Effects of 6-Benzyladenine, γ -Aminobutyric Acid, and Nitric Oxide on Plant Growth, Photochemical Efficiency, and Ion Accumulation of Perennial Ryegrass Cultivars to Salinity Stress

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Key words. growth, Fv/Fm, ion concentration, perennial ryegrass, PGR, salinity

Abstract. Plant growth regulators (PGRs) can mediate plant response to salinity stress. Perennial ryegrass (*Lolium perenne*) cultivars of BrightStar SLT, Catalina, Inspire, and SR4660ST were exposed to 0, 100, or 200 mM NaCl for 14 d. 6-benzyladenine (6-BA, 10 μ M), γ -aminobutyric acid (GABA, 500 μ M), nitric oxide (NO, 200 μ M), and H₂O were applied to the foliage every day for 3 days before stress and then every 2 days during salinity stress. Averaged across the four cultivars, a foliar spray of NO increased leaf fresh weight (FW) and dry weight (DW) at 0 mM NaCl, whereas application of 6-BA increased DW and GABA reduced Na⁺ concentration at 100 mM NaCl, compared with H₂O application. Plants treated with 6-BA, GABA, and NO had less chlorotic and necrotic leaf tissue than plants treated with H₂O at 200 mM NaCl. Spray of 6-BA and NO increased FW and DW, but application of all three PGRs maintained higher leaf photochemical efficiency and lower leaf Na⁺ concentration compared with H₂O treatment at 200 mM NaCl. Across salinity and PGR treatments, ‘Catalina’ exhibited higher plant height than the ‘Inspire’ and SR4660ST, and SR4660ST had relatively higher Na⁺ concentration than ‘Catalina’ but not ‘BrightStar SLT’ and ‘Inspire’. The results demonstrate that 6-BA, GABA, and NO ameliorated salinity tolerance of perennial ryegrass by improving growth and photochemical efficiency or reducing Na⁺ accumulation.

Salinity stress can severely inhibit plant growth and development. The adverse effects of salinity stress on plants are generally imposed through osmotic stress by limiting water uptake and excessive uptake of sodium (Na⁺) ions (Munns and Tester, 2008). High Na⁺ accumulation can disturb plant ionic homeostasis, decrease plant growth, and increase the senescence rate of older leaves. Early senescence caused by salinity stress is often accompanied by decline in chlorophyll content, reduced photosynthetic efficiency, and increased oxidative injury (Parihar et al., 2015). Plants can adjust metabolism to cope with salinity stress. This includes, but is not limited to, accumulation of water-soluble sugars and organic acids for osmotic adjustment, reduced Na⁺ uptake, and maintenance of K⁺ uptake (Munns and Tester, 2008). Hence, salinity tolerant plants often have

higher K⁺ and lower Na⁺ accumulation under salinity stress (Tang et al., 2013a, 2013b)

PGRs are strongly involved in the regulation of plant developmental processes, signaling networks, and biotic and abiotic stress responses of plants (McSteen and Zhao, 2008; Upreti and Sharma, 2016). 6-benzyladenine (6-BA) is a synthetic compound of cytokinin that plays multiple biological roles in plant growth and development, similar to naturally occurring cytokinins (Choi and Hwang, 2007). Application of 10 μ M 6-BA reduced growth suppression in two eggplant (*Solanum melongena*) genotypes at 90 mM NaCl (Wu et al., 2014). Moreover, 6-BA (25 μ M) promoted leaf elongation rate and decreased Na⁺ concentration in perennial ryegrass cultivar Pinnacle exposed to 14, 21, and 28 d of 250 mM NaCl (Ma et al., 2016). The results suggested a positive role of 6-BA in improving plant growth and salinity tolerance.

