J. Agr. Sci. Tech. (2019) Vol. 21(5): 1235-1248

Nitric Oxide Effect on Mowing Tolerance of Tall Fescue (*Festuca Arundinacea* Schreb.) Roots via Antioxidant Defense and Plant Hormones

X. H. Zhang[1], R. Chai[1], Y. F. Ma[1], W. W. Meng[1], Y. W. Qi[1], X. T. Chu[2], and Y. F. Xu[1*]

ABSTRACT

Mowing is important in maintaining quality of the turf. The objective of this study was to determine the effect of Nitric Oxide (NO) on tall fescue after mowing, and provide theoretical basis for molecular breeding of turf grass. In our study, exogenous NO significantly increased the relative growth rate compared to the control. The higher relative growth rate was associated with higher activity of Peroxides (POD), SuperOxide Dismutase (SOD), Catalase (CAT) and Ascorbate Peroxidase (APX). Expression of *CuZn-SOD*, *CAT-A*, and *APX4* increased in roots treated with NO after mowing. In addition, exogenous NO significantly increased the concentration of Indole-3-Acetic Acid (IAA), Gibberellic Acid (GA), Cytokinin (CK) and Abscisic Acid (ABA) compared to the control, which likely linked to an increase in Nitric Oxide Synthase (NOS)-like activity and endogenous NO release in tall fescue roots. These results suggested that mowing increased NOS-like activity, leading to elevated endogenous NO levels. NO might act as a signaling molecule, increasing plant hormone content, as well as up-regulating gene expression and enhancing the activity of antioxidant enzymes, thereby protecting against injuries caused by mowing and promoting re-growth of tall fescue.

Keywords: Antioxidant enzymes, Molecular breeding, Nitric oxide synthase, Turf grass.

INTRODUCTION

Turf is an important part of urban horticulture landscape. To maintain the quality of turf, maintenance of turf through irrigation, fertilizing, and mowing is necessary. Mowing influences the metabolism and growth of the turf, which can produce both positive and negative influences on turfgrass (Emmons, 2008). On the one hand, mowing is a mechanical stress to turfgrass that can result in the accumulation of Reactive Oxygen Species (ROS), such as Hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet -}$), Hydroxyl radicals (·OH), and singlet Oxygen ($^1O_2$), which causes oxidative damage to cell membrane (Apel and Hirt, 2004). On the other hand, correct mowing can improve the quality of the turf (Fu *et al.*, 2005), promote rapid growth with higher relative growth rate compared to before mowing (Wang *et al.*, 2018), and enhance its tolerance to other stresses, such as drought (Elansary and Yessoufou, 2015) and salinity stress (Shahba, 2010). Therefore, it is important to develop optimal strategies to lessen negative effect and enhance positive influences of mowing.

Plant has a complicated signal network to respond to environmental change and regulate growth and development (Sanz *et al.*, 2015). Traditional plant hormones, such

[1] College of Grassland Agriculture, Northwest A&F University, Yangling, Shaanxi province, 712100, P. R. China.
[2] Centre for Integrative Legume Research (CILR), University of Queensland, St. Lucia, Brisbane, QLD, 4072, Australia.
[*]Corresponding author; e-mail: xuyuefei@nwsuaf.edu.cn

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

as Indole-3-Acetic Acid (IAA), Gibberellic Acid (GA), Cytokinin (CK), and Abscisic Acid (ABA), are known to play critical roles in regulating plant physiological processes (Bari and Jones, 2009). In addition, NO, as an inter- and intracellular Reactive Nitrogen Species (RNS), is also an important gaseous signaling molecule involved in various physiological processes in plants, such as growth, development, and other regulating processes (Egbichi *et al.*, 2013). Moreover, NO mediates the transcription of genes encoding antioxidant enzymes and activates antioxidant enzymes (Delledonne *et al.*, 1998; Fu *et al.*, 2015) and has a complex interaction with plant hormones (Sanz *et al.*, 2015), involved in plant responses to a wide range of biotic and abiotic stresses. Despite available reports on the effects of NO on improving abiotic stress tolerance, the mechanisms of antioxidant defense and plant hormones induced by NO under mowing have not been clearly reported.

Tall fescue (*Festuca arundinacea* Schreb.) is one of the widely used cold season turf grasses. Tall fescue has good drought and heat tolerance and it is suitable for a wide range of soils and climatic conditions (Yang *et al.*, 2014). In this study, we hypothesized that NO could alleviate oxidative damage and promot the growth of tall fescue after mowing via antioxidant enzymes and plant hormones metabolism. Understanding how oxidative defense and plant hormones are involved in root responses to mowing would help developing better turf management strategies and breeding new varieties of tall fescue.

MATERIALS AND METHODS

Plant Material and Treatments

Seeds of tall fescue (*Festuca arundinacea* Schreb. cv. Arid3) were obtained from Beijing Clover Seed & Turf Co. Ltd., Beijing, P. R. China. Seeds were surface sterilized in 0.1% (w/v) sodium hypochlorite for 1 min, rinsed several times in distilled water, and germinated on moistened filter paper for 7 days at room temperature. From each pot, three seedlings with uniform height were selected and transferred into clear plastic pots (9 cm diameter×15 cm height) containing moistened silica sand. All seedlings were cultured by irrigating with Hoagland nutrient solution once every 3 days. All plants were incubated in an incubator with a day/night temperature of 25/15°C, relative humidity of 80%, day/night regime of 14/10 hours, and photosynthetic photon flux density at the plants' top of 200 μmol m^{-2} s^{-1} using fluorescent lamps.

Mowing and Sodium NitroPrusside (SNP, NO donor; Sigma, USA) were carried out after 28 days of pre-culture. Firstly, the mowing height was 1, 3, 5, and 7 cm to detetmine the correct mowing height of tall fescue. The plants were hand-clipped once. Secondly, the seedlings were grouped into 4 sets subjected to different treatments: (1) Treatment with distilled water (Control, CK); (2) Treatment with 0.1 mM SNP (CK+); (3) Treatment with correct mowing height (5); and (4) Treatment with correct mowing height and 0.1 mM SNP (5+). A 0.1 mM SNP was applied to tall fescue seedlings through the roots incubated with nutrient solution.

Relative Growth Rate and Root Morphology Index

After 7 days of treatment, 10 healthy plants were randomly chosen from each treatment group for determination of plant Height (H_1). The relative growth rate was calculated as $(H_1-H_0)/7$, where H_0 represents the mowing Height. In another 5 healthy plants randomly chosen from each group, roots were cut and dried at 80°C for 24 hours to determine the dry weight. Root surface area, root volume, root diameter, and the number of root tips were determined using a root automatic scan apparatus (*Perfection V700 Photo*, Epson, Japan), equipped with *WinRHIZO* (Regent Instruments, Canada).

