

Effects of foliar application of glycine betaine and chitosan on *Puccinellia distans* (Jacq.) Parl. subjected to salt stress

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Original Article

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Introduction: Using brackish water for irrigation may expose turfgrasses to salinity stress. Employing the best treatments to maintain high-quality turfs under saline conditions is an important requirement for turfgrass management. **Methods:** We tested the response of a halophyte grass, *Puccinellia distans*, to irrigation with saline solutions and to foliar application of two osmoprotectants, such as glycine betaine (GB) or chitosan (CH). Plants were grown in pots under controlled conditions and irrigated with 200 mM or 600 mM of NaCl solutions. The response to salinity treatments and osmoprotectant application was evaluated after 90 days by measuring leaf firing, leaf density, shoot length and biomass, root length, and shoot water potential. **Results:** Increasing salinity reduced shoot density, shoot and root length, shoot water potential, and increased leaf firing and shoot solute potential at 200 mM of NaCl. These effects were more pronounced at 600 mM of NaCl. Application of GB greatly increased shoot growth traits at 200 mM of NaCl and also showed beneficial effects on most traits at 600 mM. Application of CH showed positive effects only on leaf firing and leaf water potential at 600 mM. **Conclusions:** Our results show that *P. distans* can tolerate high levels of salt stress, which can be best alleviated by GB treatment.

INTRODUCTION

Turfgrass management often requires irrigation with poor-quality water, to avoid the use of valuable fresh water resources (Yang et al., 2009) and this practice may expose turfgrasses to salt stress. In coastal areas, salt stress may also be due to direct exposure to salt spray. For this reason, the ability to maintain a positive water balance, growth, photosynthesis, chlorophyll content, and good visual quality is an important trait for the selection of turfgrass species (Fry & Huang, 2004; Scalia et al., 2009). Moreover, the application of osmoprotectants that may alleviate drought stress is a further requirement to maintain turf quality when brackish water must be used for irrigation (Scalia et al., 2014).

Puccinellia distans, or weeping alkali grass, is a C3 perennial, cool-season halophytic grass, which grows in saline environments throughout the world. Salt stress affects plant growth and metabolism mainly through ion toxicity and osmotic stress. To counteract osmotic stress, tolerant genotypes may accumulate compatible solutes, such as polyamines, betaines, and polyols (Munns & Tester, 2008). One of the most widely present betaines is glycine betaine (GB), a quaternary ammonium compound that has a well-known activity as a natural compatible solute (Chen & Murata, 2011; Kurepin et al., 2015; Yancey, 2005). In addition to endogenous GB accumulation, this osmoprotectant can be taken up after exogenous application and can be translocated through the phloem to the whole plant (Makela et al., 1996), so it has often been used as an osmoprotectant in agricultural practices. Exogenous foliar application of GB has been reported to increase plant resistance to drought and salt stress (e.g., Ashraf et al., 2008; Rhodes & Hanson, 1993; Zaman et al., 2015). Another compound with a protective effect is chitosan (CH), the deacetylated form of chitin, a non-toxic biodegradable polymer that has been widely used in agriculture due to several beneficial effects on plant growth and resistance

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to stress (Iriti et al., 2009; Pichyangkura & Chadchawan, 2015; Wang et al., 2015).

The aim of this work was to test the effect of foliar application of the osmoprotective compounds GB and CH on growth and appearance of potted *P. distans* plants subjected to two different levels of salt stress, achieved by irrigation with 200 and 600 mM of NaCl solutions.

MATERIALS AND METHODS

Experimental design, plant material, and treatments

Seeds of weeping alkaligrass, *P. distans* (Jacq.) Parl. (Bottos Sementi srl, Pordenone, Italy) were sown (0.25 g per pot) in plastic pots of 465.5 cm³ of volume (7 × 7 and 9.5 cm in depth) and placed in a controlled growth chamber at 25 °C temperature with 12 hr of illumination per day provided by cool white fluorescent tubes [photosynthetically active radiation (PAR) 150 μmol · m⁻² · s⁻¹]. The soil mixture was 45% sand, 45% organic rich soil (C/N = 25), 5% peat, and 5% organic manure. Maintenance fertilization was provided by adding 3 g of a slow release fertilizer (Osmocote Plus, Scotts, Europe BV, the Netherlands) per liter of soil mixture. After sowing, pots were irrigated weekly to field capacity with either distilled water, 200 mM or 600 mM of NaCl solutions. Starting 40 days after sowing, plants were kept at a cutting height of 6 cm by clipping weekly. A total of 60 pots were arranged in a total randomized design. Five pots were used as replicates for each treatment. The experiment ended 90 days after sowing, for a total of 13 saline treatments. For GB treatment, an aqueous 0.1-mol/L solution of a commercial formulation (Greenstim Verdera, Finland, 97.5% GB) was prepared. For CH treatment, a 0.1-mol/L solution was prepared dissolving 1 g of CH (Sigma-Aldrich, Milan, Italy, 85% degree of deacetylation) in distilled water acidified with acetic acid, final pH 5.5. These concentrations of osmoprotectants were chosen based on literature data and previous experimental trials (Ashraf & Foolad, 2007; Bittelli et al., 2001; Scalia et al., 2014). Osmoprotectant treatments were started 40 days after sowing. Each treated pot was sprayed weekly with 10 ml of the GB or CH solution, whereas controls were sprayed with distilled water. The effect of salinity, GB, and CH treatments on several functional traits was assessed at the end of the experiment.