Other chemical or signaling molecule such as GABA and NO also demonstrated a role in regulating plant responses to salinity stress (Ahmad et al., 2016; Liu et al., 2016; Wang et al., 2017; Ziogas et al., 2017). GABA is a nonprotein amino acid that is well recognized as an endogenous plant signaling molecule (Kinnersley and Turano, 2000). Exogenous application of GABA en-

hanced photosynthesis, leaf photochemical efficiency, and enzymatic antioxidant activity in maize (*Zea mays*) and wheat (*Triticum aestivum*) exposed to a range of NaCl stress (Li et al., 2016; Wang et al., 2017). NO is a signaling molecule that has diverse biological functions in plants, including stress tolerance (Shi et al., 2011). Less reduction of plant dry weight and chlorophyll content and lower electrolyte leakage were found in maize seedlings irrigated with 100 mM NaCl solution amended with NO (Zhang et al., 2006). Application of NO also improved germination and growth rate (Kopyra and Gwózdź, 2003), increased chlorophyll content and photosynthetic rate (Fatma et al., 2016; Kong et al., 2016; Liu et al., 2016) and antioxidant activity (Khan et al., 2012; Liu et al., 2016), and regulated K⁺ and Na⁺ concentration (Kong et al., 2016; Zhao et al., 2004) in plants exposed to salinity stress. The results suggested that GABA and NO could enhance salinity tolerance by maintaining growth, cell membrane integrity, and proper function of the photosynthetic system. Collectively, benefits of PGRs likely vary with concentration of application, stress intensity, and plant species.

Salinity stress can be a problem in turfgrass management in coastal and saline areas. Perennial ryegrass (*Lolium perenne*) is a widely cultivated cool-season grass species in temperate regions due to its high quality as a forage and turfgrass. Because of wide geographic distribution, significant natural variations of salinity tolerance existed in the germplasm of this species (Tang et al., 2013a, 2013b). Perennial ryegrass commercial cultivars have moderate salinity tolerance (Harivandi et al., 1992). Due to variations of PGR functions and stress responses of cultivars, plant growth and physiology affected by different PGRs and levels of salinity stress are not well documented in perennial ryegrass, especially for GABA and NO effects on plants. To gain a better understanding of PGR effects on salinity tolerance of perennial ryegrass, we designed the experiment to examine plant growth, photochemical efficiency, and ion accumulation of four perennial ryegrass cultivars to 6-BA, GABA, and NO applications at three concentrations of salinity. The results would be beneficial for enhancing turfgrass management at salt-affected sites.

Materials and Methods

Plant material and growing conditions. Perennial ryegrass cultivars of BrightStar SLT, Inspire, and Catalina (Scotts Miracle-Gro Co. Turf-Seed, Inc. Marysville, OH) varying in salinity tolerance were used for this study. The relative salinity tolerance was ‘Catalina’ > ‘Brightstar SLT’ > ‘Inspire’ (Tang et al., 2013b). SR4660ST is a newly developed cultivar (Seed Research of Oregon Inc., Tangent, OR) with good salinity tolerance, but no information is available for comparing its salinity tolerance with the

Received for publication 25 Mar. 2019. Accepted for publication 28 Apr. 2019.

This research is supported by the Midwest Regional Turfgrass Foundation of Purdue University.
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other three cultivars. Approximately 0.15 g of seed were sown in pots (7 cm diameter, 9 cm deep) containing sand on 7 Apr. 2017 in a greenhouse. Grasses were irrigated daily with a 50-mL half-strength Hoagland solution and cut weekly to 5 cm. The average temperatures in the greenhouse were $23 \pm 2.5/17 \pm 1.5$ °C (day/night). The average *PAR* was $\approx 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with 14-h natural and artificial lights.

PGR and salinity treatments. Plants were grown in a greenhouse for 65 d before PGR and salinity treatments were imposed. Before applying salinity stress, 9 mL of a PGR solution or an H₂O control (with 0.05% Tween 20) were sprayed on the plant foliage once a day for 3 d. This volume of solution allowed wetting shoot tissues while not draining into the soil. Plant growth regulator treatments were 6-BA (10 μM), GABA (500 μM), and NO donor sodium nitroprusside (200 μM). The concentrations of PGRs and application procedure to the plants were based on other studies (Li et al., 2016; Ma et al., 2016; Wu et al., 2014) and our preliminary results. After the third PGR application, salinity stress was imposed by irrigating daily with 50 mL of Hoagland solution amended with different concentrations of NaCl, eventually attaining concentrations of 0, 100, or 200 mM NaCl. Salinity was increased by 25 mM each day until 100 mM NaCl was reached and then 50 mM NaCl for 2 additional days to establish the 200 mM NaCl treatment. Irrigation was applied to the soil carefully to avoid contact with the plant foliage. After reaching the final NaCl concentrations, plants were cut to 5 cm. Treatments of PGRs were applied every other day thereafter. All salinity and PGR applications ended 14 d after initiation of stress and plant measurements commenced.