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

Root Viability Assay

Measurements of root viability were performed by the method of Islam *et al.* (2007) with some modifications. The absorbance of the supernatant was read at 485 nm.

Ion Leakage and Lipid Peroxidation

Ion leakage was determined as described by Song *et al.* (2008). Fresh roots (0.5 g) washed in deionized water were placed in Petri dishes with 5 mL deionized water at 25°C for 2 hours. After the incubation, the Electrical Conductivity was measured (EC_1). After that, the samples were boiled for 20 minutes and the Conductivity was read again (EC_2). The ion leakage was calculated as EC_1/EC_2 and expressed as a percent. Membrane lipid peroxidation was described by the content of MalonDiAldehyde (MDA) and determined as described by Buege and Aust (1978). The absorbance of the supernatant was measured at 450, 532, and 600 nm.

Measurement of H_2O_2 and Superoxide Radical

H_2O_2 content was measured according to the method described by Veljovic-Jovanovic *et al.* (2002). The absorbance of the solution was measured at 410 nm. $O_2^{\bullet -}$ production was measured by the modified method according to Elstner and Heupel (1976). The specific absorption at 530 nm was determined.

Quantification of Antioxidant Enzymatic Activity

Peroxidase (POD, EC 1.11.1.7) activity was assayed following the method of Upadhyaya *et al.* (1985). Total SuperOxide Dismutase (SOD, EC 1.15.1.1) activity was measured using the Nitro Blue Tetrazolium (NBT) method described by Beauchamp and Fridovich (1971). Catalase (CAT, EC 1.11.1.6) activity was measured following the consumption of H_2O_2 at 240 nm as described by Aebi (1984). The assay for Ascorbate Peroxidase (APX, EC 1.11.1.11) activity was performed according to Nakano and Asada, (1981). Protein content was determined using the method described by Bradford (1976) with BSA as standard.

Gene Expression Analysis

Expression analysis of genes codifying for antioxidant enzyme was performed by quantitative Eeverse Transcriptase Polymerase Chain Reaction (qRT-PCR). Gene name, accession number, forward and reverse primer sequences are provided in Table 1 (Xu *et al.*, 2015). The $2^{-\Delta\Delta C_T}$ method was used to calculate the relative expression level between genes of interest and reference gene, respectively.

Determination of Plant Hormones Concentrations

Measurements of the concentrations of IAA, GA, CK, and ABA were performed according to Hedden (1993). The Elisa kit (Shanghai Huyu Biotechnology Co. Ltd., Shanghai, P. R. China) was used to measure plant hormone level, using purified anti-plant hormone antibodies to coat microtiter plate wells to create solid-phase antibodies. We terminated the reaction by adding sulphuric acid solution and measured the absorbance at 450 nm.

NO Production and NOS-Like Activity

NO production determination was performed according to Murphy and Noack (1994) with some modifications. The absorbance was measured at 577 and 591 nm. The NOS-like activity was measured using a NOS colorimetric assay kit (Nanjing

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

Table 1. Primer sequences used in qRT-PCR.

Gene	Accession no.	Forward primer (5′–3′)	Reverse primer (5′–3′)
Actin	AY194227.1	*TCTTACCGAGAGAGGTTACTCC*	*CCAGCTCCTGTTCATAGTCAAG*
APX2	DT702685.1	*TTTGAGCGACCAGGACATTG*	*GGCTCCCTCAAAGCCAGATC*
APX4	DT714958.1	*TGGTTTTGAAGGTGCATGGA*	*CCCCTCAGATTCTCCCTTCAG*
POD	GT036635.1	*CACATGCCCACAAGCTGATG*	*CAGAAGCGAAGCGGCAAT*
CAT-A	DT680104.1	*CTGCTGGGCAACAACTTC*	*GACTTTGGGTTGGGCTTG*
CAT-B	DT704412.1	*TCCTACGCTGATACCCAAAG*	*GTGATGGTTGTTGTGGTGAG*
CAT-C	AJ634002.1	*GACCCACATCCAGGAGAAC*	*GTCGAAGAGGAAGGTGAACA*
CuZn-SOD	DT712833.1	*TATCCCCCTTACTGGACCACAT*	*GTGTCCACCCTTGCCAAGAT*
Mn-SOD	DT694762.1	*GGGCGCCATCAAGTTCAA*	*ACCCCCACCCTCATTAGCA*

Jiancheng Bioengineering Institute, Jiangsu, China). The absorbance was measured at 530 nm (Diao *et al.*, 2017).

Statistical Analysis

In our study, a completely randomized design with three replicates was used. Values were expressed as means±*SD*. Statistical analyses were performed by analysis of one-way ANOVA using SPSS 22 statistical software (SPSS Inc., Chicago, IL, USA). Means were separated using Duncan's multiple range tests at the 5% level of significance.

RESULTS

Relative Growth Rate

The experiment with mowing height at 1, 3, 5, and 7 cm was performed to determine the point where mowing showed the most significant on growth rate. Our results showed that mowing heights in a range from 1 to 7 cm enhanced the relative growth rate, with 5 cm being the most effective mowing height, enhancing the growth rate by 81.7% compared to the control plants (Figure 1-A), and having the highest root viability of tall fescue after mowing (Figure 1-B), while 5 and 7 cm had a minor influence on cell membrane (Figure 1-C). Therefore, we used 5 cm mowing height in all following experiments. Application of exogenous NO significantly increased the relative growth rate as well (Figure 1-D).

Root Morphology

Five cm mowing significantly reduced root dry weight by 35.2% compared to the control plants. Mowing also significantly reduced root length, diameter, and volume. Application of exogenous NO increased plant growth and enhanced root dry weight. Pretreatment with exogenous NO alleviated the deleterious effects of mowing. Mowing had no effects on root tips. However, treatments with exogenous NO significantly increased root tips (Table 2).

Oxidative Damage

The phenotype of tall fescue plants treated with or without NO and mowing are shown in Figure 2. Mowing increased ion leakage in tall fescue roots by 27.9% when compared to the control plants. However, exogenous NO significantly decreased electrolyte leakage in roots (Figure 3-A). Mowing significantly increased MDA content in tall fescue. In contrast, pretreatment of roots with exogenous NO remarkably reduced MDA contents (Figure 3-B). Mowing also caused significant accumulations of H_2O_2 and superoxide radicals in roots. Treatment with exogenous NO also effectively reduced the accumulation of H_2O_2 and superoxide radical (Figures 3-C and -D).

