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RESEARCH ARTICLE

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Effects of NaCl stress on two blue fescue varieties (*Festuca glauca*)

Abstract Blue fescue is a widely used ornamental grass worldwide. Research on its salt resistance is propitious to its application in northern China where there are large areas of saline-alkali land. This study was conducted to examine the effects of NaCl stress on two blue fescue varieties Valesiaca and Select. Percentage of green leaves (PGL) of both Valesiaca and Select responded similarly to 0.3% and 0.6% NaCl treatments. Under a higher NaCl application level (1.2% NaCl), Valesiaca was reduced by below 60% at the 12th day and reached 20% at the end of the experiment, but Select to 71.67% at the 12th day and stayed over 50% unto the end. The 0.9% and 1.2% NaCl treatments sharply increased Proline content, and then reached and maintained at a maximum level throughout the treatment. Proline content of Valesiaca increased to 2.28 $mmol \cdot L^{-1}$ at the 8th day after the 1.2% NaCl treatment, but only 0.95 $mmol \cdot L^{-1}$ in the treatment of 0.3% at the same day. For Valesiaca, 1.2% NaCl treatment significantly increased its SOD activity to 30.69 $units \cdot mg^{-1}$, nearly 50% higher than 0.3% treatment at the 8th day after treatment, and over 53% higher at the 27th day. POD activity of Valesiaca increased to 6.92 $units \cdot mg^{-1}$ at the 8th day after the 1.2% NaCl treatment, but it was 2.59 $units \cdot mg^{-1}$ for 0.3% stress treatment. In conclusion, similar changes of determined index were observed for both cultivars under low NaCl stress levels (0.3%–0.6%). However, Valesiaca was more sensitive and less tolerant to high level NaCl stress (0.9%–1.2%) than Select, indicating that Valesiaca was less resistant to NaCl compared with Select.

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1 Introduction

Blue fescue (*Festuca glauca*) is a cool season, perennial, and traditionally used ornamental grass. Its silvery-blue foliage forms a dome-shaped, porcupine-like tuft of erect to arching, with dense needle-like blades radiating upward and outward (Whipker et al., 1999). Moreover, its soft texture makes itself perfect for park ornament, city greening, home decoration, and greening of golf courses, roadsides, commercial sites, walkways or other public places. Blue fescue first came to the eyes of gardeners in the 1960s in Britain and soon it was applied in many gardens around the world (Ottesen, 1989). Presently, blue fescue is widely used abroad, especially in the United States, Australia, Canada, New Zealand and European countries. The Beijing Academy of Agriculture and Forestry Sciences first introduced the blue fescue to China in the 1990s (Liu, 2007), which is still in a small-scale application stage, but has shown a favorable development prospect.

The northern part of China has large areas of saline-alkali land, where the popularization and application of blue fescue is becoming ever increasingly problematic. Blue fescue is well adapted to stress situations, with the blue/green color developing in high stress periods. The waxy leaf covering can reduce transpiration during the stress period so that the plant can survive and retain the blue-green color. Many studies on examining the salt resistance of *Festuca* species show that the tall fescue can survive 0.5% salt stress (Li and Xie, 2007), and *Festuc arundinacea* S., *Pucclnellla diatans* L., *Festuca rubra* L., *Pucclnellla diatans* L. have the strongest salt resistance, followed by *Festuc arundinacea* S. and *Festuca rubra* L. that can even bear a 2.0% salt stress level (Stoutemyer and Smith, 1936; Dudeck et al., 1983; Torelo and Rice, 1984). However, fewer studies reveal the local adaptation research of blue fescue as well as the information about its salt resistance. In recent years, with the continuous development of breeding technology, new varieties of blue fescue have been developed consecutively. Because there exist significant differences between different varieties and simultaneously it is necessary to study the adaptability of different varieties, our research aimed at the salt resistance of a new blue fescue variety and a mature old variety by currently (i) investigating the visual quality and physiological changes of blue fescue under different NaCl treatments and (ii) investigating and comparing the NaCl resistances of the two blue fescues under different NaCl treatments.

2 Materials and methods

As experimental materials, Valesiaca (*F. valesiaca* Glaucantha) is a new variety from the United States and Select (*F. glauca* Select) is a well used variety in Baoding, Hebei Province. Two-year-old blue fescue stands were transplanted to the mixture (8∶2, v/v) of sand and soil in pots of 20 cm in diameter and 15 cm in height. Plastic trays were put on the bottoms of the pots for keeping NaCl content (Yang et al., 2008). The experiment was conducted from September 8 to October 9 in 2008 at the Field Research Center of Agricultural University of Hebei, Baoding, Hebei Province, China.