Growth and physiological measurements. At 14 d, all leaves were sprayed with tap water multiple times before harvesting to ensure any NaCl on the plant stems was

removed. Plant height (HT), leaf FW and DW, leaf water content (LWC), leaf photochemical efficiency (Fv/Fm), and leaf Na⁺ and K⁺ concentration were measured. Height was recorded from the soil surface to the top of the highest leaf blade. Fresh weight was determined by weighing all leaves, and DW was determined after leaf tissues were dried at 80 °C for 3 d. LWC was calculated according to the following equation: $\text{LWC} = (\text{FW} - \text{DW}) / \text{DW} \times 100$. The Fv/Fm was measured on randomly selected leaves in each pot in darkness using a fluorescence meter (OS-30P, OPTI-Sciences, Hudson, NH).

To assess nutrient concentrations (K⁺, Na⁺, Ca²⁺, Mg²⁺, P), we followed the method previously described by Tang et al. (2013a). Briefly, 50 mg of fine powder of dry tissue was mixed with 3 mL 18 M H₂SO₄ in a 50-mL digestion tube. Tubes were placed into a digestion block at 200 °C for 45 min. After cooling, 3 mL of 30% H₂O₂ was slowly added to each tube, and tubes were returned to the block for 45 min until the mixture became totally transparent and colorless. Distilled water was added to the extraction to reach a final volume of 50 mL. A 2-mL aliquot of extraction was taken and transferred to a new tube and distilled water added to bring the final volume to 15 mL. The Na⁺, K⁺, Ca²⁺, Mg²⁺, and P concentrations were determined with the final diluted extract using a plasma atomic emission spectrometer (ICP 9820; Shimadzu, Columbia, MD).

Experimental design and statistical analyses. Experimental design was a split-split plot design with the main plot, subplots, and sub-subplot as salinity stress, PGR, and cultivar, respectively. Each cultivar was randomly assigned within salinity and PGR treatments. All treatments had four replications (pots). The analysis of variance and differences of least squares means were performed using PROC MIXED (version 9.4; SAS Institute, Cary, NC), with PGRs,

salinity, and cultivars as fixed factors and replications as a random factor.

Results

Analysis of variance. Salinity $\times$ PGR interactions ($P < 0.05$) were observed for FW, DW, Fv/Fm, and Na⁺, whereas a salinity $\times$ cultivar interaction ($P < 0.05$) was found only for P (Table 1). For variables where treatment effects did not interact, cultivar influenced HT and Na⁺, salinity affected HT, LWC, K⁺, Ca²⁺, and Mg²⁺, and PGR influenced P. No PGR $\times$ cultivar interactions or salinity $\times$ PGR $\times$ cultivar interactions were noted for any traits (Table 1).

Main effects of salinity, cultivars, and PGRs. Across cultivars and PGRs, salinity had significant effects on all traits (Tables 1 and 2). Specifically, 100 mM and 200 mM NaCl treatments significantly decreased HT, FW, DW, LWC, K⁺, Mg²⁺, and Ca²⁺ but increased Na⁺ and P concentrations. Moreover, 200 mM NaCl caused more reductions of HT, FW, DW, LWC, K⁺, and Mg²⁺ and more Na⁺ accumulation than that of 100 mM NaCl. The Fv/Fm was unaffected by 100 mM NaCl, but was reduced by 200 mM NaCl. Salinity effects on HT were independent of differences among cultivars and PGR treatments (Tables 1 and 2). The decreased Ca²⁺ and increased P were observed at both 100 and 200 mM NaCl, but there were no differences in these two measurements between the two salinity treatments.