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

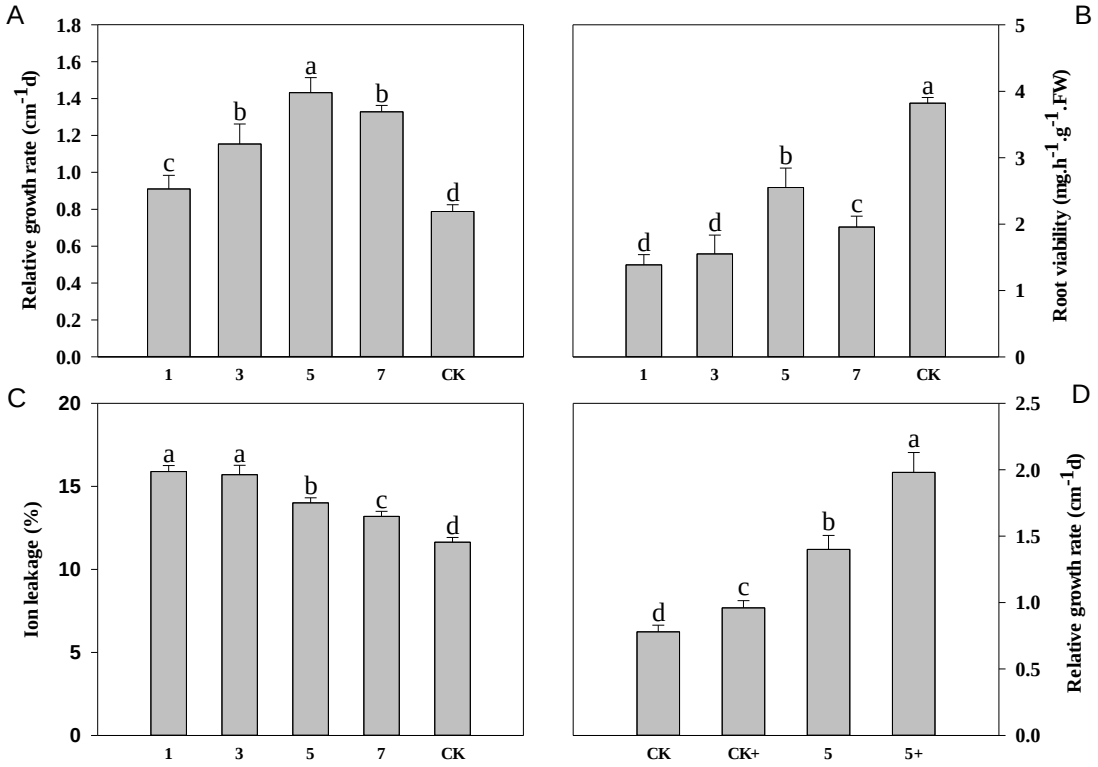

Figure 1. Changes of relative growth rate (A), root viability (B), and ion leakage (C) under different mowing heights and effect of exogenous NO application on relative growth rate combined with mowing (D). CK: Treatment with distilled water; CK+: Treatment with 0.1 mM SNP; 1: Treatment with 1 cm mowing height; 3: Treatment with 3 cm mowing height); 5: Treatment with 5 cm mowing height; 7: Treatment with 7 cm mowing height, and 5+: Treatment with 5 cm mowing height with 0.1 mM SNP application). Each value represents the mean±*SD* (n=5). Bars with different letters are significantly different at the 5% level.

Table 2. Effects of exogenous NO on root dry weight (mg), length (cm), surface area (cm^2), diameter (mm), volume (mm^3), and number of tips after mowing.[a]

Treatment	Dry weight	Length	Surface area	Diameter	Volume	Tips
CK	18.41±0.75[b]	60.29±3.51[c]	3.33±0.27[b]	0.28±0.02[b]	39.60±1.32[c]	297.33±25.54[b]
CK+	19.43±0.35[a]	72.32±1.51[a]	4.05±0.06[a]	0.34±0.02[a]	59.43±1.46[a]	849.00±57.04[a]
5	13.57±0.29[d]	43.56±3.44[d]	3.23±0.20[b]	0.21±0.01[c]	34.03±1.27[d]	304.00±14.00[b]
5+	17.43±0.35[c]	67.15±1.09[b]	2.51±0.06[c]	0.29±0.02[b]	53.20±1.25[b]	812.00±33.05[a]

[a] Each value represents mean±*SD* (n= 3). Different letters represent significant differences at the 5% level. CK, CK+, 5, and 5+ are defined previously.

Antioxidant Enzyme Activities and Transcript Levels

To evaluate whether NO and mowing affected the antioxidant enzymes, we examined their activity and expression. Mowing enhanced the activity of POD, SOD, CAT, and APX in roots by 38.7, 52.5, 99.8, and 91.8%, respectively, compared to the control plants (Figure 4). Pretreatment with exogenous NO remarkably increased POD, SOD, CAT, and APX activities compared to mowing alone.

We measured the expression of antioxidant enzymes by qRT-PCR (Figure 5). *APX2 and APX4* expressions in roots

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

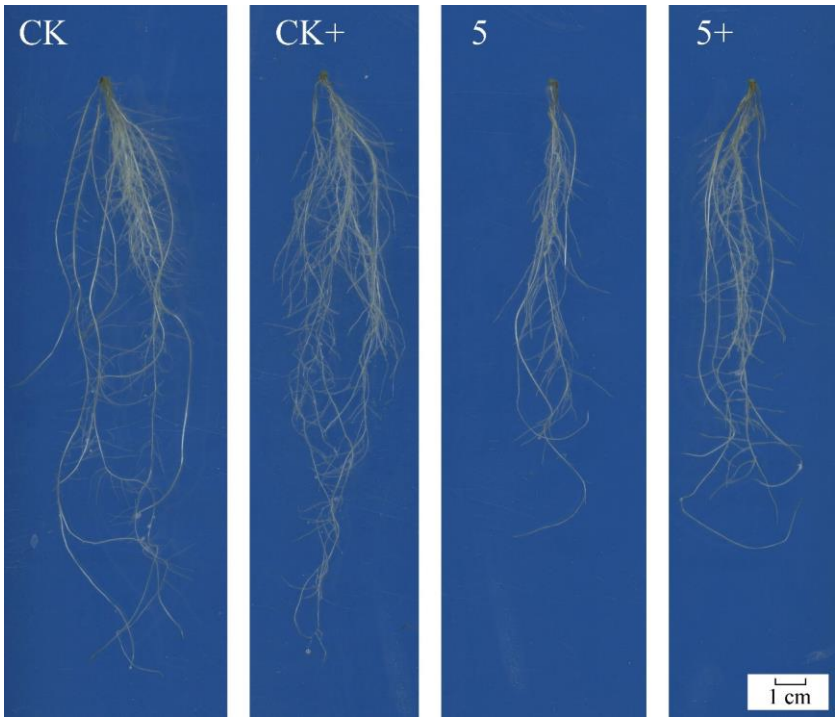

Figure 2. The phenotype of tall fescue plants treated with or without nitric oxide under control and mowing. CK, CK+, 5, and 5+ are treatments and defined previously.

