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Research Article

Effects of glycine betaine application on growth and quality of four cool-season turfgrass species under salinity stress

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Abstract

In arid and semi-arid regions, the use of saline irrigation water in landscape systems is becoming increasingly unavoidable, yet salinity adversely affects turfgrass growth and visual quality. Glycine betaine (GB), a compatible solute, has been proposed as a potential osmoprotectant to mitigate salt-induced stress. This study evaluated the responses of four cool-season turfgrass species *(Festuca rubra* subsp. *rubra*, *Lolium perenne*, *Festuca arundinacea*, and *Poa pratensis*) to foliar-applied GB under non-saline and saline (6 dS m^{-1}) conditions in a controlled greenhouse experiment. Salinity negatively influenced turf quality, turf color, chlorophyll content, and biomass parameters; however, the magnitude of these effects varied significantly among species. Significant species × salinity and species × GB interactions indicated that plant responses to both salt stress and GB application were strongly species-dependent. *F. arundinacea* and *P. pratensis* exhibited comparatively greater stability in growth and visual quality under salinity, whereas *L. perenne* showed higher sensitivity. Although GB did not exert a consistent main effect across all parameters, it partially mitigated salinity-induced reductions in specific species and treatment combinations. These findings highlight that turfgrass tolerance to salinity and the effectiveness of GB application are governed primarily by genotype-dependent responses rather than uniform treatment effects. The results underscore the importance of species selection as a primary strategy for saline turf management, with GB serving as a potentially supportive but species-specific intervention.

Keywords: glycine betaine; growth performance; osmoprotectant; saline conditions; turfgrass irrigation; turfgrass management

Introduction

In arid and semi-arid climates with limited water resources, using salt-tolerant turfgrass species and alternative water sources for irrigation is an effective cultural practice that helps alleviate pressure on freshwater resources (Alshammary *et al*., 2004; Marcum, 2006). Salinity leads to osmotic stress, ion toxicity, and nutrient imbalances, which disrupt cellular homeostasis, inhibit photosynthesis, and ultimately reduce both aesthetic and functional turfgrass quality (Parida and Das, 2005; Liu *et al*., 2023). The effects of salt stress vary by species,

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variety, salt source, and exposure duration, with genotypic differences leading to varied responses in saline environments (Dajic, 2006).

Among the strategies developed to mitigate salinity stress, the exogenous application of compatible solutes-especially glycine betaine (GB) has received growing attention. GB is a naturally occurring osmoprotectant found in many halophytic plants (Scalia *et al*., 2009; Yang *et al*., 2012; Oddo *et al*., 2019; Siddiqui *et al*., 2021). It plays a crucial role in stabilizing proteins and membranes, protecting the photosynthetic apparatus, and maintaining osmotic balance under salt stress (Yang and Lu, 2005; Pei *et al*., 2022). Numerous studies highlight the positive effects of exogenous GB on plant growth and yield under stress conditions (Makela *et al*., 1998; Rajashekar *et al*., 1999; Rezaei *et al*., 2012; Dai *et al*., 2024; Lamlom *et al*., 2025). However, the capacity to synthesize or translocate GB varies among species. Thus, external supplementation becomes particularly useful for species with low endogenous GB accumulation or limited transport to developing tissues (Scalia *et al*., 2014). Despite this, relatively few studies have focused on turfgrass responses to foliar GB application, especially under controlled saline conditions (Alshammary *et al*., 2004; Scalia *et al*., 2009; Yang *et al*., 2012; Hu *et al*., 2012; Scalia *et al*., 2014; Oddo *et al*., 2019; Mohammadi *et al*., 2024). Among the species evaluated in this study, previous research has shown that foliar-applied GB alleviated salt-induced damage by enhancing membrane stability and antioxidant activity in *Poa pratensis* (Yang *et al*., 2012) and improved ionic balance and oxidative stress tolerance in *Lolium perenne* (Hu *et al*., 2012). In *Festuca arundinacea*, improvements in growth performance under salinity have been reported following exogenous GB application (Scalia *et al*., 2014; Liu *et al*., 2023). Although direct evidence is limited for *Festuca rubra* subsp. *rubra*, its physiological similarity to other fine fescues suggests a comparable response to exogenous GB.

