$$\mathrm{WI} = \frac{R_{900}}{R_{970}}$$

…where λi is the value of band i; $R\lambda i$ is the spectral reflectance, and $\Delta\lambda$ is the difference between $\lambda(i\text{-}1)$ and λi, which is determined by the sampling interval. ChlNDI, PRI, WI and SDr/SDb represent the chlorophyll content, xanthophyll cycle, water content and ratio of the differential area of the red edge to the differential area of the blue edge of *S. validus*, respectively, where SDr is the sum of the first-order differential spectra in the 680-755 nm region, and SDb is the sum of first-order differential spectra in the 490-530-nm region [25].

Data Analysis

The data were entered into Microsoft Excel 2013 and Origin 9.0 spreadsheets. All representative values are presented as the mean and standard deviation (S.D.). Significant differences between treatments were determined by an analysis of variance (ANOVA) and the Duncan test as implemented in SAS 9.0. A P-value less than 0.05 was considered significant.

Results and Discussion

Growth Measures

Nitrate is the dominant form of the mineral N and is readily mobile in the xylem, little nitrate accumulation is beneficial to the growth of plants, but high nitrates are toxic to plants [26]. Excessive concentrations of nitrates do not entail an increase in the fresh weight of the plant [27] and caused a significant effect on stem [28] (Table 1).

The influences of the different nitrate treatments on new stem height, Δroot length, and FW are shown in Table 1. All of the values first increased and then decreased with increasing nitrate concentration, with the "watershed" concentration being 10 mmol·L^{-1}. Nitrate promoted the growth of *S. validus* at concentrations <10 mmol·L^{-1}; at 10 mmol·L^{-1}, the fresh weight reached 43.83 g, representing 46.10% and 34.65% increases over

Table 1. Effect of nitrate treatments on stem height, root length, and fresh weight of *Scirpus validus*. Values are the means of three replications±SD. Means in columns within the different concentrations of nitrate followed by different letters are significantly different (LSD test, $P<0.05$).

Nitrate/mmol·L^{-1}	New stem height/cm	Fresh weight/g	ΔRoot length/cm
0	30.56±1.48c	32.55±4.45c	9.65±2.81b
0.5	31.07±2.11c	35.13±2.38bc	9.66±1.43b
1.0	31.25±3.81c	36.20±2.60bc	10.57±1.35b
2.5	40.70±1.03ab	37.10±7.81bc	14.86±1.19a
5	43.38±3.12a	41.87±3.14ab	17.33±0.25a
10	44.86±3.18a	43.83±4.40a	18.07±2.49a
20	36.71±3.33b	38.17±2.91bc	10.34±2.00b

Table 2. Effect of nitrate treatments on the total root surface area, total root volume, and average diameter of *Scirpus validus*. Values are the means of three replications±SD. Means in columns within the different concentrations of nitrate followed by different letters are significantly different (LSD test, $P<0.05$).

Nitrate/ mmol·L^{-1}	Total root surface area/cm^2	Total root volume/cm^3	Average diameter/mm
0	2.21±0.3c	0.02±0.001g	0.24±0.01a
0.5	6.36±0.47cd	0.05±0.001e	0.30±0.00d
1.0	8.57±0.44bc	0.05±0.002d	0.38±0.02c
2.5	9.52±3.21b	0.08±0.003c	0.38±0.01c
5	11.00±1.53b	0.08±0.009b	0.41±0.04b
10	16.54±1.27a	0.10±0.007a	0.47±0.02a
20	5.20±0.47d	0.05±0.002f	0.29±0.01d

the initial and control values, respectively. In addition, at 10 mmol·L^{-1}, Δroot length and new stem height were 18.07 and 44.86 cm, respectively, which represent 87.25% and 46.79% increases compared with the control levels, respectively. In contrast, at 20 mmol·L^{-1}, the fresh weight, new stem height and Δroot length declined by 12.91%, 18.17% and 42.79%, respectively, compared with that at 10 mmol·L^{-1}.