NaCl treatments were arranged in a completely randomized design with three replicates in repeated measurements. Random sampling was done at each time as described by Xu and Huang (2004). When growing well and conformably, plants were treated with 0% (control), 0.3%, 0.6%, 0.9% and 1.2% of NaCl respectively at the 1st, 3rd, 8th, 12th, 17th, 22nd day after initiating the experiment. For the assay of Proline (Pro), superoxide dismutase (SOD), and peroxidase (POD), leaf samples were collected from each pot at the 3rd, 8th, 12th, 17th, 22nd, and 27th day after the treatments, frozen immediately in liquid nitrogen and stored at $-70°C$ until use.

Percentage of green leaves (PGL) is the plant green leaf percentage account in all leaves and determined using a visual estimate method.

Pro content was measured according to the method of Bates et al. (1973). 0.5 g sample of fresh leaves was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman #2 paper. Then, 2 mL filtrate was mixed with 2 mL acid-ninhydrin and 2 mL glacial acetic and heated at 100°C for 1 h. The reaction was terminated in an ice bath, and then 4 mL toluene was added to the mixture, stirred for 15–20 s. The chromophore was aspirated from the aqueous phase, and the absorbance was read at 520 nm.

The activity of SOD was determined using the photochemical method described by Giannopolitis and Ries (1977). About 0.5 g fresh sample (<2 mm) was homogenized in an ice bath in 5 mL 100 $mmol \cdot L^{-1}$ phosphate buffer (pH 7.8) containing 0.1 $mmol \cdot L^{-1}$ EDTA. The homogenate was centrifuged at 10000 $r \cdot min^{-1}$ for 20 min at 4°C. One unit SOD activity was defined as the amount of enzyme required for 50% inhibition of the rate of NBT (Nitrotetrazolium Blue chloride) reduction measured at 560 nm.

Activities of POD were measured following the method of Xu and Ye (1989). For this, 0.5 g fresh sample (<2 mm) was homogenized in an ice bath in 5 mL 100 $mmol \cdot L^{-1}$ phosphate buffer (pH 7.8). The homogenate was centrifuged at 10000 $r \cdot min^{-1}$ for 20 min at 4°C. The supernatant was used as enzyme extract. 0.1 mL of the enzyme extract was added to the substrate mixture containing 2.91 mL phosphate buffer (10 $mmol \cdot L^{-1}$, pH 7.0), 0.05 mL guaiacol (20 $mmol \cdot L^{-1}$) and 0.05 mL H_2O_2 (40 $mmol \cdot L^{-1}$). The absorbance change of brown guaiacol (1-hydroxy-2-methoxybenzene) at 460 nm was recorded for calculating POD activity.

3 Results

3.1 Percentage of green leaves (PGL)

Our results showed that the PGL of both blue fescue cultivars decreased under NaCl stress treatment for 3 days throughout the stress period compared with that of the CK treatment. The results from Fig. 1 illustrated that the higher NaCl concentration was applied, the lower green leaf percentage was shown and the worse visual quality was presented, which indicated that the high salinity of soil was supposed to affect the visual quality of blue fescue.

The green leaf percentage of both cultivars could be kept over 80% under 0.3% NaCl treatment and over 70% under 0.6% NaCl treatment, which indicated that blue fescue could appear well and show an acceptable quality under 0.6% NaCl treatment.

Both Valesiaca and Select responded similarly to 0.3% and 0.6% NaCl treatment, with higher PGL compared to higher NaCl level (1.2% NaCl) in this experiment. The PGL of Valesiaca was reduced by below 60% 12 days after treatment and reached 20% at the end of the experiment. However, the PGL of Select reached 71.67% 12 days after treatment, staying over 50% unto the end.

3.2 Proline (Pro) content

The NaCl stress resulted in an increase of Pro content in *F. valesiaca* Glaucantha and *F. glauca* Select compared to that in the control plant (Fig. 2). The 0.9% and 1.2% NaCl treatment sharply increased the Pro content, which then reached and stayed at a maximum level throughout the treatment. The Pro content in Valesiaca increased to 2.28 $mmol \cdot L^{-1}$ in 8 days in the 1.2% NaCl treatment, but only reached 0.95 $mmol \cdot L^{-1}$ in the 0.3% treatment in the same time period. Valesiaca got the maximum level of Pro