Growth measurements

The overall condition of plants was monitored and the degree of leaf firing were visually estimated with the attribution of a percentage of chlorotic leaves compared to the total of each pot. After measuring shoot density, plants were removed from the pots, roots were washed with deionized water and blotted dry, and root length was measured as root extension from the stem bases to the farthest extending root. After measuring shoot length, leaves and shoots were removed and weighed for the determination of total shoot biomass fresh weight (FW). After recording FW, shoots were dried to constant weight at 70 °C for 48 hr, and dry weight (DW) was determined.

Water potential measurements

At the end of the experiment, before removing plants from the pots, shoots were sampled for leaf water potential (Ψ_L) determination. Measurements were carried out with a pressure chamber (SKPM 1400, Skye Instruments Ltd., Powys, UK). To measure leaf solute potential (Ψ_s), samples of fresh shoots were taken from each pot and used for the extraction of cell sap. Leaf samples were rinsed in distilled water, blotted dry, and placed in plastic hypodermic syringes. After freezing in liquid nitrogen and thawing, sap was expressed by hand and collected in an Eppendorf vial. The sap samples were centrifuged (Eppendorf Microfuge, Hamburg, Germany) to precipitate cell debris, and 50 μl of the supernatant was used for analysis of Ψ_s of cell sap. Osmolality of expressed shoot sap was measured with a cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Leaf turgor potential (Ψ_p) was calculated as the difference between Ψ_L and Ψ_s .

Data analysis

All data are presented as mean ± standard deviation. Data were analyzed with one-way analysis of variance (ANOVA), using the software package SigmaPlot 12 (Systat Software, Inc., San Jose, USA). Fisher's least significant difference multiple comparisons test ($p = .05$) was used to compare means when ANOVA was significant.

RESULTS

Growth measurements

Leaf firing is commonly used in grasses as an easily measured index of the survival rate of leaves. A certain degree of leaf firing was observed in all treatments: control plants at the end of the experiment showed a leaf firing of about 30%. Saline treatment increased the level of leaf firing from 30% to 40% only at the highest concentration tested, 600 mM, while irrigation with 200 mM of NaCl did not show any effect. GB application showed a beneficial effect on turf appearance even without saline treatment, slightly reducing leaf firing of the controls to 25% and of 600 mM-treated plants to 35%. Plants sprayed with CH showed in all treatments a more intense pigmentation and a 30% level of leaf firing, so this osmoprotectant had a slightly greater effect on leaf firing reduction at 600 mM than GB.

Shoot density is related to wear tolerance, an important trait for recreational turfgrass use. Shoot density did not differ significantly in non-salt controls with or without application of either GB or CH, and was on average of 17 shoots/cm² (Fig. 1a). Under 200 mM of saline treatment, shoot density was reduced to 11.8 shoots/cm², and spraying with GB significantly increased this value to 14 shoots/cm², whereas treatment with CH increased shoot density slightly but not significantly. Increasing saline concentration further decreased shoot density, which was reduced to 9.7 shoots/cm² at 600 mM of NaCl. In addition, in this case, GB treatment significantly increased shoot density to 10.7 shoots/cm², whereas CH treatment had no significant effect.