Root Measurements

Roots are the major organs for uptake of nitrate, which affect some morphological characteristics of plants [29] and increase the root surface area, average diameter and volume [30, 31]. These imbalances will affect root function and cause premature aging in the aerial parts of the leaves. Under such conditions, the plant will adapt by increasing the root-shoot ratio, stimulating lateral root growth, and reducing the number of axes [32].

Table 2 shows the influences of different nitrate concentrations on the root surface area, average diameter and volume in *S. validus*. All increased significantly with increasing nitrate concentrations. At 10 mmol·L^{-1}, root surface area, average diameter and volume were 16.54 cm^2, 0.47 mm, and 0.098 cm^3, respectively. These values were 7.50, 1.99 and 5.76 times, respectively, those of the control treatment. At 20 mmol·L^{-1}, the values of the three measures decreased. Root surface area was reduced to 5.20 cm^2, representing a 68.58% decrease, even though this value was higher (2.36 times greater) than that of the control treatment ($P<0.05$). In addition, at 20 mmol·L^{-1}, root average diameter was only 0.29 mm, i.e., 61.65% of the maximum value, and root volume was significantly reduced to only 45.92% of the maximum value.

In this study, in the range of 0-10 mmol·L^{-1} of nitrate, the root growth of the plants increased with increasing nitrate concentration. This result indicates that the ability to utilize nitrate is positively correlated with root length, surface area, volume, and average diameter. However, at 20 mmol·L^{-1}, the root parameters showed various degrees of decline, decreasing by more than 40%. These findings are consistent with those in *Rhus typhina* and *Pinus ponderosa* [33], *Nicotiana tabacum* [34], *Populus* [31], and *Triticum aestivum* [35]. This change affects the nitrate uptake rate of the roots, thereby affecting the accumulation of nitrogen (Fig. 3). The main reason for these results is that low concentrations of nitrate stimulate the elongation of lateral root [36], whereas high nitrate concentrations increase abscisic acid levels in root tips [37] and inhibit

Table 3. Total nitrogen, ammonium, and nitrate contents of *Scirpus validus* under different nitrate concentrations. Values are the means of three replications±SD. Means in columns within the different concentrations of nitrate followed by different letters are significantly different (LSD test, $P<0.05$).

Nitrate/ mmol·L^{-1}	Ammonium/mg·g^{-1}	Nitrate/mg·g^{-1}	Total nitrogen/mg·g^{-1}
0	3.43±0.10a	4.64 ±0.70b	8.06 ±0.79b
0.5	3.29±0.69a	5.21±1.25b	8.50±0.61b
1.0	3.50±0.54a	6.58± 0.84ab	10.08± 1.01ab
2.5	3.27±0.89a	7.87±1.47a	11.14±1.66a
5	3.31±1.12a	8.96±0.12a	12.27±1.16a
10	3.85±0.85a	7.90±0.97a	11.74±1.61a
20	3.85±0.89a	7.54±0.21ab	11.39±1.07a

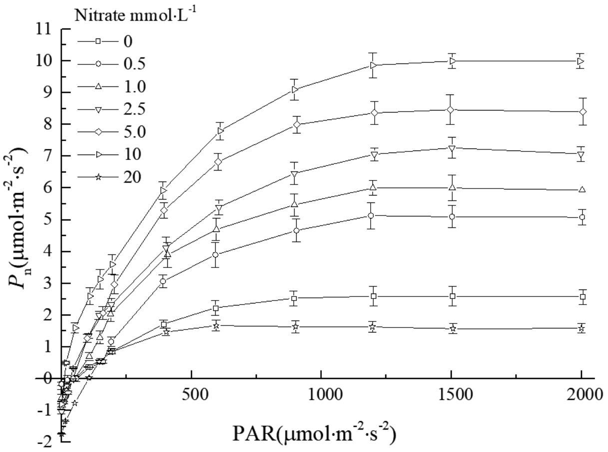

Fig. 1. Photosynthetic rate-light response curves and simulation of *Scirpus validus* under different nitrate concentrations. Values are the means of three replications±SD.

this activity [38]; this inhibition is more evident at concentrations of 10 mmol·L^{-1} or more, which leads to the reduced accumulation of nitrogen, especially at 20 mmol·L^{-1}. These observations suggest that the ability of plants to utilize nitrate varies not only with the nitrogen content of the plant but also with root length, diameter, volume and surface area.