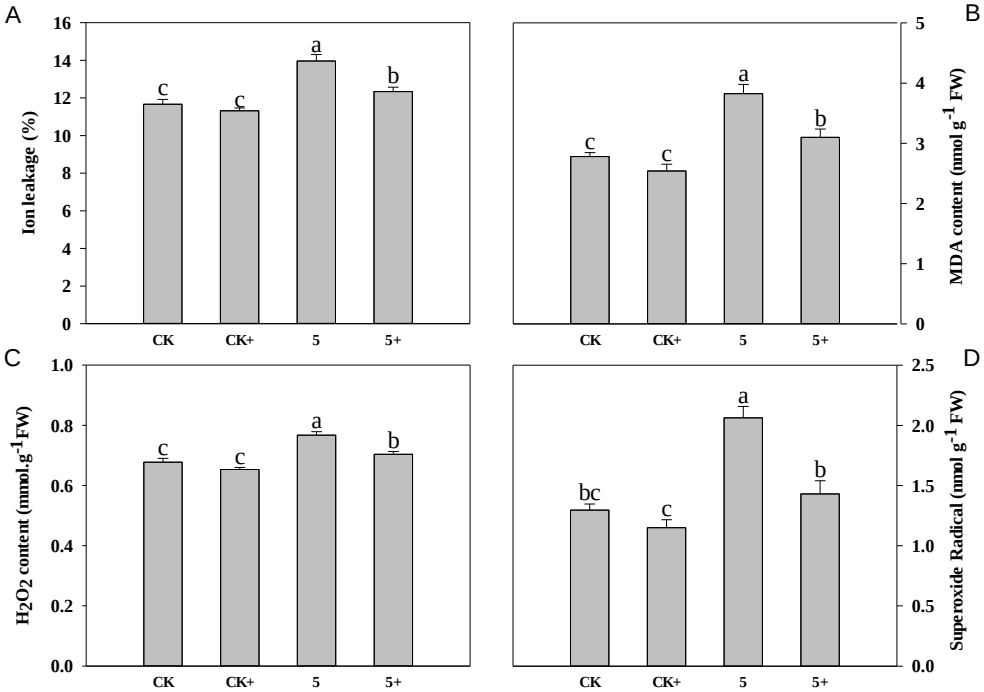

Figure 3. Effects of exogenous NO application on oxidative damage after mowing on ion leakage (A), MDA (B), H_2O_2 (C), and superoxide radical (D). CK, CK+, 5, and 5+ are treatments and defined previously. Each value represents the mean±*SD* (n=3). Bars with different letters are significantly different at the 5% level.

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

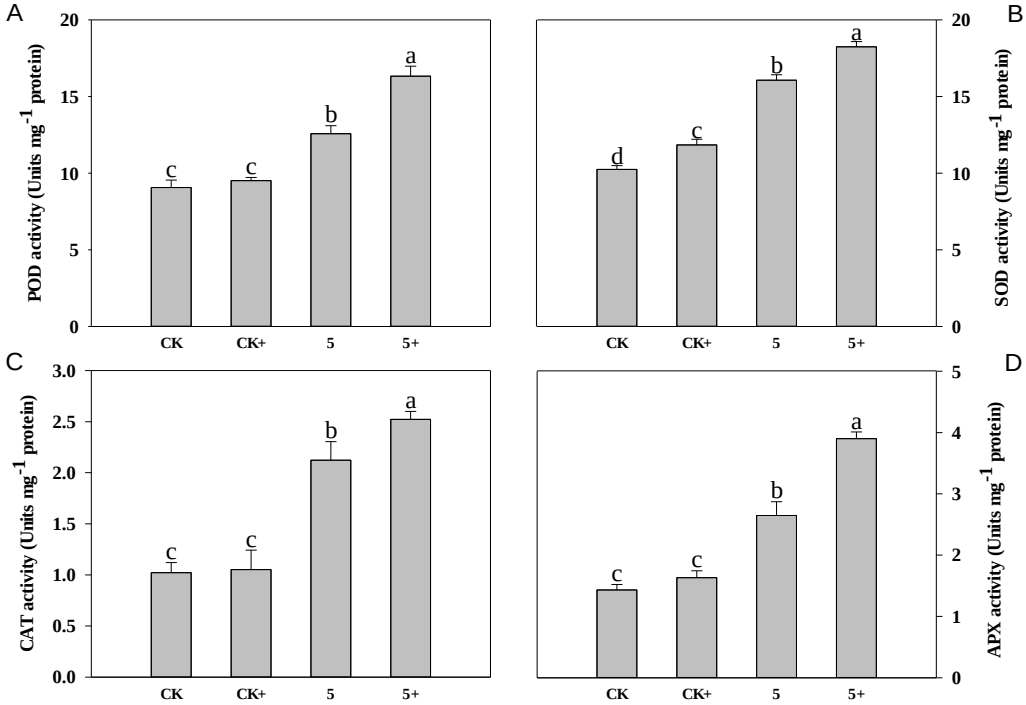

Figure 4. Effects of exogenous NO application on POD (A), SOD (B), CAT (C), and APX (D) enzyme activity after mowing. CK, CK+, 5, and 5+ are treatments and defined previously. Each value represents the mean±SD (n= 3). Bars with different letters were significantly different at the 5% level.

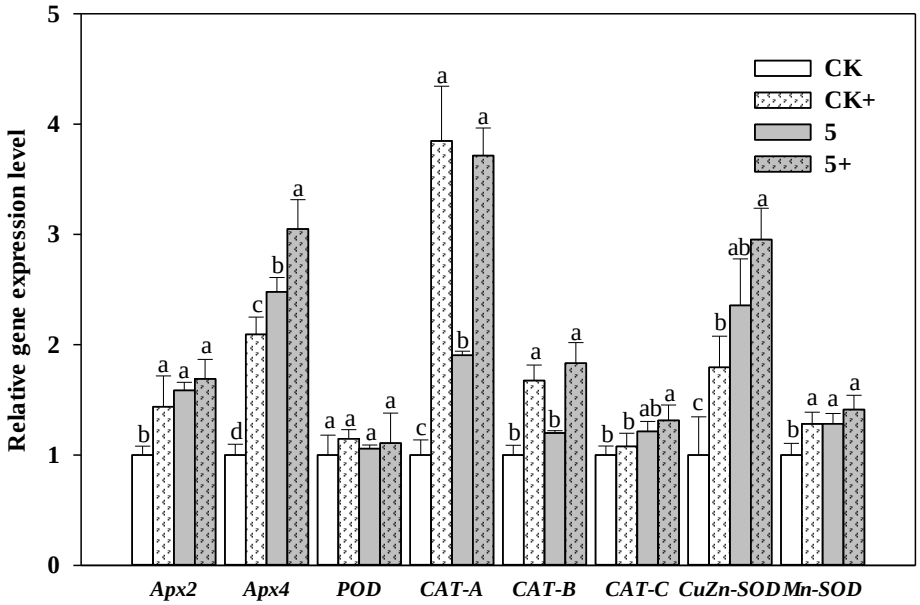

Figure 5. Effects of exogenous NO application on the expression of *POD*, *CuZn-SOD*, *Mn-SOD*, *CAT-A*, *CAT-B*, *CAT-C*, *Apx2*, and *Apx4* after mowing. CK, CK+, 5, and 5+ are treatments and defined previously. Each value represents the mean±*SD* (n=3). Bars with different letters are significantly different at the 5% level.

