

The Effects of Silicon on *Festuca Arundinacea* under PH Stress

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Abstract. Experiments were performed to study effects of exogenous silicon (0, 2, and 8 mM) and pH stress (pH 3.0, 5.5, 8.0) on *Festuca arundinacea*. The effect on growth, photosynthetic pigments content and antioxidant enzymes activities were researched. Our results indicated that under pH 3.0 stress, above ground dry weight, the activities of catalase (CAT), peroxidase (POD) and chlorophyll content were maximum under the 2mM Si treatment and restrained under 8mM Si treatment. Under pH8.0 treatment, the plant height, the activities of APX, POD and chlorophyll content increased significantly with increase of the concentration of silicon treatment which were maximum under 8mM Si treatment, but the POD, dry weights of roots and above ground declined a little compared to 2mM Si treatment. It illustrates that under acid stress, the lower concentration of Si treatment is effective in alleviating the injury of plants under acid and alkali stress.

Introduction

pH stress is considered one of the most detrimental environmental stresses in light of the intensified acid rain and salinization. Acid rain as a global environmental issue can cause soil acidification and inhibit the growth of plants [1,2]. Excessive alkaline salt concentrations can damage or even kill plants by generating osmotic, ionic, and oxidative stress [3]. Many studies have investigated the injuries effect and forms of the plants [4,5]. Tall fescue (*Festuca arundinacea* cv. Barlexas) is a major species of cool-season forages and turf grasses. They are widely used in warm temperate to subtropical regions all over the world because they are easy to plant [6]. but its persistence is impaired by biotic and abiotic stresses [7].

Silicon (Si) is the second most abundant element after oxygen in soil. Si plays an important role in biological process, primarily it provides mechanical strength and elasticity to cell walls [8]. Si mainly in the form of silicic acid, H_4SiO_4 , is prominent in the soil solution [9]. Several beneficial effects of Si have been reported, including increased photosynthetic activity, insect and disease resistance, reduced mineral toxicity, improved nutrient imbalance, and enhanced drought and frost tolerance [10-14]. But researches showed different expression patterns and tissue or cellular localization that are associated with different levels of Si accumulation in different plant species [15]. Currently, N, P as the main nutrition applied in the lawn. Impacts from unremittingly elevated N, P pollution rates can extend beyond the directly affected plant-soil systems, and impinge on ecosystem services such as provision of clean drinking water [16]. Therefore, it is vital to find an environmental-friendly fertilizer to substitute N and P partly. Si is one of the elements will not damage soil with excessive extension [17]. The previous experiment showed that especially the plants may be able to substitute carbon compounds in a relevant share by silicon compounds [18]. Investigating the effect of Si in *Festuca arundinacea* under different pH treatment will provide basis for the Si application in the grass planting.

Materials and Methods

Plant Materials and Treatments. The *Festuca arundinacea* seeds were sterilized for 20 min with 0.5% (w/v) permanganate solution, rinsed three times with sterile distilled water, then soaked in Na_2SiO_3 9H₂O₂ solution for 24 h at 25°C. The seeds were planted in the plastic pots filled with perlite. Three treatments of pH values (pH 3.0, 5.5, 8.0) and three concentrations of Si (0, 2, 8 mM) were selected in the present work. There were nine treatment and every treatment repeated three times. Na_2SiO_3 9H₂O₂ was used as donor of Si. Tap water used to prepare different Si concentration of solution, then H₂SO₄ and NaOH were used to adjust pH value of solution using the PHS-3C type pH instrument. The solution was added in order to keep the perlite moist with at fixed time and with fixed quantity everyday. 30-days-seedlings were harvested to determine data.

Determination of Growth. Plant height were measured before drying. Plant materials dried at 105 °C for 30 min and then 80 °C to constant weight in oven were used to measure dry weight [5].

Determination of Antioxidant Enzymes Activities. Approximately 0.20 g of fresh leaves was extracted in

50 mM potassium phosphate buffer (PBS, pH 7.8). The homogenates were centrifuged at 15,000×g and 4°C for 20 min, and the supernatants were used for assaying enzyme activity[19].