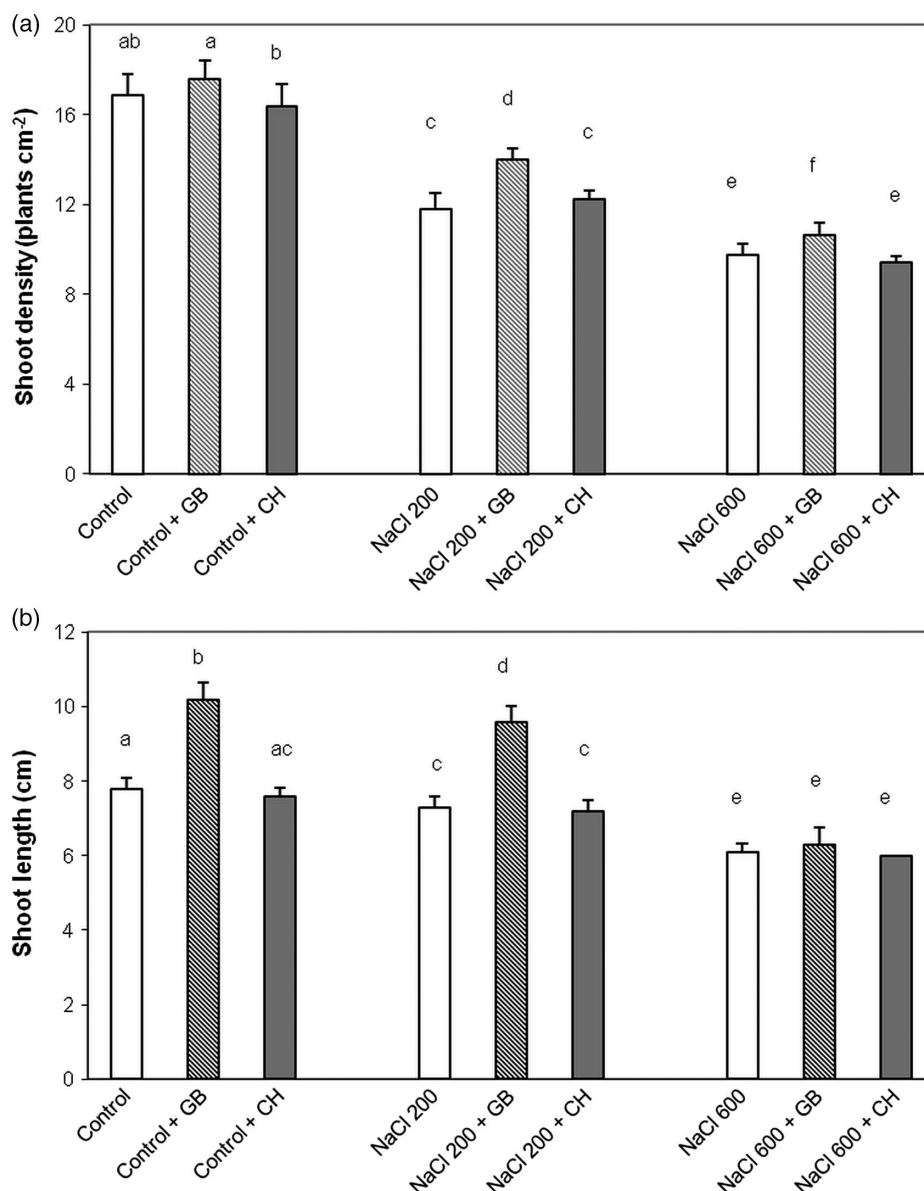


Fig. 1. Effects of salt stress and GB or CH application on shoot density (a) and shoot length (b) of *Puccinellia distans* at the end of a 90-day experimental cycle. The values are represented as mean \pm SD of five replicates. Different letters indicate significant differences (Fisher's LSD, $p < .05$)

Shoot length, root length, and fresh or dry biomass are often used to evaluate growth responses of grasses. Shoot length at the end of the experiment, before clipping, was on average 8 cm in no-salt control plants (Fig. 1b). Application of GB greatly increased shoot length, up to 10 cm, whereas the application of CH reduced shoot length slightly but not significantly compared to controls. Similar results were found for 200 mM of NaCl-treated plants: saline treatment alone or combined with CH application reduced shoot length to 7 cm, while GB application increased shoot length to 9.6 cm. A further increase in saline concentration reduced growth in all treatments: shoot length at the end of the experiment was 6 cm and neither GB nor CH application affected this parameter at 600 mM of NaCl (Fig. 2). The effect of saline treatment on shoot biomass depended on NaCl concentration (Fig. 3): at 200 mM, there was no significant effect on fresh shoot biomass, whereas at 600 mM, there was a reduction in FW and DW of 42% and 27%, respectively. Application of

GB significantly increased FW in all treatments, 48% when compared to no-salt controls, 35% with respect to 200 mM of NaCl treatment, and up to 80% when compared to 600 mM of NaCl treatment. There was also a slight increase in DW with GB application, particularly evident in the 600 mM treatment where a 40% increase was measured. Application of CH resulted in a small increase in FW when compared to controls, between 10% and 20%, but a 20% decrease in DW in both saline treatments, which was significant for 600 mM treatment.

Root length decreased under saline treatment, and this decrease was greater with rising NaCl concentrations, going from 10 cm in no-salt controls to 7 cm in the 600-mM treatment (Fig. 4). A reduction in root length with increasing saline concentration was also observed in GB-treated plants; this reduction was significant when compared with no-salt controls, but not at 200 and 600 mM of NaCl treatments. The application of CH also reduced root length, but this did

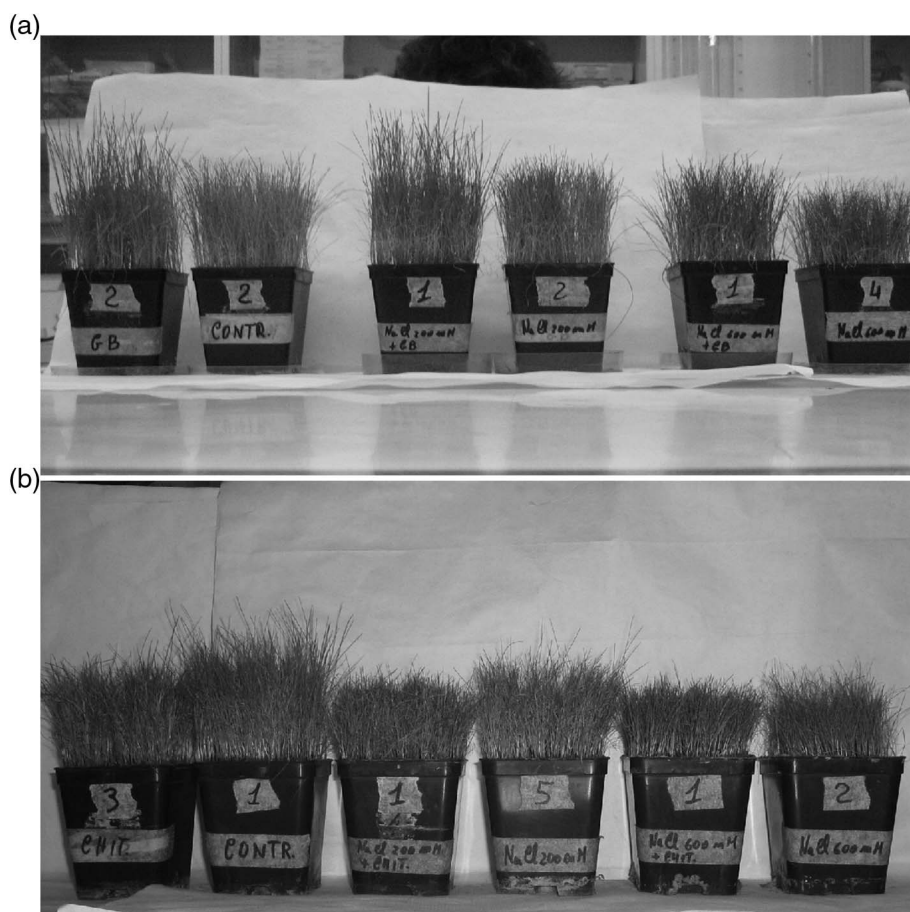


Fig. 2. Appearance of representative samples of *Puccinellia distans* at the end of the experiment, 90 days after sowing, treated with (a) GB or (b) CH