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

were significantly higher in mowed plants when compared to the control plants. Mowing and NO treatments were more effective in inducing *APX4* expression than that of *APX2* and had no cumulative effect on *APX2* expression. *POD* expression was not affected by either mowing or exogenous NO. In contrast, *CAT-A* transcription in roots significantly increased after NO treatment and mowing. The transcription levels of *CAT-B* in roots were also 67.5% higher after NO treatment when compared to the control plants. Similarly, the transcript levels of *CAT-C* in roots were 21.4% higher after NO treatment when compared with the control plants. Expression of *Mn-SOD* and *CuZn-SOD* was significantly higher after NO treatment and mowing, respectively.

Plant Hormones Concentrations and Ratio

To elucidate whether NO and mowing influenced the levels of plant hormones, we measured the concentrations of CK, IAA, GA, and ABA in roots (Figure 6), finding 169.9, 107.6, 162.1, and 104.3% increase after mowing, respectively. Treatment with exogenous NO remarkably increased the contents of CK, IAA, GA, and ABA compared to mowing that resulted in 8.0, 12.5, 12.7, and 10.0% increment, respectively.

NO Release and NOS-Like Activity

After mowing, NO release increased significantly in tall fescue (Figure 7-A). NO release was significantly affected by mowing, with a 136.1% increase compared to the control plants. Application of exogenous NO also increased NO release by 46.8% compared to mowed plants. Mowing also significantly increased Nitric Oxide Synthase (NOS)-like activity in tall fescue (Figure 7-B). However, exogenous NO treatment decreased NOS-like activity compared to mowing.

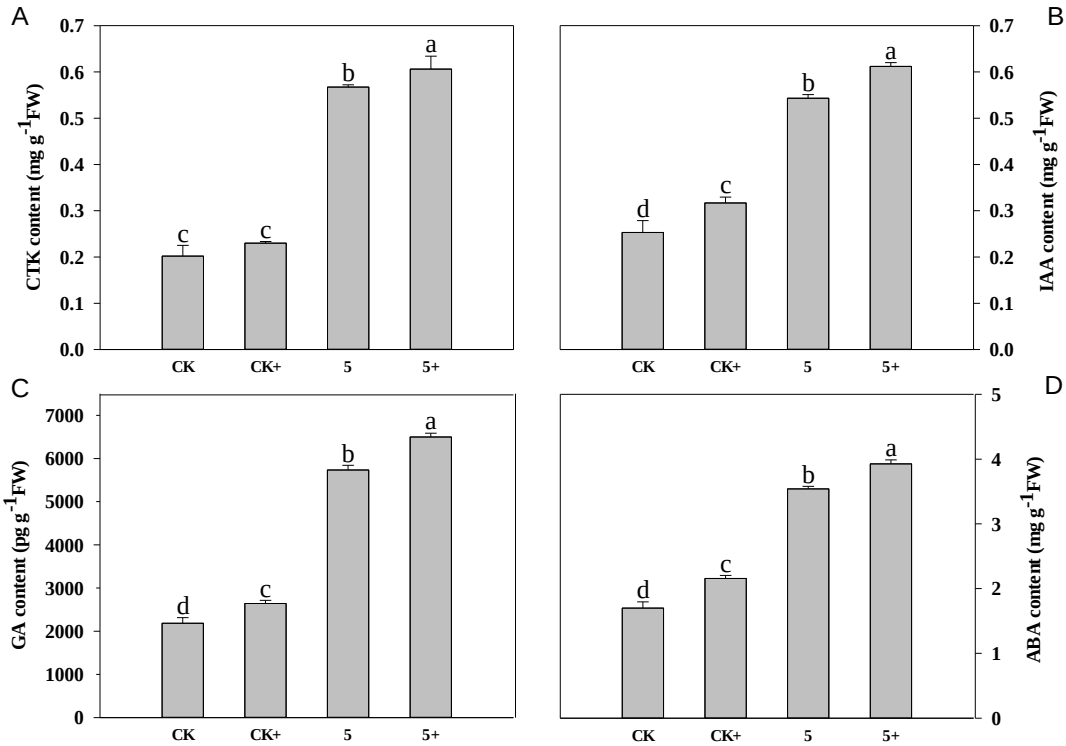

Figure 6. Effects of exogenous NO application on the levels of CK (A), IAA (B), GA(C), and ABA (D) after mowing. CK, CK+, 5, and 5+ are treatments and defined previously. Each value represents the mean±*SD* (n= 3). Bars with different letters are significantly different at the 5% level.

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

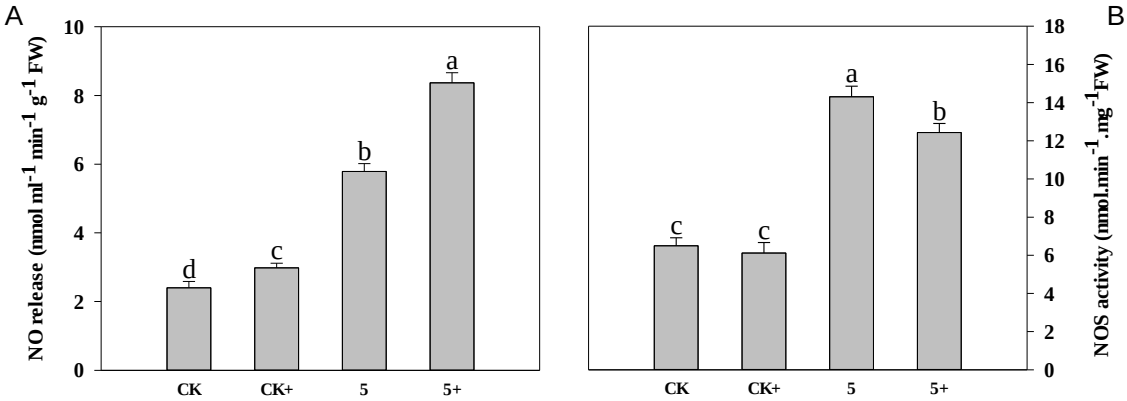

Figure 7. Effects of exogenous NO application on the content of NO release (A) and NOS-like activity (B) after mowing. CK, CK+, 5, and 5+ are treatments and defined previously. Each value represents the mean±*SD* (n= 3). Bars with different letters are significantly different at the 5% level.