CAT (EC 1.11.1.6) activity was measured by determining the decrease in the absorbance at 240 nm due to H₂O₂ consumption, as described by Azevedo et al[20]. The reaction mixture contained 0.3 ml of H₂O₂ (0.1 M), 1.5 ml of PBS (50 mM pH 7.8), 0.2 ml of the sample and 1 ml of distilled water.

POD (EC 1.11.1.7) activity was determined according to Bai et al[21]. with some modifications. The reaction mixture contained 1.0 ml of 0.3% H₂O₂, 1.0 ml of 0.05 M PBS (pH 7.8), 0.8 ml of 0.2% guaiacol, and 0.2 ml of the sample. The increase in absorbance at 470 nm during a 1min period was determined.

AXP (EC 1.11.1.11) activity was determined by measuring the decrease in the absorbance of ascorbic acid (ASA) at 290 nm[22]. The reaction mixture contained 2.7 ml of a mixture containing PBS (25 mM, pH 7.0) 0.1mL AsA (7.5 mM), 0.1 ml of the sample, and 0.1ml of H₂O₂ (300mM). The oxidation of ascorbate was initiated by H₂O₂ and was monitored for 3 min. One unit of APX activity was defined as the amount of enzyme oxidizing 1μM of ascorbate, and the extinction coefficient was 2.8 mM⁻¹cm⁻¹.

Determination of Malondialdehyde (MDA). The thiobarbituric acid (TBA) test was used to determine MDA content for the measurement of membrane damage[23]. Plant samples (0.3 g) were homogenized in 2 ml cold phosphate buffer (pH 7.8, 50 mM), and then the solution was homogenized in 3 ml of 0.5% TBA in 5% trichloroacetic acid (w/v). Samples were heated at 100 °C for 10 min, and quickly cooling, the homogenate was centrifuged at 4500×g for 10 min again. The absorbance was measured at 440, 532 and 600 nm, distilled water being used as a blank control. The concentration of MDA was calculated using the extinction coefficient of 155 mM⁻¹cm⁻¹ and by correcting the specific absorbance at 600 nm of wavelength.

Determination of Photosynthetic Pigments Content. Photosynthetic pigments were extracted using ethanol-acetone solution (v/v 1:2). The absorbance were read at 663nm, 645nm and 470nm respectively. The content of total chlorophyll, chlorophyll a, chlorophyll b and carotenoid were calculated by using the formula described by Lichtenthaler [24].

Statistical Analysis. Data were analysed using of Student's t-test (Spss 19.0) and analysis of variance (ANOVA) and comparisons between the mean values were made by the least significant difference (LSD) test at p< 0.05, and a standard error (SE) was calculated.

Result

Effects of Si on the Growth of Festuca Arundinacea under Ph Stress. For pH8.0-treatment, the plant height significantly increased with the increase of Si concentration, but for pH3.0-treatment, the plant height showed lower increase (Table1). The plant height firstly increased and then decreased with the increase of Si concentration under the condition of pH5.5-treatment. When the Festuca arundinacea seedlings were treated with Si and pH 3.0 (or 8.0), the height augmented insignificantly compared with the plants were treated with the pH 5.5- treatment.

The acid stress and alkali stress affected dry weight (DW) of roots, stems and leaves insignificantly (Table 1). Under all pH treatment, the DW of above ground showed the tendency which increased first and then decreased with the increase of Si concentration and increased significantly in a moderate concentration of Si (2 mM) (P<0.05). The DW of above ground increased 54.2% under pH3.0 treatment, 48.7% under pH 5.5 treatment and 10.24% under pH8.0 treatment compared with those of the corresponding single treatments with pH (Table 1). Si interacting with pH 3.0 (or 8.0) treatment has augmented plant height significantly compared with the plants were treated with the single pH 5.5.