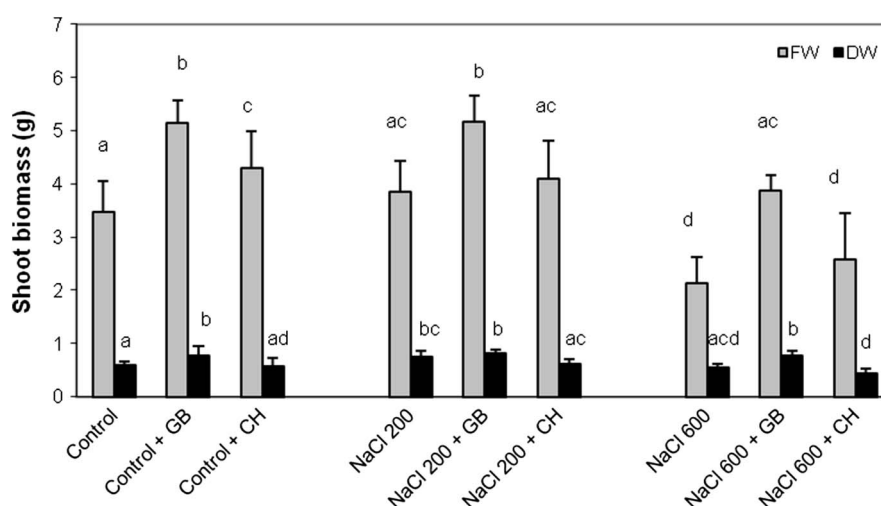


Fig. 3. Effects of salt stress and GB or CH application on shoot biomass of *Puccinellia distans* at the end of a 90-day experimental cycle. The values are represented as mean \pm SD of five replicates. Different letters indicate significant differences within either DW or FW data (Fisher's LSD, $p < .05$)

not appear to be related to saline concentration, as in all treatments, root length was 6 cm (Fig. 5).

Water potential measurements

To adapt to saline environments, plants must be able to adjust their water potential in order to maintain water uptake

from the soil. Shoot water potential was on average -0.3 MPa in no-salt control plants, and was not affected by GB or CH application (Fig. 6a). Under 200 mM of saline treatment, Ψ_L decreased to -1.5 MPa. GB treatment allowed partial recovery of Ψ_L that reached -0.8 MPa, whereas CH application had no significant effect. A further drop in Ψ_L was measured under saline 600 mM treatment, down to

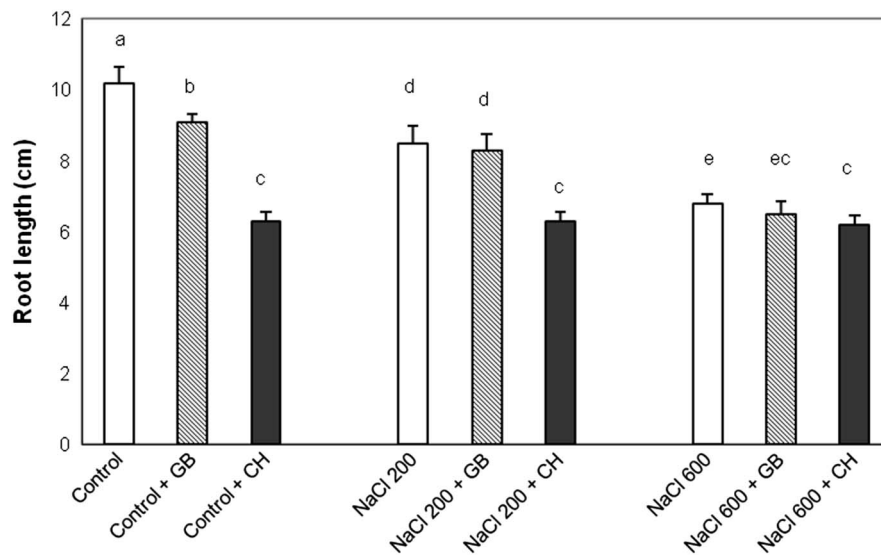


Fig. 4. Effects of salt stress and GB or CH application on root length of *Puccinellia distans* at the end of a 90-day experimental cycle. The values are represented as mean \pm SD of five replicates. Different letters indicate significant differences (Fisher's LSD, $p < .05$)