Although GB has been applied to the cool-season turfgrass species mentioned above, the findings across studies remain inconsistent. While Hu *et al*. (2012) demonstrated significant increases in chlorophyll content and biomass production in *L. perenne* under salinity following GB application, Scalia *et al*. (2014) and Yang *et al*. (2012) reported species-dependent responses in *F. arundinacea* and *P. pratensis*, respectively. Similarly, Oddo *et al*. (2019) observed only partial mitigation of salt-induced reductions in shoot growth in *Puccinellia distans*, suggesting that the magnitude of GB effectiveness may vary among species and stress levels. Root growth responses also appear variable; Hu *et al*. (2012) reported improved root biomass in *L. perenne*, whereas Scalia *et al*. (2014) indicated that below-ground responses were less pronounced and dependent on salinity severity. Differences in experimental conditions, including salinity intensity (e.g., moderate EC levels around 6 dS m^{-1} as reported by Alshammary *et al*., 2004 versus high NaCl concentrations used in other controlled experiments such as Oddo *et al*., 2019), growth systems (soil-based vs. controlled environments), and GB concentration, likely contribute to these variations. Furthermore, most previous investigations have evaluated individual turfgrass species independently (Hu *et al*., 2012; Yang *et al*., 2012; Scalia *et al*., 2014), limiting standardized interspecific comparisons under uniform salinity stress. Consequently, it remains unclear whether GB consistently enhances both above-ground and below-ground growth parameters across different cool-season turfgrass species exposed to similar saline stress levels.

The reported variability in GB responses may be related to its multifaceted physiological roles under salinity stress. Exogenous GB has been suggested to enhance stress tolerance through mechanisms such as membrane stabilization, improved ion homeostasis, protection of the photosynthetic apparatus, and regulation of antioxidant systems (Ashraf and Foolad, 2007; Hu *et al*., 2012; Yang *et al*., 2012). Although these mechanisms were not directly evaluated in the present study, they provide a conceptual framework for interpreting potential GB-mediated responses under saline conditions. The proposed pathways are illustrated in Figure 1.

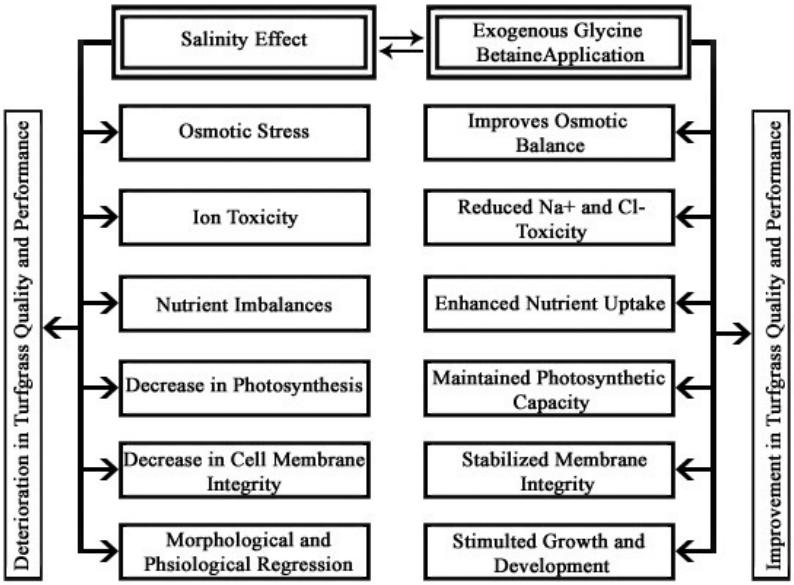

Figure 1. Conceptual illustration of the proposed physiological mechanisms by which exogenous GB may alleviate salinity stress in plants, based on previous literature

Despite the growing body of research on GB application in turfgrass species, most previous studies have evaluated individual species separately and under heterogeneous experimental conditions, limiting direct comparison of species-specific responses under standardized salinity stress. In addition, empirical evidence regarding the response of *F. rubra* subsp. *rubra* to exogenous GB application remains limited. The present study was designed to help address these limitations by employing a uniform factorial design (species × salinity × GB) under a controlled salinity level (6 dS m^{-1}), thereby enabling direct interspecific comparison of both visual quality parameters and biomass allocation traits within the same experimental framework. By integrating aboveground and belowground growth responses, this study provides a more comprehensive assessment of species-dependent tolerance patterns and the species-specific effectiveness of GB under saline irrigation. We hypothesize that GB application may improve growth and quality parameters under saline conditions; however, the magnitude and direction of this response are expected to vary among species. Accordingly, the objective of this study was to systematically evaluate species-specific responses to salinity and GB application within a standardized experimental context.