N content

With increasing nitrate concentration, total nitrogen, nitrate and ammonium of *S. validus* first increased and then decreased; the differences in ammonium content were not significant (P>0.05), whereas those in total nitrogen and nitrate content were significant (Table 3). Nitrate had the largest contribution to the total nitrogen content of *S. validus*. At 5 mmol·L^{-1}, total nitrogen reached a maximum of 12.27 mg·g^{-1}, 1.52 times higher than the value in control plants. At 20 mmol·L^{-1}, total nitrogen content decreased by 13.67% to 1.31 times the control level. Nitrate content showed a similar trend as total nitrogen. At 5 mmol·L^{-1}, the maximum value of 8.96 mg·g^{-1} was reached, which was 1.93 times the control level. At 20 mmol·L^{-1}, the minimum content of 7.54 mg·g^{-1} was observed, which was 1.63 times that of the control plants. In contrast, the ammonium content of *S. validus* showed little change with increasing nitrate concentration, ranging from 3.05-3.86 mg·g^{-1}.

Light Response Parameters

Photosynthesis is the basis for the physiological processes underlying plant activity, growth and development [39] and can serve as an indicator of plant growth. The role of nitrate as an osmoticum affecting stomatal opening was elucidated, stomatal movement is highly regulated by multiple pathways to reduce excess water loss and maintain CO_2 uptake for photosynthesis [40], and the nitrate uptake and assimilation depends on photosynthesis [41]. Nitrate can promote the photosynthetic rate of plants and increase electron fixation of CO_2 [42] and stimulate nitrate assimilation into amino acid [43], but in high NO_3^- supply condition, the photosynthesis is reduced and a substantial proportion of the NO_3^- taken up is not assimilated [44]. However, at high nitrate concentrations, the lack of water in plant tissue will cause stomatal closure, impairments to chlorophyll, light-related enzyme inactivation or denaturation, and photosynthetic rate and assimilation reductions and the deceased PRI.

As shown in Fig. 1 and Table 4, both the process and characteristic parameters of the optical response of photosynthesis in leaves of *S. validus* differed significantly among the different nitrate concentrations. With increasing nitrate concentrations, the light-saturated net photosynthetic rate (P_{nmax}), light saturation point (*LSP*) and quantum yield at the light compensation point (Φc) first increased and then decreased. However, the light compensation point (*LCP*) and respiration rate (R_d) showed no obvious changes with increasing nitrate concentration. The decrease of *LSP* and the increase of *LCP* in plant photosynthesis indicate that the utilization ability and degree of light energy (strong light and dim light) decreased. The decrease of Φ indicates that the conversion efficiency of light energy utilization under low light intensity is decreased [45]. The decrease of P_{nmax} and the increase of R_d indicate that the synthesis

Table 4. Model-fitted values of photosynthesis-light response parameters of *Scirpus validus*.

Light response model	Nitrate concentration (mmol·L^{-1})	Φ	LSP	P_{nmax}	LCP	R_d	R^2
Rectangular hyperbolic modified model	0	0.0053	893	2.6	34.58	-0.18	0.9913
	0.5	0.0061	1190	5.1	39.60	-0.25	0.8994
	1.0	0.0137	1198	6.08	47.99	-0.66	0.9941
	2.5	0.018	1204	7.27	43.99	-0.79	0.9607
	5.0	0.0188	1495	8.47	44.54	-0.837	0.9931
	10	0.0245	1503	10	28.38	-0.70	0.9598
	20	0.0137	595	1.84	113.90	-1.56	0.9792