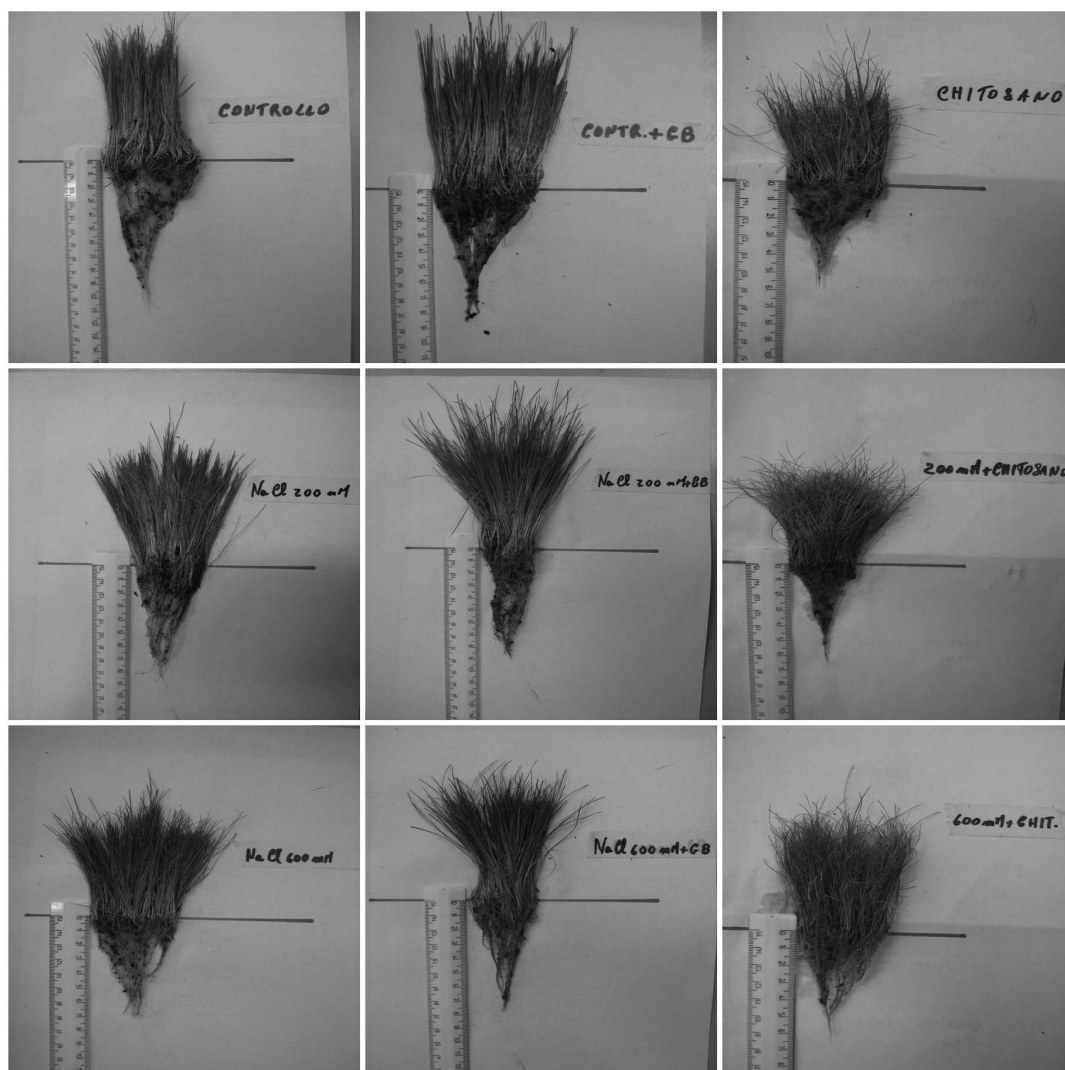


Fig. 5. Representative samples of *Puccinellia distans* at the end of the experiment, 90 days after sowing, showing root and shoot length