Materials and Methods

Plant materials, treatments, and experimental design

The experiment involved four cool-season turfgrass species commonly used in landscape areas, particularly in transitional and cool-climate regions: *Festuca rubra* L. subsp. *rubra* 'Navigator II', *Lolium perenne* L. 'Blackcat', *Festuca arundinacea* (syn. *Schedonorus arundinaceus*) L. 'Barlexas II', and *Poa pratensis* L. 'Baron'. Hereafter, FRR is referred to *Festuca rubra* L. subsp. *rubra*, LP to *Lolium perenne* L., FA to *Festuca arundinacea* L., and PP to *Poa pratensis* L. in the context of this study. The study was conducted at Akdeniz University, Antalya, Türkiye, in 2019. The experiment utilized 18 cm diameter x 20 cm deep plastic pots filled with a growing medium of 50% sand, 25% peat, and 25% perlite. Seeds were planted in pots at 20, 35, 45, and 7.5 g m^{-2} for FRR, LP, FA, and PP, respectively. Pots were maintained in a greenhouse for three months with an average temperature of 25/20 °C (day/night), a 13-14-hour photoperiod, and supplemental lighting to achieve photosynthetically active radiation level of 400 µmol/m^2s. During the establishment period, the pots

received 2.5 N g m^{-2} from a balanced fertilizer (20-20-20; N-P_2O_5-K_2O) to ensure adequate turf development. Fertilization was discontinued before the initiation of salinity treatments, and no fertilizer was applied during the salinity or GB application period in order to avoid confounding effects on plant stress responses. Turfgrass was mowed weekly to a uniform height of 5 cm for all species. This mowing height was selected to maintain standard management conditions for cool-season turfgrasses while avoiding additional mechanical stress during salinity treatment.

The treatments for each of the species were as follows: control (SAL-, GB-; no GB or salinity stress was applied), foliar sprayed GB (SAL-, GB+), saline irrigation (SAL+, GB-) and saline irrigation with foliar sprayed GB (SAL+, GB+). Each treatment was replicated three times for each species in a completely randomized design, totaling 48 pots. Control (SAL−, GB−) and GB-only (SAL−, GB+) pots were irrigated with tap water having an electrical conductivity (EC) of 0.54 dS m^{-1} throughout the experiment. Salinity treatment (SAL+) was initiated on 05 February 2019 by gradually increasing the EC of the irrigation solution by 1 dS m^{-1} per day to avoid osmotic shock. The target salinity level of 6 dS m^{-1} was reached on 19 February 2019. After reaching this level, irrigation of the salinity treatments (SAL+) was continued at 6 dS m^{-1} for the remainder of the experimental period, whereas non-saline treatments (SAL−) continued to receive tap water (0.54 dS m^{-1}).

GB treatment was applied four times at weekly intervals as a foliar spray. The first application was initiated two weeks after the salinity level had reached and stabilized at 6 dS m^{-1}. A GB solution was prepared at a concentration of 2.28 g L^{-1}, selected based on effective ranges reported in previous studies investigating the mitigation of abiotic stresses in turfgrass and other plant species (Gibon *et al*., 1997; Allard *et al*., 1998; Díaz-Zorita *et al*., 2001; Raza *et al*., 2014; Keough, 2016). The solution was applied at a volume of 150 mL per pot, corresponding to the amount required to uniformly wet the plant canopy in pots with a surface area of 0.025 m^2. This resulted in 0.342 g GB per pot, equivalent to an application rate of 13.44 g m^{-2}. To maintain consistency across treatments, the same volume of distilled water was sprayed on non-GB control groups. Following the completion of the four GB applications, measurements were initiated and conducted weekly for five consecutive weeks. Therefore, Week 1 represents the first week after the final GB application.