Perennial ryegrass cultivars differed in HT and Na⁺ concentrations averaged across salinity and PGR treatments (Tables 1 and 3). SR4660ST and 'Inspire' had lower HT than 'Catalina', whereas 'BrightStar SLT', 'Inspire', and SR4660ST did not differ in HT. SR4660ST had the higher level of Na⁺ than 'Catalina', but not 'BrightStar SLT' and 'Inspire'. 'Catalina', 'BrightStar SLT' and 'Inspire' did not differ in leaf Na⁺ concentration.

PGR effects on P were independent of effects of salinity and differences among cultivars (Table 1). 6-BA had lower P (2.66 mg·g⁻¹) than H₂O, GABA, and NO, which did not differ (mean = 2.80 mg·g⁻¹) (data not shown).

Interactive effects of salinity and plant growth regulators. Averaged across the four cultivars, PGRs had some effects on plant traits at 0 and 100 mM NaCl (Table 4). Compared with a foliar spray of H₂O, application of NO increased FW 11% and DW 9.1% at 0 mM NaCl, while 6-BA increased DW 9.6% and GABA reduced Na⁺ 20% at

Table 1. Analysis of variance for plant height (HT), leaf fresh weight (FW), leaf dry weight (DW), leaf water content (LWC), leaf photochemical efficiency (Fv/Fm), leaf potassium (K⁺), sodium (Na⁺), calcium (Ca²⁺), magnesium (Mg²⁺), and phosphorus (P) concentrations across four perennial ryegrass cultivars exposed to plant growth regulators (PGR) under 0, 100, and 200 mM NaCl treatments.

	HT	FW	DW	LWC	Fv/Fm	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	P
Salinity (S)	*	*	*	*	*	*	*	*	*	*
PGR (P)	NS	NS	*	NS	*	NS	*	NS	NS	*
Cultivar (C)	*	NS	NS	NS	NS	NS	*	NS	NS	NS
S $\times$ P	NS	*	*	NS	*	NS	*	NS	NS	NS
S $\times$ C	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
P $\times$ C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S $\times$ P $\times$ C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *Nonsignificant or significant at $P < 0.05$, respectively.

Table 2. Salinity effects on plant height (HT), leaf fresh weight (FW), leaf dry weight (DW), leaf water content (LWC), leaf photochemical efficiency (Fv/Fm), leaf concentrations of potassium (K⁺), sodium (Na⁺), calcium (Ca²⁺), magnesium (Mg²⁺), and phosphorus (P) across perennial ryegrass cultivars and plant growth regulators.

Salinity (mM)	HT (cm)	FW (g)	DW (g)	LWC (%)	Fv/Fm	K ⁺ (mg·g ⁻¹)	Na ⁺ (mg·g ⁻¹)	Ca ²⁺ (mg·g ⁻¹)	Mg ²⁺ (mg·g ⁻¹)	P (mg·g ⁻¹)
0	6.6 a ^c	3.7 a	0.79 a	77.9 a	0.821 a	30.3 a	2.26 c	6.2 a	4.6 a	2.3 b
100	4.8 b	2.1 b	0.53 b	74.4 b	0.823 a	22.0 b	11.4 b	5.0 b	2.7 b	3.0 a
200	3.5 c	1.1 c	0.31 c	72.8 c	0.803 b	20.3 c	20.1 a	4.2 b	2.4 c	3.0 a

^cMeans followed by the same letter within three salinity treatments for a given measurement are not significantly different at $P < 0.05$.

100 mM NaCl. Otherwise, there were no effects of PGRs on any trait at 0 and 100 mM NaCl.

At 200 mM NaCl, application of 6-BA, GABA, and NO improved salinity tolerance of all four cultivars. Plants treated with 6-BA, GABA, and NO had less chlorotic and necrotic leaf tissue than plants treated with H₂O (Fig. 1). Compared with H₂O application, spray of 6-BA increased FW by 63% and DW by 70%, and NO increased FW by

34% and DW by 35%; GABA did not affect FW and DW at 200 mM NaCl (Table 4). Foliar-applied 6-BA, GABA, and NO significantly improved Fv/Fm at 200 mM NaCl, although only a 3% to 4% increase in Fv/Fm was observed, compared with H₂O (Table 4). The plants sprayed with 6-BA, GABA, and NO showed decreased Na⁺ by 20%, 28%, and 26%, respectively, compared with the H₂O application at 200 mM NaCl (Table 4).