DISCUSSION

Mowing has different effects on the growth of turfgrass plants. It increases shoot density, but it also hinders root growth (Liu and Huang, 2002; Cutulle *et al.*, 2014). Here, mowing significantly decreased root biomass. Additionally, MDA content is an indicator of lipid peroxidation and oxidative damage to the membrane (Asada, 2006). In this study, mowing increased MDA content and ion leakage. These results showed that mowing caused certain oxidative damage to roots of tall fescue. To alleviate ROS injuries, plants employ various enzymatic and non-enzymatic antioxidants (Apel and Hirt, 2004). Here, exogenous NO application increased activities of POD, SOD, CAT, and APX, but significantly decreased ROS in different cellular compartments. These results indicated that NO protected tall fescue from oxidative damage caused by mowing through enhancing antioxidant enzymes activities. This protective effect of NO was also found in high-light stress (Xu *et al.*, 2013), chilling stress (Zhou *et al.*, 2005), and drought stress (Lu *et al.*, 2009).

NO also affected the expression of a number of genes involved in stress resistance, metabolism, and signal transduction (Parani *et al.*, 2004). In our study, transcription of these three antioxidant enzymes (CuZn-SOD, CAT-A, and APX4) was significantly increased after mowing. This suggests that these enzymes may play an important role in antioxidant protection during root growth in tall fescue from transcriptional level. From the above, NO might act as a signaling molecule enhancing the expression of genes codifying antioxidant enzymes in roots of tall fescue to enhance activities of antioxidant enzymes, alleviating oxidative damage. However, expression data and enzymatic activity measurements do not always directly correlate. This might be induced by post-translational modification such as phosphorylation or deubiquitinating (Mann and Jensen, 2003).

Many studies have demonstrated that there is a complex interplay between plant hormones and different abiotic stresses (Davies *et al.*, 1986). In this study, the contents of these four hormones were all increased after mowing. Interestingly, these results were different from some previous reports. Environmental stress generally led to increase in the content of ABA but decrease in the contents of CK, IAA, and GA, such as salt (Zhang *et al*., 2018) and drought (Davies *et al.*, 1986; Zhang and Schmidt, 2000; Figueiredo *et al*., 2008). The reason for this difference may be that

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

environmental stresses often inhibit plant growth (Zhu, 2016), however, mowing is not a sustained injury to plant, which can promote faster growth (Wang *et al*., 2018). In addition, exogenous NO increased the content of four kinds of plant hormones. CK can promote cell division and bud differentiation (Wilkinson *et al.*, 2012). This may explain why mowing increased number of root tips (Table 2). In addition, IAA is one of the most important plant hormones that promotes the growth of coleoptiles and stems and induces root growth (Davies, 2010). An increase in IAA content may lead to an increase in root dry weight. Likewise, GA can promote cell elongation and induce the synthesis of many hydrolytic enzymes that can supply more nutrient for the plant to use (Davies, 2010). Higher GA content may increase length and dry mass of root after mowing. ABA is called a "stress hormone" because it enhances resistance to stress. Previous studies indicated that ABA induces the expression of antioxidant enzymes and enhances the activities of POD, SOD, CAT, and APX (Xu *et al.*, 2013; Verma *et al.*, 2016). In this study, exogenous NO significantly increased the content of ABA, which may be related to higher antioxidant enzymes activities.

NO and plant hormones are both important signaling molecules involved in many physiological processes. Recent research provided evidence for a cross-talk between NO and plant hormones (Sanz *et al*., 2015). NO might act downstream of ABA signaling under drought stress (Lu *et al.*, 2009), even though NO is an upstream regulator of ABA signaling under osmotic stress (Xing *et al.*, 2004). Moreover, auxin also acts upstream signal of NO to trigger root ferric-chelate reductase activity in response to iron deficiency in Arabidopsis (Chen *et al.*, 2010). Crosstalk of NO with H_2S and H_2O_2 improve salinity and drought stress tolerance in citrus plants (Ziogas *et al.*, 2015; Liang *et al.*, 2018). Here, exogenous NO significantly increased the content of CK, IAA, GA, and ABA under mowing. These results indicated that NO might act as a signaling molecule increasing the contents of hormones. However, the interaction between hormones and NO signal pathways after mowing remains unclear.

Both mowing and SNP treatment increased endogenous NO release in tall fescue roots, even though with different efficiency. SNP treatment alone induced minor changes compared to the control. However, mowing combined with SNP increased NO release more than mowing alone (Figure 7). This is an interesting result that suggested that endogenous NO release is stable when plants are not under stress. Tall fescue might respond to mowing by increasing NOS activity, enhancing endogenous NO levels (Xu *et al*., 2013). However, mowing combined with SNP treatment slightly reduced NOS activity. This result is similar to prior studies and may be explained by the negative feedback regulation of NOS by exogenous NO (An *et al.*, 2005; Qu *et al.*, 2006).

It is often assumed that the appropriate mowing height of tall fescue is about 3-8 cm (Emmons, 2008). The relative growth rate is generally used as an index to estimate lawn mowing resistance. In our study, we found that tall fescue had higher relative growth rate, root viability, and lower ion leakage when mowing height was 5 cm. Under this mowing height, there might be a better balance between mowing injury and protective effect of nitric oxide. We also demonstrated that mowing significantly increased NO release through NOS-like enzyme. And NO, as a bioactive antioxidant, protects tall fescue roots against mowing damage by up-regulating gene expression of antioxidant enzymes and increasing plant hormone content to promote re-growth of tall fescue. Further investigations are necessary to understand the effect of NO on tall fescue after mowing and the cross-talk between NO and other components in response to mowing.

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

ACKNOWLEDGEMENTS

This work was supported by National Key R&D Program of China (No. 2017YFE0111000) and the Fundamental Research Funds for the Central Universities (No. 2452019080).