Growth measurements and data analysis

Turfgrass resistance to salt is assessed through root-shoot growth metrics and overall quality (Marcum and Kopec 1997). Weekly data were collected on turfgrass quality (TQ), turfgrass color (TC), chlorophyll content (CC), clipping dry weight (CDW), starting one week after GB application. The clippings were then dried at 72 °C for 48 hours (until constant weight was achieved) to determine weekly CDW.

TQ includes factors such as color, density, uniformity, texture, disease resistance, and tolerance to environmental pressures. TQ was recorded weekly on a 1-9 scale (1 = poorest quality, 6 = acceptable, 9 = best) and TC was assessed weekly using a 1-9 visual scale (1 = straw brown, 6 = light green, 9 = dark green) (Sever Mutlu *et al*., 2011; Sever Mutlu *et al*., 2017).

CC was weekly measured using a Field Scout CM 1000 Chlorophyll Meter (Spectrum Techn., Inc., Plainfield, IL, USA), which calculates relative chlorophyll concentration by assessing the ratio of reflected red and far-red light. This meter produces a unitless index ranging from 0 to 999 (Çakır *et al*., 2024). Measurements were taken between 12:00 and 14:00 on sunny days, holding the meter approximately 1,3 meters from the leaf surface with the sun behind the device (Geng *et al*., 2014). Ten readings per plot were averaged to determine CC.

At the end of the experiment, the plants were removed from the pots, and the roots were carefully separated from the canopy. The roots were washed with distilled water to remove growing medium residues. The weighed shoots and roots were dried at 72 °C for 48 hours (until constant weight was achieved) and then weighed to determine shoot dry weight (SDW) and root dry weight (RDW).

TQ, TC, CC, and CDW were collected every week and averaged on a pot-by-pot basis to condense and simplify the data presentation. RDW and SDW were measured only once at the end of the experiment

following harvest. Statistical analyses were conducted using SPSS version 29.0.2.0 (IBM Corp., Armonk, NY, USA). The experiment followed a completely randomized factorial design with three fixed factors: species (4 levels), salinity (2 levels), and GB application (2 levels). Data were analysed using three-way analysis of variance (ANOVA) to test main effects and interaction effects. Normality was assessed using Shapiro–Wilk test and Q–Q plots; homogeneity by Levene's test. For weekly measurements (TQ, TC, CC, and CDW), separate three-way ANOVA models were performed for each evaluation week. In addition, weekly values were averaged per pot across the five evaluation weeks, and the same factorial ANOVA structure was applied to the averaged (AVG) data to summarize overall treatment effects across time. RDW and SDW, measured once at harvest, were analyzed using the same factorial ANOVA structure. Prior to ANOVA, assumptions of normality and homogeneity of variances were verified. When significant main or interaction effects were detected ($p < 0.05$), means were separated using Duncan's multiple range test. Data are presented as mean ± standard deviation ($n = 3$). Pearson correlation coefficients were calculated to assess relationships among the measured variables using averaged data per pot.

Results

Species significantly affected CC in all five evaluation weeks and in the overall average (Table 1). Salinity showed significant effects in three weeks and in the average values, whereas GB application was significant in four weeks and in the overall average. No species × salinity or salinity × GB interactions were detected. However, species × GB interaction was significant in two weeks and in the average values. A three-way interaction (species × salinity × GB) was detected in one week.

Table 1. Effects of turfgrass species, salinity, and GB on relative chlorophyll content (CC), measured weekly for five weeks after the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1})