Interactive effects of cultivars and salinity. Leaf P concentration was the only trait for which the effects of salinity differed among cultivars, albeit subtly (Tables 1 and 5). The range in P concentration across cultivars was 0.3, 0.4, and 0.1 mg·g⁻¹ at 0, 100, and 200 mM NaCl, respectively (Table 5). Increasing salinity from 0 to 100 mM NaCl increased P concentration in all cultivars. The magnitude of the increase was 0.1, 0.7, 0.4, and 0.5 mg·g⁻¹ for cultivars Catalina, BrightStar SLT, Inspire, and SR4660ST, respectively. Increasing salinity

from 100 to 200 mM NaCl affected P concentration only slightly. Across PGRs at 0 mM NaCl, 'Inspire' had higher P concentration than 'Catalina' and 'BrightStar SLT', and P concentration did not differ in SR4660ST, compared with other three cultivars (Table 5). At 100 mM NaCl, 'Catalina' showed higher P concentration than other cultivars, which did not differ in P concentration. At 200 mM NaCl, no differences in P concentration were observed in four cultivars across PGR treatments.

Discussion

Perennial ryegrass accessions exposed to salinity stress showed reductions in plant elongation rate, dry weight and K⁺ concentration, and increased Na⁺ concentrations (Song et al., 2017; Yin et al., 2017). Our results in four cultivars supported these observations. Fv/Fm is a major indicator for photochemical efficiency of Photosystem II, and decline in Fv/Fm was observed in perennial ryegrass at ≥150 mM NaCl (Tang et al., 2013a). However, salinity did not affect the Fv/Fm of warm-season grasses, such as salt hay (*Spartina patens*), smooth cordgrass (*Spartina alterniflora*), denseflower cordgrass (*Spartina densiflora*), and seashore saltgrass (*Distichlis spicata*) (Maricle et al., 2007). In this study, Fv/Fm remained unchanged at 100 mM NaCl but decreased at 200 mM NaCl across PGR treatments, suggesting that the lower level of salinity stress had little inhibition of photochemical efficiency in perennial ryegrass. It might also indicate that the growth reduction of perennial ryegrass caused by low salinity stress (e.g., 100 mM NaCl) could result from excessive Na⁺ accumulation. However, growth inhibition under high salinity stress (e.g., 200 mM NaCl) could be due to a combination of impaired photosynthesis and accumulation of Na⁺. The Na⁺ and K⁺ concentrations are important traits associated with salinity tolerance in plants (Azadi et al., 2011; Song et al., 2017). High level of Na⁺ in perennial ryegrass under salinity stress can also interfere with accumulation of other ions such as Ca²⁺ and Mg²⁺ (Song et al., 2017), as we also noted. Plant tolerance to osmotic stress and Na⁺ toxicity ultimately determines the overall plant response to salinity stress (Munns and Tester, 2008). Our results were consistent with these findings regardless of PGR applications.

Phosphorus is an essential nutrient element for plant growth and development. Salinity stress decreased, increased, or had no effect on P content in plant tissues (Champagnol, 1979; Fageria, 1985; Grattan and Grieve, 1999; Nemati et al., 2011; Sharpley et al., 1992). Addition of P to the saline soil improved crop yield in many crop species but did not necessarily increase crop salt tolerance (Champagnol, 1979). In barley (*Hordeum vulgare*), plants exposed to a combination of salinity stress and P deficiency showed better salinity tolerance compared with plants grown under salinity

Table 3. Cultivar differences in plant height (HT) and leaf sodium (Na⁺) concentration across salinity treatments and plant growth regulators.

Cultivar	HT (cm)	Na ⁺ (mg·g ⁻¹)
Catalina	5.34 a ²	10.6 b
BrightStar SLT	5.31 ab	11.0 ab
Inspire	4.84 b	10.7 ab
SR4660ST	4.39 b	12.0 a

²Means followed by the same letter within cultivars for a given measurement are not significantly different at *P* < 0.05.