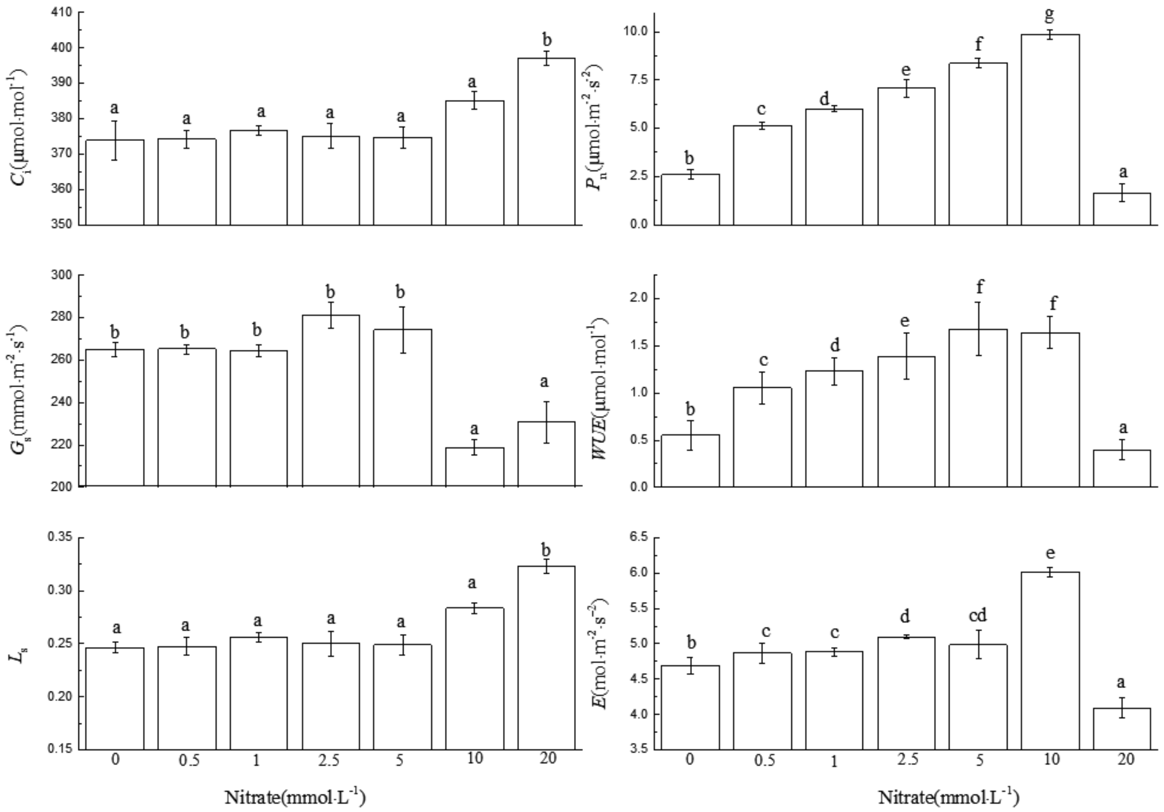

Fig. 2. The response of photosynthetic physiological parameters of *Scirpus validus* to nitrate concentrations (mean±SD) under the same photosynthetically active radiation (PAR = 1200 μmol·m^{-2}·s^{-1}) .Values are the means of three replications ± SD. Means in columns within the different concentrations of nitrate followed by different letters are significantly different (P<0.05).

ability of organic compounds decreased and respiration consumption increased. Taken together, the results show that the highest concentration of nitrate had an inhibitory effect on photosynthetic efficiency and on light energy utilization efficiency in the leaves of *S. validus*. P_{nmax} was lowest at the nitrate concentration of 20 mmol·L^{-1}, indicating that photosynthesis is sensitive to nitrate stress.

Photosynthetic Gas-Exchange Parameters

The variation in gas exchange parameters is shown in Fig. 2. With increasing nitrate concentration, P_n, E, G_s, and WUE first increased and then decreased. At 0-10 mmol·L^{-1}, they increased with increasing nitrate concentration. However, at 20 mmol·L^{-1}, P_n decreased significantly to 1.63 μmol·m^{-2}·s^{-2}, which was decreased by 37.18% and 83.45% compared with the control and highest values, respectively. E and WUE decreased significantly to 4.09 mmol·m^{-2}·s^{-1} and 0.40 μmol·mol^{-1}, respectively, which correspond to 68.05% and 72.06%, respectively, of the control values. G_s first increased with increasing nitrate concentration (<5 mmol·L^{-1}) but then decreased to 218.75 mmol·m^{-2}·s^{-1} at 10 mmol·L^{-1}. C_i and L_s continued to increase with increasing nitrate concentration; at 20 mmol·L^{-1}, C_i and L_s reached 397 μmol·mol^{-1} and 0.32, respectively, representing increases of 6.2% and 31.38% compared with the control levels.