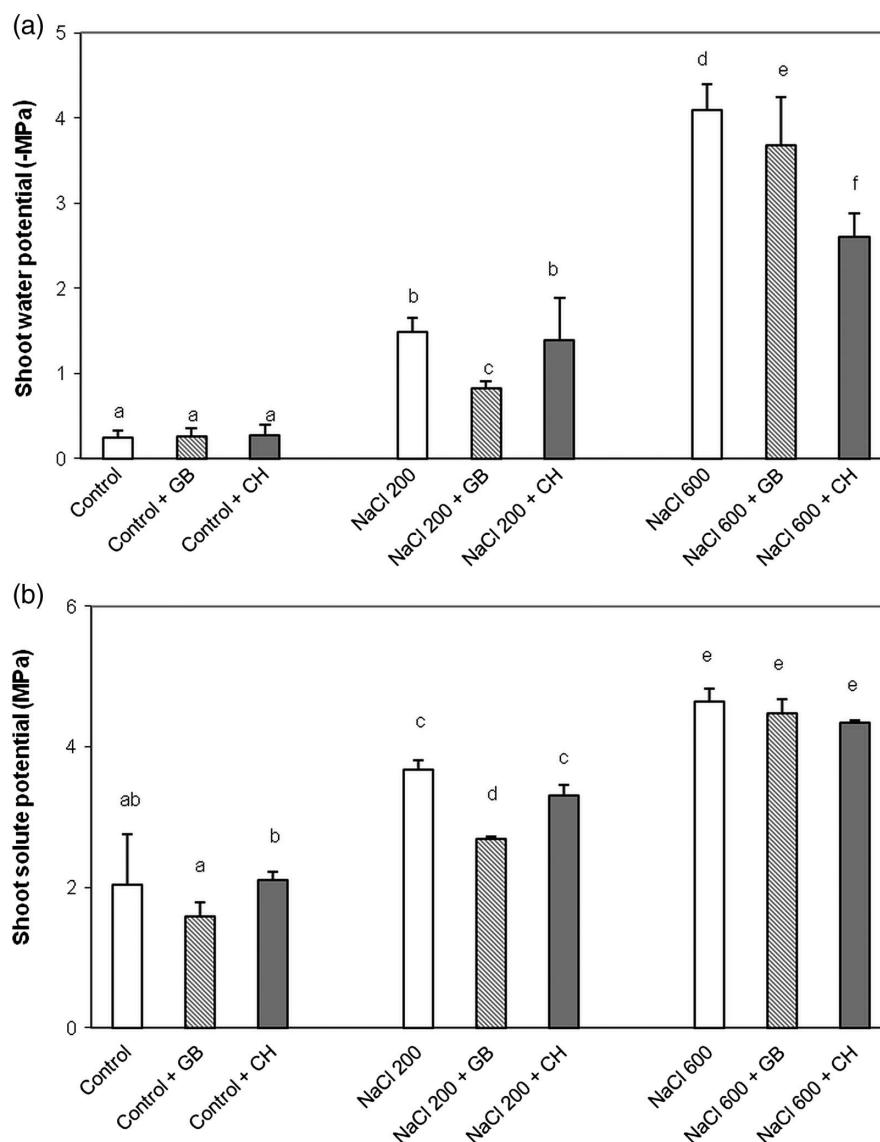


Fig. 6. Effects of salt stress and GB or CH application on shoot water potential (a) and shoot solute potential (b) of *Puccinellia distans* at the end of a 90-day experimental cycle. The values are represented as mean \pm SD of five replicates. Different letters indicate significant differences (Fisher's LSD, $p < .05$)

–4 MPa, and also in this case GB treatment significantly improved plant water status. At this saline concentration, application of CH resulted in an increase in Ψ_L up to –2.6 MPa. Solute potential of expressed sap from the leaves was on average 2.0 MPa in no-salt controls, and application of GB or CH did not significantly affect this parameter (Fig. 6b). Solute potential increased significantly with saline concentration. At 200 mM, Ψ_s was 3.7 MPa; GB application reduced Ψ_s to 2.7 MPa, while the effect of CH application on Ψ_s was not significant. At 600 mM, Ψ_s ranged from 4.6 to 4.3 MPa and no significant differences were evident with either GB or CH application. Pressure potential values were around 1.3–1.8 MPa in no-salt controls with or without GB or CH treatment. Turgor pressure was maintained at positive values also at 200 mM, while Ψ_p was closer to turgor loss point at 600 mM for controls (0.5 MPa), increased slightly with GB treatment (0.8 MPa), and reached values comparable to no-salt controls with CH treatment (1.7 MPa).

DISCUSSION

Leaf firing, shoot biomass, and shoot and root length have been widely used as traits to determine the response to salinity of turfgrasses (Guo et al., 2016; Marcum, 1999). Healthy appearance and high shoot density are key factors in the evaluation of turfgrass quality for recreational purposes, and irrigation with brackish water or the direct effect of salt spray in coastal areas can cause leaf firing and shoot fade. *P. distans* can tolerate relatively high saline concentrations (Akhzari et al., 2012; Tarasoff et al., 2007), and our data showed a significant increase in leaf firing only at the highest concentration tested, that is 600 mM of NaCl. Leaf firing recorded in no-salt controls can be explained by the length of the experimental period, quite longer than for other reported data (e.g., Guo et al., 2016). The degree of injury with GB or CH application was lower than that of a widely used halophyte grass, *Zoysia matrella*, which showed 39%

leaf firing at a salinity level of approximately 480 mM (Uddin et al., 2012). Shoot density was negatively affected already at 200 mM, and while CH application had no effect on shoot density, GB increased it at all saline concentrations, which is a desirable effect as this trait is not only an important component of turf visual quality, but it is also related to wear tolerance (Głab et al., 2015). On the other hand, the effect of CH on leaf firing was more pronounced than that of GB at 600 mM of saline treatment, resulting in greener, less damaged shoots. CH application has been reported to induce plant defense mechanisms, mediated by an oxidative burst response, and in several species CH application resulted in increased chlorophyll content (Pichyangkura & Chadchawan, 2015).

One of the requirements of turf management is to reduce the frequency of cuts, so the cost-benefits trade-off of osmoprotectant application must be evaluated. GB consistently increased shoot length by 30% in control treatments and 200 mM of saline treatment, thus increasing the frequency of cuts to maintain the desired turf height. At higher saline concentration, instead, there was no increase in shoot length, so the positive effects on turf color and density were obtained without further costs in terms of more frequent cuts. CH did not affect shoot length in any treatment, but on the other hand did not increase turf density under saline conditions.

P. distans showed osmotic adjustment with increasing salinity, as would be expected for a halophyte and as reported also for another species of the genus, *Puccinellia tenuiflora* (Guo et al., 2010). The natural adaptability of *Puccinellia* to salt stress conditions was further enhanced by GB application. GB has a well-documented effect as osmoprotectant, and maintaining a better water status allows greater gas exchange levels and photosynthesis, and higher turgor driving cell expansion and shoot growth. Furthermore, GB application has been reported to increase leaf nitrogen content (Zhang et al., 2014), thus contributing to growth enhancement. Indeed, foliar application of GB resulted in higher levels of fresh biomass, due to greater tissue water content of the shoots, which can reduce the toxic effects of high levels of NaCl (Marcum & Murdoch, 1990). The better water status was confirmed by less negative Ψ_L values than in the saline treatments, particularly at 200 mM. The effect of GB in increasing water content was also evident from the osmotic potential of expressed sap, which was lower under this treatment except for 600 mM. At the higher saline concentration, GB treatment did not avoid the decrease in average cell turgor pressure, which was comparable to that of the 600 mM of NaCl control. The positive effect of GB on growth in all saline treatments was confirmed by the increase in dry biomass. The ability to maintain higher growth rates under saline conditions is a typical trait for salt-tolerant turfgrasses (Marcum & Murdoch, 1994), and in *P. distans* GB application indeed reestablished shoot growth back to control conditions. Many halophytes show a typical enhancement of growth with low levels of salinity (Greenway & Munns, 1980) but in this case it was not evident. This is however in agreement with data reported for other species of *Puccinellia* that did not show stimulation of growth at low salt concentrations (Dashtebani et al., 2014; Flowers & Colmer, 2008;