Table 4. Interactive effects of plant growth regulator (PGR) of 6-benzyladenine (6-BA), γ-aminobutyric acid (GABA), nitric oxide (NO), and salinity on leaf fresh weight (FW), leaf dry weight (DW), leaf photochemical efficiency (Fv/Fm), and leaf sodium (Na⁺) concentration across four perennial ryegrass cultivars.

Salinity (mM)	PGR	FW (g)	DW (g)	Fv/Fm	Na ⁺ (mg·g ⁻¹)
0	H ₂ O	3.46 b ²	0.76 b	0.820	2.48
	6-BA	3.40 b	0.77 b	0.821	1.47
	GABA	3.53 b	0.79 b	0.820	2.91
	NO	3.88 a	0.83 a	0.822	2.18
100	H ₂ O	2.11	0.52 b	0.824	12.5 a
	6-BA	2.20	0.57 a	0.819	12.0 ab
	GABA	2.07	0.52 b	0.825	10.0 b
	NO	1.89	0.50 b	0.824	11.1 ab
200	H ₂ O	0.86 b	0.23 c	0.781 c	24.7 a
	6-BA	1.40 a	0.39 a	0.805 b	19.8 b
	GABA	1.12 ab	0.29 bc	0.813 ab	17.7 b
	NO	1.15 a	0.31 b	0.814 a	18.3 b

²Within each salinity treatment (0, 100, and 200 mM NaCl), means followed by the same letter among PGRs for a given measurement are not significantly different at *P* < 0.05.

Fig. 1. Appearance of perennial ryegrass cultivars sprayed with water, 6-benzyladenine (6-BA, 10 μM), γ-aminobutyric acid (GABA, 500 μM), and nitric oxide (NO, 200 μM) at 14 d of 200 mM NaCl.

Table 5. Effects of salinity on P concentration in each cultivar across plant growth regulators.

NaCl (mm)	Catalina	BrightStar SLT	Inspire	SR4660ST
	(mg·g ⁻¹)			
0	2.2 bB ²	2.3 bB	2.5 bA	2.3 cAB
100	3.2 aA	3.0 aB	2.9 aB	2.8 bB
200	3.1 aA	3.0 aA	3.0 aA	3.0 aA

²Within a cultivar or salinity, means followed by the same lowercase letter are not significantly different at $P < 0.05$. Lowercase letters indicate comparisons made within three salinity treatments for a given cultivar, and uppercase letters represent comparisons made among cultivars for a given salinity treatment (0, 100, or 200 mM NaCl).

with sufficient P supply (Talbi et al., 2011). In perennial ryegrass, the salinity-tolerant group of accessions exhibited increased P and lower Na⁺ concentrations, but the moderately tolerant and the sensitive group of accessions showed reduced P and increased Na⁺ concentrations under high salinity stress (Song et al., 2017). Our results showed that salinity stress increased P concentration across PGRs and cultivars, but foliar applications of 6-BA, GABA, and NO did not affect P concentration at 100 or 200 mM NaCl. The results suggested a complex relationship among P nutrition, salinity tolerance, and PGRs.

Foliar application of 6-BA enhanced FW, DW, and Fv/Fm and reduced Na⁺ concentration at 200 mM NaCl but also increased DW at 100 mM NaCl across four perennial ryegrass cultivars. The results indicated 6-BA (10 µM) enhanced salinity tolerance of perennial ryegrass by improving growth and photochemical efficiency and limiting Na⁺ accumulation under high salinity stress. The application of 6-BA also ameliorated plant growth in eggplant (*Solanum melongena*) genotypes exposed to 90 mM NaCl (Wu et al., 2014) and in perennial ryegrass cultivar of Pinnacle at 250 mM NaCl (Ma et al., 2016). The results indicated that 6-BA strongly promoted plant growth under a range of salinity stress. The synthesis and/or transport of cytokinin from root to shoot could be inhibited by salinity stress, leading to lower cytokinin content in the shoot and closure of stomata (Ghanem et al., 2011). Therefore, foliar spray of 6-BA could supplement the lack of cytokinin provision from the roots, contributing to sustained shoot growth and physiological activities of plants.