The observations of photosynthetic gas exchange in the leaves (Fig. 2) show that at a fixed light intensity, P_n, E, G_s, LSP and P_{nmax} had greater degrees of decline in the nitrate treatments compared with the control values (Table 4 and Fig. 2). These findings suggest that high concentrations of nitrate (≥10 mmol·L^{-1}) can inhibit photosynthesis, mainly due to the effects of infiltrated nitrate on transmembrane transport of substances of plant cells [44] or the nitrogen content of leaves [46]. When photosynthesis increases, the availability of both carbohydrate and organic acids rises and these accumulate in the vacuole, constituting an alternative to the osmoregulatory function of nitrate [27].

In this study, nitrate stress significantly inhibited Φ, especially at 20 mmol·L^{-1} (Table 4). At this concentration, light energy utilization decreased, and P_{nmax} decreased rapidly (Fig. 2). The reduction in photosynthetic rate was mainly due to stomatal and non-stomatal limitation [47]. Stomatal limitation is caused by a decrease in the CO_2 supply to chloroplasts, which result from decreased C_i and reduced stomatal aperture, whereas non-stomatal limitation is due to stomatal diffusion resistance involving mesophyll cells. The results show that at low nitrate concentrations, G_s decreased, resulting in C_i decline; however, at much higher nitrate concentrations,

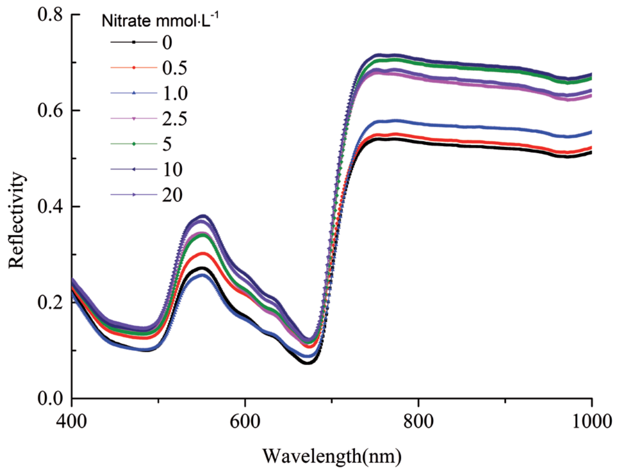

Fig. 3. The reflection spectrum of *Scirpus validus* under different nitrate concentrations.

C_i and L_s increased. These results indicate that the effect of nitrate on photosynthetic rate occurs primarily through stomatal limitation. Li reported similar results: WI and WUE are low, and the plant opens the stomata to maintain the CO_2 supply and normal growth [48]. This indicated high NO_3^- can reduce the stomatal aperture and transpiration rates [44].

Spectral Parameters

Several spectral models have been established to characterize the physiological state of plants [25, 49, 50] and to monitor plant nutrition [15, 51]. The spectral reflectance values of the *S. validus* leaves under different treatments are shown in Fig. 3. In the wavelength range of 400-1000 nm, a strong reflectance in the visible spectral region was observed from the leaves from all of the treatments, with strong reflectance peaks at 550 nm. At 700-750 nm, the reflection rapidly increased; at 750-1000 nm, the rate of increase slowed, with the values eventually forming a high "reflection platform". At 550 nm, where the reflection intensity decreased in the order 10 mmol·L^{-1} > 20 mmol·L^{-1} > 2.5 mmol·L^{-1} > 5 mmol·L^{-1} > 0.5 mmol·L^{-1} > 0 mmol·L^{-1} > 1.0 mmol·L^{-1}, the reflection intensity also increased substantially with increasing nitrate concentration, and no variations were shown. In the 750-1000-nm range, where the reflection intensity was in the order 10 mmol·L^{-1} > 5 mmol·L^{-1} > 20 mmol·L^{-1} > 2.5 mmol·L^{-1} > 1.0 mmol·L^{-1} > 0.5 mmol·L^{-1} > 0 mmol·L^{-1}, the reflection strength tended to increase substantially with increasing nitrate concentration. In the infrared range, the direct reflection spectrum showed obvious variation. This result indicates that it is possible to use infrared light Poor's diagnosis to determine the influence of nitrate on *S. validus* [52].