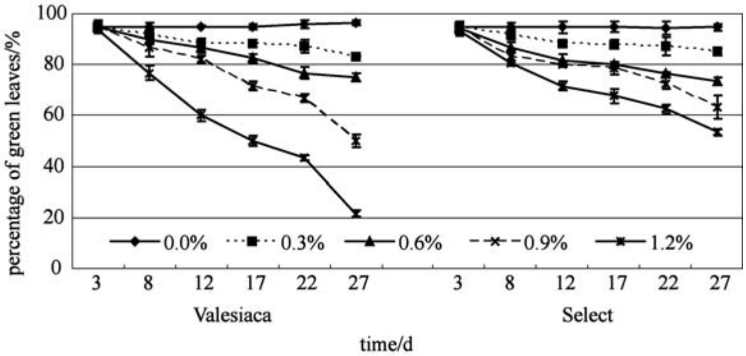

Fig. 1 The effects of NaCl stress on the PGL

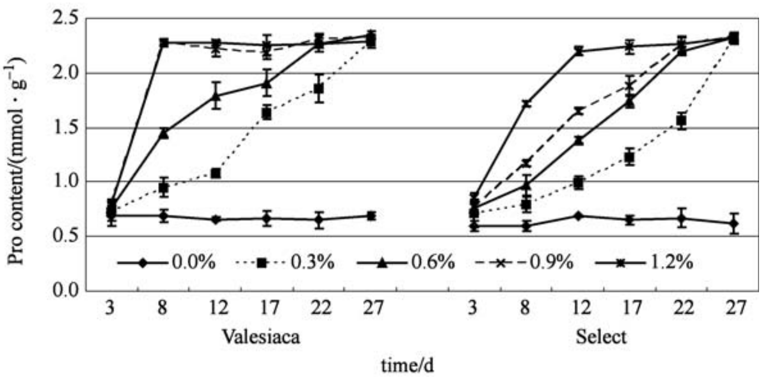

Fig. 2 The effects of NaCl stress on Pro content

content at the 8th day in both 0.9% and 1.2% NaCl treatments but Select did on the 12th day in the 1.2% NaCl treatment and the 22nd day in the 0.9% NaCl treatment. This revealed higher NaCl stress levels could cause Pro accumulation in Valesiaca much faster than that in Select. Therefore, Valesiaca was more sensitive than Select.

3.3 Superoxide dismutase (SOD) activity

SOD activity was increased by NaCl treatment, with a peak occurring at the 8th day after the treatment, and thereafter it dropped to the initial value until the 22nd day when it began to increase again (Fig. 3). Valesiaca, was significantly increased by 1.2% NaCl treatment in SOD activity to 30.69 units·mg^{-1} 8 days after treatment, nearly 50% higher than that in 0.3% NaCl treatment, with over 53% higher 27 days after treatment. Between the two varieties, the SOD activity of Valesiaca declined by 60% at the 12th day lower than that at the 8th day while Select only declined by 38% and reached a higher SOD activity under each NaCl stress level at the end of the trial.

3.4 Peroxidase (POD) activities

POD activity increased gradually in response to blue fescue's NaCl stress before reaching the POD peak. In this experiment Valesiaca at the 17th day and Select at the 22nd reached the maximum POD activity under all NaCl stresses compared with the CK, and thereafter, POD began to decline under the durative NaCl stress (Fig. 4). POD activity in Valesiaca increased to 6.92 units·mg^{-1} only 8 days after the 1.2% NaCl treatment, but under the 0.3% NaCl stress it increased to 2.59 units·mg^{-1}. Significant NaCl stress effects on the two varieties were observed 8 days after the treatment. Valesiaca had a significantly higher POD activity than Select under 0.9 and 1.2% NaCl treatments but the differences were not significant under 0.3% and 0.6% stress levels ($P < 0.05$). In addition, the average POD activity of Select at the end of the experiment under each NaCl stress was 17% higher than that in Valesiaca.

4 Discussion and conclusions

Our results demonstrated that Select and Valesiaca had better resistance to NaCl stress. Select was more tolerant to high NaCl concentration compared with Valesiaca.