The nonprotein amino acid GABA rapidly accumulates in plant tissues in response to biotic and abiotic stress (Kinnersley and Turano, 2000). In maize, application of GABA enhanced plant height and leaf water content, increased leaf FW and DW, and alleviated damage to membranes, and mitochondrial and chloroplast function in plants at 150 and 300 mM NaCl (Wang et al., 2017). In wheat, exogenous GABA application improved leaf photosynthesis and chlorophyll fluorescence parameters, increased antioxidant enzyme activity, and reduced malondialdehyde accumulation in plants exposed to 50 to 300 mM NaCl (Li et al., 2016). In this study, GABA-treated perennial ryegrass plants had higher Fv/Fm at 200 mM NaCl and lower Na⁺ concentration at both 100 and 200 mM NaCl. Together with results of other

studies, it appears that application of GABA plays a strong role in reducing Na⁺ accumulation while maintaining photosynthetic activities during salinity stress. Because GABA is a major metabolic component in the interface between carbon and nitrogen (Michaeli and Fromm, 2015), its regulation of carbon metabolism or signaling pathway could modulate photosynthesis and nutrient uptake to improve salinity tolerance of plants.

Nitric oxide (NO) is a signaling molecule that plays an important role in delaying leaf senescence and increasing abiotic stress tolerance (Freschi, 2013). Our results in perennial ryegrass showed that plants treated with NO maintained higher FW, DW, and Fv/Fm and lower Na⁺ concentration at 200 mM NaCl, compared with foliar application of H₂O. Lower reductions of leaf and root DW and chlorophyll content and lower electrolyte leakage were also found in maize seedlings irrigated with 100 mM NaCl solution amended with NO (Zhang et al., 2006). Application of NO also maintained higher water content, chlorophyll content, and K⁺ to Na⁺ ratio, alleviating salinity damage on plant growth and ionic balance in bermudagrass (*Cynodon dactylon*) (Liu et al., 2016). The results suggested that NO could facilitate salinity tolerance by maintaining growth, cell membrane integrity, and proper function of the photosynthetic system. The results suggested that SNP could improve salinity tolerance by maintaining growth, cell membrane integrity, and proper function of the photosynthetic system. Reduced Na⁺ concentration might be partially due to SNP increasing plasma membrane H⁺-ATPase activity to decrease the uptake of Na⁺ and increase uptake of K⁺, as was seen in two ecotypes of reed (*Phragmites communis*) at 200 mM NaCl (Zhao et al., 2004). The lower levels of Na⁺ concentration in all four perennial ryegrass cultivars suggested a positive role of SNP in controlling Na⁺ uptake under high salinity stress. Additionally, exogenous NO delayed salinity-induced leaf senescence in cotton (*Gossypium hirsutum*) (Kong et al., 2016). Delayed leaf senescence with NO application could arise from reduced expression of genes involved in chlorophyll breakdown pathway and senescence (Liu and Guo, 2013; Ma et al., 2010). Collectively, NO-enhanced salinity tolerance of perennial ryegrass could be associated with delayed leaf senescence, increased photosynthetic capacity, and reduced Na⁺ toxicity.

Perennial ryegrass germplasm or cultivars varied substantially in salinity tolerance

(Harivandi et al., 1992; Tang et al., 2013a, 2013b). In this study, four cultivars exhibited some variations in response to 100 and 200 mM NaCl across PGRs. The relatively higher HT and lower level of Na⁺ found in 'Catalina' indicated its better salinity tolerance. The results were consistent with a previous study conducted under 300 mM NaCl (Tang et al., 2013b). Nevertheless, application of 6-BA, GABA, and NO promoted salinity tolerance of four cultivars.

Conclusion

Salinity stress significantly impaired the perennial ryegrass growth, decreased K⁺ and increased Na⁺ concentrations in four perennial ryegrass cultivars. Foliar spray of 6-BA, GABA, and NO ameliorated salinity tolerance by improving plant growth, maintaining photochemical efficiency, or reducing Na⁺ concentration at 200 mM NaCl. 6-BA also showed positive effects on DW at 100 mM NaCl. The results provided an important basis for enhancing management practices and exploring molecular mechanisms of salinity tolerance mediated by PGRs.

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