Shabala & Mackay, 2011). In previous experiments, we found an increase in growth at low levels of salinity only up to 40 days after sowing (Scalia et al., 2009), while data here reported were taken 90 days after sowing. CH application did not result in significantly increased growth in any of the treatments, either in terms of fresh or dry shoot biomass. CH treatment gave better results than GB for some traits only under 600 mM conditions, improving leaf firing and overall shoot color, and increasing leaf water potential, which was found to be less negative than in the other treatments, possibly due to the effect of CH on the reduction of transpiration (Bittelli et al., 2001). Halophytes are generally able to increase reactive oxygen species scavenging systems under salt stress, but the extent of this response is dependent on genotype and salinity level (Dashtebani et al., 2014). As CH application has also been reported to increase antioxidant activity, the contribution to salt tolerance could occur through a protective effect on cell membrane integrity (Yang et al., 2009, 2012).

The relation between root growth and salt tolerance is not straightforward, as extensive root growth is often (Dudeck & Peacock, 1985; Marcum & Kopec, 1997) but not always (Marcum & Murdoch, 1990) related to high salt tolerance. In *P. distans*, we found that root length gradually decreased with increasing saline concentration, as reported, for example, in maize varieties (Hussain et al., 2014). Application of osmoprotectants determined a reduction of root length in the no-salt controls. In the case of GB application, this could be explained as a result of increased allocation of resources to the shoots, which increased in both length and biomass. This however could not explain the reduction of root length with CH application, as in this case there was only a slight and not significant increase in shoot biomass. Furthermore, the effect of CH application on root length was constant and did not vary with saline concentration. In *Paspalum* accessions grown under salt stress, water status traits were more strongly related to shoot than to root growth, suggesting that different factors were necessary to explain root growth (Lee et al., 2005).

Our data confirm that *P. distans* is a well-suited choice for turfs exposed to salt stress. While CH was more effective at improving shoot water status and visual quality at the highest saline concentration tested, a comprehensive evaluation of the different traits showed that treatment with GB gave the best overall results as an osmoprotectant, improving health, growth, and visual quality of the turf under both levels of salinity tested.

CONCLUSION FOR FUTURE BIOLOGY

Turfgrass industry requires sustainable management and efficient water and resource use. Availability and cost of irrigation water are serious threats to landscape development and to the expansion and maintenance of sport fields, golf courses, and other turfgrass areas, especially in saline environments (Aamlid et al., 2016; Kurepin et al., 2015). Although extrapolation of results from controlled conditions to field conditions requires careful attention and is not always straightforward (Poorter et al., 2016; Yancey, 2005), screening for responses in controlled systems is a first step

toward the selection of species, varieties, and treatments best suited for turfs exposed to salt stress.