REFERENCES

1. Aebi, H. 1984. Catalase *In Vitro*. *Methods Enzymol.*, **105**: 121-126.
2. An, L. Z., Liu, Y. H., Zhang, M. X., Chen, T. and Wang, X. L. 2005. Effects of Nitric Oxide on Growth of Maize Seedling Leaves in the Presence or Absence of Ultraviolet-B Radiation. *J. Plant Physiol.*, **162(3)**: 317-326.
3. Apel, K. and Hirt, H. 2004. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. *Annu. Rev. Plant Biol.*, **55**: 373-399.
4. Asada, K. 2006. Production and Scavenging of Reactive Oxygen Species in Chloroplasts and Their Functions. *Plant Physiol.*, **141(2)**: 391-396.
5. Bari, R. and Jones, J. D. 2009. Role of Plant Hormones in Plant Defence Responses. *Plant Mol. Biol.*, **69(4)**: 473-488.
6. Beauchamp, C. and Fridovich, I. 1971. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Anal. Biochem.*, **44(1)**: 276-287.
7. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.*, **72(1)**: 248-254.
8. Buege, J. A. and Aust, S. D. 1978. Microsomal Lipid Peroxidation. *Methods Enzymol.*, **52**: 302-310.
9. Chen, W. W., Yang, J. L., Qin, C., Jin, C. W., Mo, J. H., Ye, T. and Zheng, S. J. 2010. Nitric Oxide Acts Downstream of Auxin to Trigger Root Ferric-Chelate Reductase Activity in Response to Iron Deficiency in Arabidopsis. *Plant Physiol.*, **154(2)**: 810-819.
10. Cutulle, M., Derr, J., McCall, D., Nichols, A. and Horvath, B. 2014. Effect of Mowing Height and Fertility on Bermudagrass (*Cynodon dactylon*) Encroachment and Brown Patch Severity in Tall Fescue. *Weed Technol.*, **28(1)**: 225-232.
11. Davies, P. J. 2010. *The Plant Hormones: Their Nature, Occurrence, and Functions.* Springer, Dordrecht. doi: 10.1007/978-1-4020-2686-7_1
12. Davies, W., Metcalfe, J., Lodge, T. and da Costa, A. R. 1986. Plant Growth Substances and the Regulation of Growth under Drought. *Funct. Plant Biol.*, **13(1)**: 105-125.
13. Delledonne, M., Xia, Y. J., Dixon, R. A. and Lamb, C. 1998. Nitric Oxide Functions as a Signal in Plant Disease Resistance. *Nature*, **394**: 585-588.
14. Diao, Q., Song, Y., Shi, D. and Qi, H. 2017. Interaction of Polyamines, Abscisic Acid, Nitric Oxide, and Hydrogen Peroxide under Chilling Stress in Tomato (*Lycopersicon esculentum* Mill.) Seedlings. *Front. Plant Sci.*, **8**: 203.
15. Egbichi, I., Keyster, M., Jacobs, A., Klein, A. and Ludidi, N. 2013. Modulation of Antioxidant Enzyme Activities and Metabolites Ratios by Nitric Oxide in Short-Term Salt Stressed Soybean Root Nodules. *S. Afr. J. Bot.*, **88**: 326-333.
16. Elansary, H. O. and Yessoufou, K. 2015. Growth Regulators and Mowing Heights Enhance the Morphological and Physiological Performance of Seaspray Turfgrass during Drought Conditions. *Acta Physiol. Plant.*, **37(11)**: 1-11.
17. Elstner, E. F. and Heupel, A. 1976. Inhibition of Nitrite Formation from Hydroxylammoniumchloride: A Simple Assay for Superoxide Dismutase. *Anal. Biochem.*, **70(2)**: 616-620.
18. Emmons, R. D. 2008. *Turfgrass Science and Management*. Thomson Delmar, Clifton Park, NY.
19. Figueiredo, M. V., Burity, H. A., Martínez, C. R. and Chanway, C. P. 2008. Alleviation of Drought Stress in the Common Bean (*Phaseolus vulgaris* L.) by Co-Inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl. Soil Ecol.*, **40(1)**: 182-188.
20. Fu, J., Chu, X., Sun, Y., Miao, Y., Xu, Y. and Hu, T. 2015. Nitric Oxide Mediates 5-Aminolevulinic Acid-Induced Antioxidant Defense in Leaves of *Elymus nutans* Griseb. Exposed to Chilling Stress. *PLoS One*, **10**: e0130367.
21. Fu, J. M., Koski, A. J. and Qian, Y. L. 2005. Responses of Creeping Bentgrass to Salinity

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

and Mowing Management: Growth and Turf Quality. *HortScience,* **40(2)**: 463-467.
22. Hedden, P. 1993. Modern Methods for the Quantitative-Analysis of Plant Hormones. *Annu. Rev. Plant Biol.,* **44(1)**: 107-129.
23. Islam, E., Yang, X., Li, T., Liu, D., Jin, X. and Meng, F. 2007. Effect of Pb Toxicity on Root Morphology, Physiology and Ultrastructure in the Two Ecotypes of *Elsholtzia argyi*. *J. Hazard. Mater.,* **147(3)**: 806-816.
24. Liang, Y., Zheng, P., Li, S., Li, K. Z. and Xu, H. N. 2018. Nitrate Reductase-Dependent NO Production Is Involved in H_2S-Induced Nitrate Stress Tolerance in Tomato *via* Activation of Antioxidant Enzymes. *Sci. Hortic.,* **229**: 207-214.
25. Liu, X. Z. and Huang, B. R. 2002. Mowing Effects on Root Production, Growth, and Mortality of Creeping Bentgrass. *Crop Sci.,* **42(2)**: 1241-1250.
26. Lu, S., Su, W., Li, H. and Guo, Z. 2009. Abscisic Acid Improves Drought Tolerance of Triploid Bermudagrass and Involves H_2O_2 and NO Induced antioxidant Enzyme Activities. *Plant Physiol. Biochem*., **47(2)**: 132-138.
27. Mann, M. and Jensen, O. N. 2003. Proteomic Analysis of Post-Translational Modifications. *Nat. Biotechnol.,* **21(3)**: 255-261.
28. Murphy, M. E. and Noack, E. 1994. Nitric Oxide Assay Using Hemoglobin Method. *Methods Enzymol.*, **233**: 240-250.
29. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol*., **22(5)**: 867-880.
30. Parani, M., Rudrabhatla, S., Myers, R., Weirich, H., Smith, B., Leaman, D. W. and Goldman, S. L. 2004. Microarray Analysis of Nitric Oxide Responsive Transcripts in Arabidopsis. *Plant Biotechnol. J*., **2(4)**: 359-366.
31. Qu, Y., Feng, H. Y., Wang, Y. B., Zhang, M. X., Cheng, J. Q., Wang, X. L. and An, L. 2006. Nitric Oxide Functions as a Signal in Ultraviolet-B Induced Inhibition of Pea Stems Elongation. *Plant Sci.,* **170(5)**: 994-1000.
32. Sanz, L., Albertos, P., Mateos, I., Sanchez-Vicente, I., Lechon, T., Fernandez-Marcos, M. and Lorenzo, O. 2015. Nitric Oxide (NO) and Phytohormones Crosstalk during Early Plant Development. *J. Exp. Bot*., **66(10)**: 2857-2868.
33. Shahba, M. A. 2010. Interaction Effects of Salinity and Mowing on Performance and Physiology of Bermudagrass Cultivars. *Crop Sci.,* **50(6)**: 2620.
34. Song, L. L., Ding, W., Shen, J., Zhang, Z. G., Bi, Y. R. and Zhang, L. X. 2008. Nitric Oxide Mediates Abscisic Acid Induced Thermotolerance in the Calluses from Two Ecotypes of Reed under Heat Stress. *Plant Sci.,* **175(5)**: 826-832.
35. Upadhyaya, A., Sankhla, D., Davis, T. D., Sankhla, N. and Smith, B. 1985. Effect of Paclobutrazol on the Activities of Some Enzymes of Activated Oxygen Metabolism and Lipid Peroxidation in Senescing Soybean Leaves. *J. Plant Physiol.,* **121(5)**: 453-461.
36. Veljovic-Jovanovic, S., Noctor, G. and Foyer, C. H. 2002. Are Leaf Hydrogen Peroxide Concentrations Commonly Overestimated? The Potential Influence of Artefactual Interference by Tissue Phenolics and Ascorbate. *Plant Physiol. Biochem.,* **40(6)**: 501-507.
37. Verma, V., Ravindran, P. and Kumar, P. P. 2016. Plant Hormone-Mediated Regulation of Stress Responses. *BMC Plant Biol.,* **16**: 86.
38. Wang, M., Xu, Z., Song, J., Liu, X. and Jiao, X. 2018. Effects of Different Mowing Treatments and Stubble Heights on the Compensatory Growth and Quality of Lettuce (*Lactuca sativa* L.). *J. Hortic. Sci. Biotechnol.,* **93(5)**: 537-544.
39. Wilkinson, S., Kudoyarova, G. R., Veselov, D. S., Arkhipova, T. N. and Davies, W. J. 2012. Plant Hormone Interactions: Innovative Targets for Crop Breeding and Management. *J. Exp. Bot*., **63(9)**: 3499-3509.
40. Xing, H., Tan, L. L., An, L. H., Zhao, Z. G., Wang, S. M. and Zhang, C. L. 2004. Evidence for the Involvement of Nitric Oxide and Reactive Oxygen Species in Osmotic Stress Tolerance of Wheat Seedlings: Inverse Correlation between Leaf Abscisic Acid Accumulation and Leaf Water Loss. *Plant Growth Regu*., **42(1)**: 61-68.
41. Xu, Y., Xu, Q. and Huang, B. 2015. Ascorbic Acid Mitigation of Water Stress-Inhibition of Root Growth in Association with Oxidative Defense in Tall Fescue