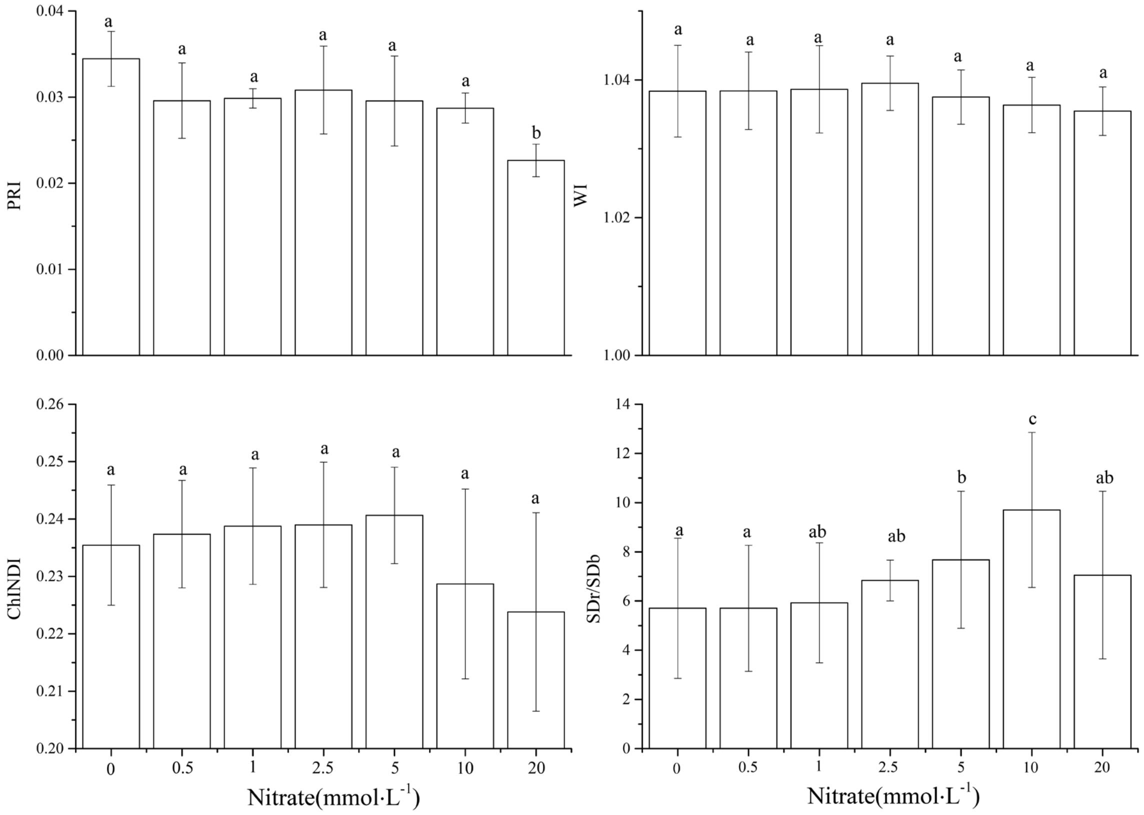

Fig. 4. The response of spectral parameters (PRI, WI, ChINDI, SDr/SDb) of *Scirpus validus* to nitrate concentrations. Values are the means of three replications±SD. Means in columns within the different concentrations of nitrate followed by different letters are significantly different (*P*<0.05).