When the plants were treated pH 3.0, the DW of roots decreased with addition of Si, but the diversity was not significant (P<0.05). When the plants were treated with pH 5.5(or 8.0), the DW of roots showed the tendency which increased first and then decreased with the increase of Si concentration, but the diversity was not significant. The result shows supplying Si affects insignificantly to the DW of roots, but is significantly positive with the DW of above ground.

Table 1 Effects of Si on the growth of Festuca arundinacea under pH stress

pH	Si (mM)	Plant height [cm]	Root dry weight [g/5stock]	Above ground dry weight [g/5stock]
3.0	0	4.766±0.428b	0.0107±0.0047ab	0.0110±0.0032c
	2	4.923±0.574b	0.0100±0.0015b	0.0170±0.0021a
	8	4.944±0.500b	0.0078±0.0014b	0.0154±0.0011ab
5.5	0	5.061±0.489ab	0.0109±0.0015ab	0.0093±0.0016c
	2	5.601±0.962a	0.0142±0.0338a	0.0139±0.0014b
	8	5.473±0.732ab	0.0124±0.0033ab	0.0108±0.0019c
8.0	0	4.359±0.537b	0.0137±0.0017ab	0.0117±0.0024bc
	2	4.904±0.297b	0.0140±0.0247a	0.0129±0.0010bc
	8	5.140±0.441ab	0.0136±0.0043ab	0.0113±0.0018c

Significant differences at p < 0.05 were showed with different letters in the same line

Effects of Si on the Antioxidant Enzymes Activities of Festuca Arundinacea under pH Stress. When Si was not applied, the POD, CAT and APX activities in leaves of tall fescue treated with pH 5.5 solutions were the highest than the pH3.0 or 8.0 treatments. and for pH8.0-treatment, the POD, CAT and APX activities was lowest. Under pH3.0 stress, POD activities declined with the increase of Si concentration, but the difference is not significant ($P<0.05$). for pH5.5-treatment, POD activities in Si2 concentration were maximum and caused negative impact with Si8 concentration. Under pH8.0 stress, POD activities significantly improved with the increase of Si concentration (Figure 1).

When Si was not applied, CAT activities were significantly restrained with pH 3.0 (or 8.0) treatment compared with pH 5.5 treatment (Figure 2). Under the same pH value condition, the CAT activities increased first and then decreased with the increase of Si concentration. for pH5.5-treatment, the CAT activities increased 24.7% and under pH8.0 treatment increased by 11.3%, but under pH3.0 treatment barely increased 1.3% compared with those of the control and the corresponding single treatments of pH. When the combined treatment of 2 mM Si and pH 3.0 (or 5.5) was used caused negative impact. The pH3.0 treatment decreased 15.5% and pH5.5 treatment decreased 2.3% (Figure 2). Si interacting with pH 8.0 treatment has significantly enhanced CAT activities.

When Si was not applied, APX activities were insignificantly restrained under pH 3.0 (or 8.0) stress ($P<0.05$). Under pH3.0 stress, APX activities enhanced in 2mM Si treatment and restrained in 8mM Si treatment, but the diversity was not significant. under pH5.5 treatment, APX activities improved 21.9% significantly in Si2 concentration and the APX activities in 8mM Si treatment were less than in 2mM Si treatment (Figure 3). It illustrates it will cause negative impact with the increase of Si concentration. The APX activities improved when the combined treatment of 2 mM (8 mM) Si and pH 8.0 was used and in 8mM Si treatment significantly improved 10.1% compared with those of the control and the corresponding single treatments of pH. The result indicate Si interacting with pH3.0 (or 8.0) treatment can enhance APX activities, but it is not significant with pH3.0 treatment.