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REFERENCES

- Aamlid, T. S., Knox, J. W., Riley, H., Kvalbein, A., Pettersen, T. (2016) Crop coefficients, growth rates and quality of cool-season turfgrasses. *J. Agro. Crop Sci.* 202, 69–80.
- Akhzari, D., Sepehry, A., Pessarakli, M., Barani, H. (2012) Studying the effects of salinity stress on the growth of various halophytic plant species (*Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans*). *World Appl. Sci. J.* 16, 998–1003.
- Ashraf, M., Foolad, M. R. (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 59, 206–216.
- Ashraf, M., Nawaz, K., Athar, H. R., Raza, S. H. (2008) Growth enhancement in two potential cereal crops, maize and wheat, by exogenous application of glycinebetaine. In: Abdelly, C., Ozturk, M., Ashraf, M., Grignon, C. (eds.) *Biosaline Agriculture and High Salinity Tolerance*. Birkhauser, Switzerland, pp. 21–35.
- Bittelli, M., Flury, M., Campbell, G. S., Nichols, E. J. (2001) Reduction of transpiration through foliar application of chitosan. *Agric. Forest Meteorol.* 10, 67–175.
- Chen, T. H. H., Murata, N. (2011) Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ.* 34, 1–20.
- Dashtebani, F., Hajiboland, R., Aliasgharzad, N. (2014) Characterization of salt-tolerance mechanisms in mycorrhizal (*Claroideoglomus etunicatum*) halophytic grass, *Puccinellia distans*. *Acta Physiol. Plant.* 36, 1713–1726.
- Dudeck, A. E., Peacock, C. H. (1985) Effects of salinity on seashore paspalum turf grasses. *Agron. J.* 11, 47–50.
- Flowers, T. J., Colmer, T. D. (2008) Salinity tolerance in halophytes. *New Phytol.* 179, 945–963.
- Fry, J., Huang, B. (2004) *Applied Turfgrass Science and Physiology*. Wiley, Hoboken, NJ.
- Glab, T., Szewczyk, W., Dubasc, E., Kowalika, K., Jezierski, T. (2015) Anatomical and morphological factors affecting wear tolerance of turfgrass. *Sci. Hort.* 185, 1–13.
- Greenway, H., Munns, R. (1980) Mechanism of salt tolerance in nonhalophytes. *Annu. Rev. Plant Phys.* 31, 149–190.
- Guo, H., Wang, Y., Dandan, L., Chena, J., Zonga, J., Wang, Z., Chenc, X., Liu, J. (2016) Growth response and ion regulation of seashore paspalum accessions to increasing salinity. *Environ. Exp. Bot.* 131, 137–145.
- Guo, L. Q., Shi, D. C., Wang, D. L. (2010) The key physiological response to alkali stress by the alkali-resistant halophyte *Puccinellia tenuiflora* is the accumulation of large quantities of organic acids and into the rhizosphere. *J. Agric. Crop Sci.* 196, 123–135.
- Hussain, I., Arslan Ashraf, M., Anwar, F., Rasheed, R., Niaz, M., Wahid, A. (2014) Biochemical characterization of maize (*Zea mays* L.) for salt tolerance. *Plant Biosyst.* 148, 1016–1026.
- Iriti, M., Picchi, V., Rossoni, M., Gomarasca, S., Ludwig, N., Gargano, M., Faoro, F. (2009) Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. *Environ. Exp. Bot.* 66, 493–500.
- Kurepin, L. V., Ivanov, A. G., Zaman, M., Pharis, R. P., Suleyman, I., Allakhverdiev, S. I., Hurry, V., Hüner, N. P. A. (2015) Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. *Photosynth. Res.* 126, 221–235.
- Lee, G., Carrow, R. N., Duncan, R. R. (2005) Growth and water relation responses to salinity stress in halophytic seashore paspalum ecotypes. *Sci. Hort.* 104, 221–236.
- Makela, P., Pelttonen-Sainio, P., Jokinen, K., Pehu, E., Setälä, H., Hinkkanen, R., Somersalo, S. (1996) Uptake and translocation of foliar-applied glycinebetaine in crop plants. *Plant Sci.* 121, 221–230.
- Marcum, K. B. (1999) Salinity tolerance mechanisms of grasses in the subfamily *Chloridoideae*. *Crop Sci.* 39, 1153–1160.
- Marcum, K. B., Kopec, D. M. (1997) Salinity tolerance of turfgrasses and alternative species in the subfamily *Chloridoideae* (*Poaceae*). *Int. Turfgrass Soc. Res. J.* 8, 735–742.
- Marcum, K. B., Murdoch, C. L. (1990) Growth responses, ion relations, and osmotic adaptations of eleven C4 turfgrasses to salinity. *Agron. J.* 82, 892–896.
- Marcum, K. B., Murdoch, C. L. (1994) Salinity tolerance mechanisms of six C4 turfgrasses. *J. Am. Soc. Hortic. Sci.* 119, 779–784.
- Munns, R., Tester, M. (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681.
- Pichyangkura, R., Chadchawan, S. (2015) Biostimulant activity of chitosan in horticulture. *Sci. Hortic.* 196, 49–65.
- Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., van der Putten, W. H., Kleyer, M., Schurr, U., Postma, J. (2016) Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytol.* 212, 838–855.
- Rhodes, D., Hanson, A. D. (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Biol.* 44, 357–384.
- Scalia, R., Oddo, E., Russo, G., Saiano, F., Grisafi, F. (2014) Effectiveness of glycinebetaine foliar application in relieving salt stress symptoms in two turf-grasses. *Grassl Sci.* 60, 92–97.
- Scalia, R., Oddo, E., Saiano, F., Grisafi, F. (2009) Effect of salinity on *Puccinellia distans* (L.) Parl. treated with NaCl and foliarly applied glycinebetaine. *Plant Stress* 3, 49–54.

- Shabala, S., Mackay, A. (2011) Ion transport in halophytes. *Adv. Bot. Res.* 57, 151–199.
- Tarasoff, C. S., Mallory-Smith, C. A., Ball, D. A. (2007) Comparative plant responses of *Puccinellia distans* and *Puccinellia nuttalliana* to sodic versus normal soil types. *J. Arid Environ.* 70, 403–417.
- Uddin, K., Juraimi, A. S., Ismail, M. R., Hossain, Md. A., Othman, R., Rahim, A. A. (2012) Physiological and growth responses of six turfgrass species relative to salinity tolerance. *Sci. World J.* 2012, 1–10.
- Wang, M., Chen, Y., Zhang, R., Wang, W., Zhao, X., Du, Y., Yin, H. (2015) Effects of chitosan oligosaccharides on the yield components and production quality of different wheat cultivars (*Triticum aestivum* L.) in Northwest China. *Field Crops Res.* 172, 11–20.
- Yancey, P. H. (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* 208, 2819–2830.
- Yang, F., Hu, J., Li, J., Wu, X., Qian, Y. (2009) Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. *Plant Growth Regul.* 58, 131–136.
- Yang, Z., Yu, J., Merewitz, E., Huang, B. (2012) Differential effects of abscissic acid and glycine betaine on physiological responses to drought and salinity stress for two perennial grass species. *J. Am. Soc. Hort. Sci.* 137, 96–106.
- Zaman, M., Kurepin, L. V., Catto, W., Pharis, R. P. (2015) Enhancing crop yield with the use of N-based fertilizers co-applied with plant hormones or growth regulators. *J. Sci. Food Agric.* 95, 1777–1785.
- Zhang, L. X., Lai, J. H., Gao, M., Ashraf, M. (2014) Exogenous glycinebetaine and humic acid improve growth, nitrogen status, photosynthesis, and antioxidant defense system and confer tolerance to nitrogen stress in maize seedlings. *J. Plant Interact.* 9, 159–166.