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

(*Festuca arundinacea* Schreb.). *Front. Plant Sci*., **6**: 807.
42. Xu, Y. F., Fu, J. J., Chu, X. T., Sun, Y. F., Zhou, H. and Hu, T. M. 2013. Nitric Oxide Mediates Abscisic Acid Induced Light-Tolerance in Leaves of Tall Fescue under High-Light Stress. *Sci. Hortic*., **162**: 1-10.
43. Yang. W. Z., Fu, J. J., Yang, L.Y., Zhang, X., Zheng, Y. L., Feng, F. and Xu, Y. F. 2014. Protective Effects of Complementary Ca^{2+} on Low-Light-Induced Oxidative Damage in Tall Fescue. *Russ. J. Plant Physl.*, **61(6)**: 818-827.
44. Zhang, X., Wu, W., Ervin, E. H., Shang, C. and Harich, K. 2018. Salt Stress-Induced Injury Is Associated with Hormonal Alteration in Kentucky Bluegrass. *HortScience*, **53(1)**: 97-101.
45. Zhang, X. Z. and Schmidt, R. E. 2000. Hormone-Containing Products' Impact on Antioxidant Status of Tall Fescue and Creeping Bentgrass Subjected to Drought. *Crop Sci*., **40(5)**: 1344-1349.
46. Zhou, B., Guo, Z., Xing, J. and Huang, B. 2005. Nitric Oxide Is Involved in Abscisic Acid-Induced Antioxidant Activities in *Stylosanthes guianensis*. *J. Exp. Bot.*, **56(442)**: 3223-3228.
47. Ziogas, V., Tanou, G., Belghazi, M., Filippou, P., Fotopoulos, V., Grigorios, D. and Molassiotis, A. 2015. Roles of Sodium Hydrosulfide and Sodium Nitroprusside as Priming Molecules during Drought Acclimation in Citrus Plants. *Plant Mol. Biol*., **89(4)**: 433-450.
48. Zhu, J. K. 2016. Abiotic Stress Signaling and Responses in Plants. *Cell*,**167(2)**: 313-324.

اثر اکسید نیتریک روی مقاومت به چیدن در ریشه Tall Fescue (*Festuca Arundinacea* Schreb.) از طریق دفاع آنتی اکسیدانی و هورمون های گیاهی

ز. ه. ژانگ، ر. چایی، ی. ف. ما، و. و. منگ، ی. و. کی، ز. ت. چو، و ی. ف. زو

چکیده

چیدن چمن در حفظ کیفیت آن اهمیت دارد. هدف این پژوهش تعیین اثر اکسید نیتریک (NO) روی Tall Fescue (Festuca Arundinacea Schreb.) بعد از چیدن و فراهم آوردن مبانی نظری برای بهنژادی مولکولی چمن علفی بود. در این پژوهش، NO بیرونی (exogenous) نرخ رشد نسبی را در مقایسه با شاهد به طور معناداری افزایش داد. این نرخ رشد نسبی بالاتر با فعالیت بیشتر پراکسیدها (POD)، سوپراکسید دیسموتاز(SOD)، کاتالاز (CAT)، و آسکوربات پراکسیداز(APX) همراه بود. بیان *CuZn-SOD*، *CAT-A*، و *APX4* در ریشه تیمار شده با NO بعد از چیدن افزایش یافت. افزون بر این، NO بیرونی به طور معناداری غلظت indole-3-acetic acid (IAA) ، اسید جیبرالیک (GA)، سیتوکینین(CK)، وآبسه اسید (ABA) را در مقایسه با شاهد افزایش داد که احتمالا در پیوند با افزایش فعالیت مشابه (NOS)اکسید نیتریک سنتاز و رها سازی NO درونی(endogenous) در ریشه Tall Fescue بود. این نتایج چنین اشارت دارد که چیدن باعث افزایش فعالیت مشابهNOS شده و منجر به زیاد شدن NO درونی شد. NO احتمالا به عنوان یک مولکول علامت دهنده (signaling) منجر به افزایش محتوای هورمونی و همچنین تنظیم مجدد بیان ژن

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]

و ارتقای فعالیت آنزیم های آنتی اکسیدان شده و در نتیجه از گیاه در برابر جراحات ناشی از چیدن حفاظت کرده و در جهت بهبود رشد مجدد Tall Fescue عمل میکند.

[Downloaded from jast.modares.ac.ir on 2024-04-26]

[DOR: 20.1001.1.16807073.2019.21.5.18.5]