Effects of Si on the MDA Content of Festuca Arundinacea under pH stress. When Si was not applied, MDA levels were significantly improved under pH3.0 stress, but reduced under pH8.0 stress. for pH 3.0-treatment, the application of Si significantly reduced MDA content which decreased by 11.0% in 2mM Si treatment and 20.6% in 8mM Si treatment (Table 2). Under pH5.5 treatment, the application of Si also reduced MDA content, but it was not significant ($P<0.05$), which decreased by 2.6% in Si2 concentration and 10.5% in Si8 concentration. However, Under pH8.0 treatment, the MDA level significantly increased in 2mM Si treatment and 8mM Si treatment compared with the single pH treatment and it was approximate in 2mM Si treatment and 8mM Si treatment. Si interacting with pH3.0 treatment significantly decreased MDA content.

Table 2 Effects of Si on POD, CAT, APX activity and MDA content in Festuca arundinacea leaves under pH stress

pH	Si [mM]	POD [mmol/L]	CAT [mmol/L]	APX [mmol/L]	MDA [nmol/g(FW)]
3.0	0	36.285 ± 0.711b	440.925 ± 2.205d	56.961 ± 0.822c	43.273 ± 1.522a
	2	34.736 ± 1.073c	447.607 ± 8.076cd	57.913 ± 0.602c	38.855 ± 0.984b
	8	31.816 ± 0.627d	371.694 ± 3.713f	56.072 ± 1.321c	34.367 ± 0.671c
5.5	0	36.523 ± 0.757b	454.677 ± 3.984c	57.153 ± 0.602c	34.561 ± 1.341c
	2	39.212 ± 1.044a	567.032 ± 7.878a	68.561 ± 1.746a	33.669 ± 1.218c
	8	26.110 ± 0.525ef	444.169 ± 1.241d	60.637 ± 1.729b	30.931 ± 0.322d
8.0	0	25.835 ± 0.958f	421.393 ± 3.254e	56.167 ± 1.528c	29.558 ± 1.342d
	2	27.493 ± 0.894e	468.826 ± 4.408b	57.729 ± 0.775c	34.555 ± 0.743c
	8	32.433 ± 0.561d	462.185 ± 7.717bc	62.025 ± 1.688b	34.662 ± 0.984c

Significant differences at $p < 0.05$ were showed with different letters in the same line

Effects of Si on the Photosynthetic Pigments Content of Festuca Arundinacea under pH stress. The Chl content and carotenoid contents decreased significantly under single pH3.0 (or 8.0) treatment compared with pH5.5 treatment, especially under pH3.0 treatment ($p<0.05$). For pH3.0-treatment, The Chl b, Chl a, total Chl and carotenoid contents increased significantly with the addition of Si, at the same time, the Chl a content in 8mM Si treatment was less than in 2mM Si treatment. The Chl a content increased by 50.4% in 2mM Si treatment and 48.7% in 8mM Si treatment compared with single pH3.0 treatment (Table 3). It showed the increase of Si concentration caused negative impact. for pH5.5-treatment, the Chl and carotenoid content in 2mM Si treatment was less than the single pH5.5 treatment and in 8mM Si treatment raised a little than it in 2mM Si treatment. Under pH8.0 treatment, the Chl and carotenoid content in 2mM Si treatment was also less than it in single pH8.0 treatment, but it in 8 mM Si treatment was more than it in single pH8.0 treatment. The Chl a content increased by 14.8% in 8mM Si treatment compared with the single pH8.0 treatment.

Table 3 Effects of Si on photosynthetic pigments content of *Festuca arundinacea* under pH stress

pH	Si[mM]	Chla [mg/g]	Chl b [mg/g]	Total Chl [mg/g]	Carotenoid[mg/g]
3.0	0	1.176±0.005g	0.559±0.005c	1.735±0.010f	0.4824±0.009g
	2	1.760±0.003a	0.693±0.002a	2.453±0.005a	0.670±0.004b
	8	1.748±0.011b	0.705±0.009a	2.453±0.019a	0.686±0.005a
5.5	0	1.703±0.003c	0.692±0.003a	2.395±0.006b	0.644±0.005 c
	2	1.495±0.004e	0.629±0.003a	2.125±0.005cd	0.591±0.005d
	8	1.509±0.001d	0.627±0.007b	2.136±0.007c	0.596±0.004d
8.0	0	1.485±0.006e	0.617±0.007b	2.102±0.024d	0.575±0.009e
	2	1.373±0.014f	0.617±0.018b	1.943±0.028e	0.544±0.005f
	8	1.705±0.004c	0.692±0.001a	2.396±0.005b	0.668±0.004b