Fig. 4 shows the influences of different nitrate concentrations on PRI, SDr/SDb, WI and ChlNDI. Generally, these parameters first increased and then decreased with increasing nitrate concentrations. WI and ChlNDI showed no pronounced changes, unlike PRI and SDr/SDb. PRI declined as nitrate concentration increased and showed no obvious variation in the range of 0-10 mmol·L^{-1}; however, at 20 mmol·L^{-1}, the PRI value was 0.02, which was 65.74% that of the control value. SDr/SDb showed a different pattern from PRI in the range of 0-10 mmol·L^{-1}; at 10 mmol·L^{-1}, SDr/SDb was 9.70, which was 1.70 times that of the control value. SDr/SDb subsequently decreased to 7.05, which was 72.68% the value at 10 mmol·L^{-1} and 1.24 times the control value.

The SDr/SDb was previously found to be significantly and positively correlated with plant nitrogen content. With increasing nitrate concentration, the SDr/SDb increased and then decreased, likely reflecting the rapid growth of the plant, which requires nitrogen; alternatively, the pattern of SDr/SDb might be related to changes in K$^+$ [53]. At 20 mmol·L^{-1}, SDr/SDb decreased, and SDr/SDb and total nitrogen content were 72.71% and 92.85%, respectively, of the highest values. This SDr/SDb decrease occurred because the plant accumulated more nitrate with increasing nitrate concentration, leading to a decrease in nitrogen metabolism activity and an increase in ammonium content (Fig. 3), which can be toxic.

ChlNDI characterizes the chlorophyll content, and nitrogen is an element that forms part of the synthesis of chlorophyll. Chlorophyll is the material basis of light absorption, transmission and conversion [54]. The results showed that chlorophyll content changed with increasing nitrate concentration. At 20 mmol·L^{-1}, the lowest chlorophyll content was observed, which was 95.06% that of the control value. This difference was not significant, similar to the results of Gromaz [27]. This result was obtained because nitrate stress can inhibit the absorption of Mg^{2+}, which in turn will cause a decrease in plant chlorophyll content.

Conclusions

The effects of different levels of nitrate stress on the growth, photosynthesis characteristics and spectral parameters of *S. validus* were investigated to elucidate the mechanisms of nitrate damage to plants and the adaptive physiological responses of plants to nitrate stress. The important findings were as follows. The inhibitory effects of nitrate stress on *S. validus* growth were greater at concentrations higher than 10 mmol·L^{-1}. Nitrate concentration greater than 10 mmol·L^{-1} inhibited the growth of *S. validus*; specifically, the fresh weight, new stem height, Δroot length, surface area, and root average diameter and volume were reduced. Nitrate stress increased the level of ammonium in the plants, whereas the total nitrogen and nitrate nitrogen were reduced. Under stress ($\geq$10 mmol·L^{-1}), nitrate damaged the photosynthetic system and highly reduced the net photosynthetic rate, transpiration rate, quantum yield at the light compensation point, light saturation point, and light-saturated net photosynthetic rate. Furthermore, nitrate increased the stomatal limitation and stomatal conductance and influenced spectral parameters, e.g., reduced both PRI and SDr/SDb. The inhibitory effect of nitrate was most pronounced at 20 mmol·L^{-1} ($P<0.05$), primarily due to penetration and non-stomatal limitation.

This study identified the physiological responses of *S. validus* to nitrate stress. The observed changes in physiological indices in *S. validus*, including photosynthetic parameters and spectral indicators, suggest that nitrate can inhibit root growth, differentiation and photosynthesis in plants, leading to an overall reduction in growth. Our study also shows that near-infrared light can be used as a diagnostic tool for measuring nitrate stress.

Abbreviations

PAR, photosynthetic active radiation;
E, transpiration rate;
G_s, stomatal conductance;
C_i, intercellular CO_2 concentration;
C_a, air CO_2 concentration;
WUE, Water-use efficiency;
L_s stomatal limitation;
P_n, net photosynthetic rate;
LSP, light saturation point;
LCP, light compensation point;
P_{nmax}, light-saturated net photosynthetic rate;
ChlNDI, chlorophyll content;
WI, water content;
PRI, xanthophyll cycle index;
SDr/SDb, ratio of the differential area of the red edge to that of the blue edge

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Conflict of Interest

The authors declare no conflict of interest.

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