NaCl treatment showed different effects on PGL, Pro content and POD in the two varieties of blue fescue. During the first 3 days in salt treatment, both Valesiaca and Select presented normal growth, and thereafter the

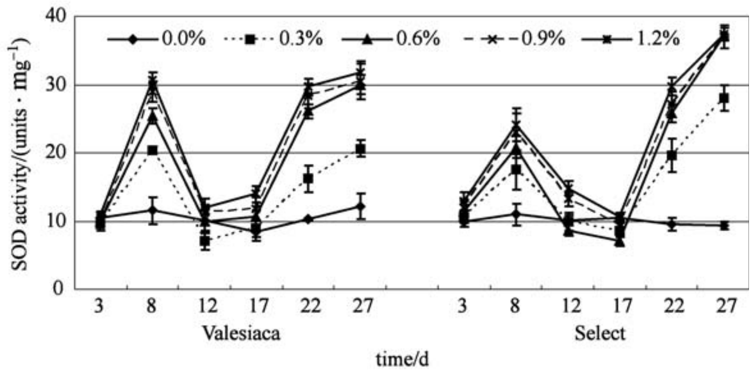

Fig. 3 The effects of NaCl stress on the SOD activity

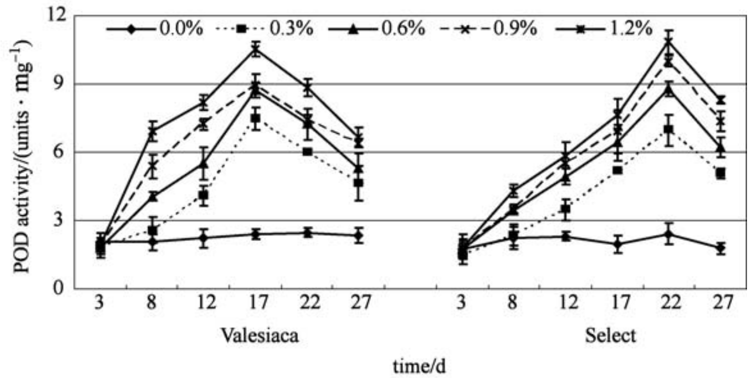

Fig. 4 The effects of NaCl stress on the POD activity

reduction in PGL occurred first in high NaCl concentration treated plants and then in the lower ones. The reason was that their roots had difficulty absorbing water because of external osmotic changes caused by NaCl stress, which then led to damage to leaves, accelerated leaf senescence and resulted in PGL reduction (Babourina et al., 2000; Ahmed et al., 2008). Valesiaca was more affected by salt than Select, which proved its lower adjustability to NaCl and lower salt resistance.

Pro, as one of the most important osmotic agents, exists widely but little in plants, badly affected by a stress of adversity (Huang et al., 1998; Li et al., 2008). The Pro content in both cultivars increased steadily to the maximum level sooner or later, which indicated that a long-term NaCl stress would make Pro accumulate and stay at a high level finally. In this experiment Valesiaca accumulated its Pro more rapidly than Select. The sensitive response of Pro content in Valesiaca to NaCl stress revealed that its primary osmotic concentration was lower than that in Select and hence it was harder to absorb water under NaCl stress.

Normally plants can develop the antioxidant defense system to scavenge active oxygen species and protect cells against stress injury, and SOD is one of the most effective antioxidant enzymes (Zhang et al., 2008). Changes in the level of antioxidants may be indicative of the levels of oxidative stress and stress resistance (Lin et al., 2008). SOD is responsible for scavenging free radicals and reactive oxygen species to produce H_2O_2 and O_2 (Jiang and Huang, 2001; Liang et al., 2001). Changes of SOD activities in our experiment indicated that the dismutation ability of SOD was able to increase transiently at NaCl stress prophase and later the plants momentarily were adapted to the stress and the SOD scavenging ability was bigger than the production of active oxygen species. As a result the SOD activity dropped to the initial value and reached a balance. However, the increases of SOD activity at the end indicated a long-term stress would cause more accumulation of active oxygen species for scavenging.

The level of POD has been used as an indicator of free radical damage to cell membranes under stress conditions (Liang et al., 2003). The POD constitutes a set of enzymes that catalyze the oxidation of substrates by H_2O_2 (Abbasi and Kushad, 2006). The POD activity may increase with NaCl stress in both blue fescue cultivars until the peak appears and thereafter may decrease, which indicates that the POD activity cannot always increase under long-term stress. Such an increase has been found in other species under salt stress (Tang et al., 2008). The increase in POD activity suggests an accelerated production of activated oxygen species in tissues. The higher POD activity in Select at the end than that in Valesiaca showed a better ability to curtail lipid peroxidation.

In summary, our results suggest that the decline of PGL and changes of physiological index in the two fescue cultivars with increasing NaCl stress level were obviously associated with NaCl stress intensities. Select was more tolerant to a high NaCl stress level than Valesiaca but under low NaCl stress the two behaved similarly. Therefore, the selection of blue fescue on the basis of these results may help to develop NaCl-tolerant blue fescue cultivars.

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