Significant differences at $p < 0.05$ were showed with different letters in the same line

Discussion

The various physiological parameters of *Festuca arundinacea* have diversities in different pH treatments, but it has less effect under acid treatment than alkali treatment with stronger acid resistance. The application of Si alleviates the harm of plants under pH stress and the effect of Si in *Festuca arundinacea* has much difference under different pH stress.

The result shows under pH3.0 treatment, DW of above ground, the activities of CAT, APX and Chl Content were maximum under the 2mM Si treatment and will be restrained under 8mM Si treatment. The increase of DW of above ground, Chl content, carotenoid content and the decrease of MDA content was extremely significant. Chl and carotenoid are the principal pigment for photosynthesis in green plants. Their content can provide valuable information on physiological state of plants. Analysis of chlorophyll a fluorescence parameters is considered as an important approach for evaluating the health or integrity of the internal apparatus during photosynthetic process within a leaf and provides a rapid and accurate technique for detecting and quantifying the tolerance of plants to stress[25]. The application of Si could increase the antioxidant enzymes activities and decrease MDA content. The POD, CAT and APX, as antioxidant enzymes, could eliminate hydrogen peroxide and reduce the toxicity, while the increase of the reactive oxygen species cause the lipid peroxidation[26]. MDA is a product of the peroxidation of unsaturated fatty acids in phospholipids. The change of MDA content has been widely used as an indicator of free radical damage to cell membranes[27]. The induced decrease of MDA content indicated Si application improved the *Festuca arundinacea* resistance. The POD activities and DW of roots decreased may caused by the too high Si concentration. Si distributed unevenly throughout the organ in different plants, some plants the Si concentration over ground is more than in the roots as rice [28]. But some plants the Si concentration over ground is far less than in the roots as the *Trifolium incarnatum*, the Si concentration in the roots is approximately eight times more than Si over ground. *Festuca arundinacea* belonging to grasses shows Si concentration underground is more than over ground. The Si *Festuca arundinacea* absorbed mostly accumulate in the roots, so the Si concentration over ground is lower and under ground is higher to cause the inhabited impact. It is unable to take up Si actively via the roots, even though the concentration in soil solution is high, and, therefore, fail to accumulate high Si in the tops and to benefit from Si. So, with low Si uptake, foliar application has been attempted [29].

Under pH5.5 treatment, the plant height, DW of roots, DW of stems and leaves, the activities of APX, POD, CAT were all maximums in 2mM Si treatment, but the chl and carotenoid content decreased in 2mM Si treatment. Previous experiences illustrated Si did not have effects in the plants chl under the normal growth condition, barely had protective effect in the plants chl under stress[30].

Under pH8.0 treatment, the plant height, the activities of APX, POD, Chl and carotenoid content increased significantly with increase of the concentration of silicon treatment which were maximum under 8mM Si treatment. The study showed under pH8.0 treatment, *Festuca arundinacea* needed more Si to alleviate the harm by the stress, because the *Festuca arundinacea* suffer more harm under alkali stress possibly. The Chl content was lower in 2 mM Si treatment than in single pH8.0 treatment as former studied, the low Si concentration applied did not improve the Chl content and as the increase of Si concentration, the Chl content increased first then decreased[31]. The lower level of CAT activities in 8mMSi treatment than 2mM Si treatment is in agreement with the results of the previous experiment that showed the activity of SOD, GPX, APX, DHAR and GR in salt-stressed cucumber leaves was increased by Si addition, however, an increase in CAT activity was not observed [32].

The study illustrated that the lower concentration of Si treatment is effective in alleviating the injury of plants under acid and alkali stress. The Si fertilization can be applied in *Festuca arundinacea* according to the environment pH value to enhance its growth.

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