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Avg.
Species (SP)						
F. rubra	204±34^{a*†}	229±50^{b}	196±24^{b}	165±50^{b}	165±51^{b}	192±29^{b}
L. perenne	177±26^{b}	207±46^{b}	178±26^{bc}	167±61^{b}	153±38^{b}	176±27^{b}
F. arundinacea	213±29^{a}	295±51^{a}	215±28^{a}	227±42^{a}	218±39^{a}	234±21^{a}
P. pratensis	196±36^{ab}	240±54^{b}	177±22^{c}	190±36^{ab}	144±37^{b}	190±24^{b}
Salinity (SAL)						
SAL (+)	201±32	234±49	185±27^{b}	168±26^{b}	149±24^{b}	188±35^{b}
SAL (-)	194±36	251±51	198±29^{a}	207±47^{a}	191±51^{a}	208±23^{a}
Glycine betaine (GB)						
GB (+)	205±26^{a}	259±41^{a}	188±32^{a}	206±26^{a}	185±47^{a}	209±54^{a}
GB (-)	190±25^{b}	226±38^{b}	195±49^{a}	169±36^{b}	155±42^{b}	187±41^{b}
SP	*	*	*	*	*	*
SAL	ns	ns	*	*	*	*
GB	*	*	ns	*	*	*
SP × SAL	ns	ns	ns	ns	ns	ns
SP × GB	*	ns	ns	*	ns	*
SAL × GB	ns	ns	ns	ns	ns	ns
SP × SAL × GB	ns	*	ns	ns	ns	ns

Data are presented as mean ± standard deviation ($n = 3$). Week 1–5 represent weekly measurements following the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1}). ANOVA was conducted using a three-way factorial design (Species (SP) × Salinity (SAL) × Glycine betaine (GB)). *: Significant at $p < 0.05$; ns: non-significant. Means within columns followed by different lowercase letters differ significantly according to Duncan's multiple range test ($p < 0.05$)

Across treatments, FA exhibited the highest average CC, whereas FRR, LP, and PP showed comparatively lower and similar values. Salinity reduced average CC by approximately 10% compared with non-saline treatments. GB application increased CC by approximately 11% relative to untreated plants.

Species significantly influenced TC in all evaluation weeks and in the average values (Table 2). Significant species × salinity and salinity × GB interactions were detected in four of the five weeks. Species × GB interaction was significant in one week, and a significant three-way interaction was observed in two weeks.

Table 2. Effects of turfgrass species, salinity, and GB on turfgrass colour (TC), measured weekly for five weeks after the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1})

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Avg.
Species (SP)						
F. rubra	7.00±0.60^{a*‡}	5.80±1.50^{b}	6.60±1.60^{a}	6.30±1.70^{a}	6.00±1.40^{a}	6.40±1.70^{b}
L. perenne	3.90±0.80^{c}	4.80±1.00^{c}	5.30±1.20^{b}	4.80±0.80^{b}	4.60±1.40^{b}	4.70±0.90^{c}
F. arundinacea	6.30±1.30^{b}	6.80±0.70^{a}	7.00±1.10^{a}	6.80±1.50^{a}	6.50±0.90^{a}	6.70±1.10^{ab}
P. pratensis	7.80±1.70^{a}	6.50±1.20^{ab}	7.20±0.90^{a}	6.90±1.40^{a}	6.30±1.20^{a}	6.90±1.20^{a}
Salinity (SAL)						
SAL (+)	6.20±0.90	4.90±1.40^{b}	5.90±1.10^{b}	5.40±1.40^{b}	5.00±0.70^{b}	5.50±0.90^{b}
SAL (-)	6.30±1.20	7.00±1.50^{a}	7.10±0.80^{a}	7.00±1.50^{a}	6.70±1.30^{a}	6.80±1.50^{a}
Glycine betaine (GB)						
GB (+)	6.40±0.80	6.40±1.80^{a}	6.40±0.90	6.10±1.30	5.80±1.10	6.20±0.90
GB (-)	6.10±1.50	5.60±1.40^{b}	6.60±0.80	6.30±0.90	5.90±1.30	6.10±1.60
SP	*	*	*	*	*	*
SAL	ns	*	*	*	*	*
GB	ns	*	ns	ns	ns	ns
SP × SAL	ns	*	*	*	*	*
SP × GB	*	ns	ns	ns	ns	ns
SAL × GB	*	*	ns	*	*	ns
SP × SAL × GB	ns	ns	*	ns	*	ns

Data are presented as mean ± standard deviation (n = 3). Week 1–5 represent weekly measurements following the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1}). ANOVA was conducted using a three-way factorial design (Species (SP) × Salinity (SAL) × Glycine betaine (GB)). *: Significant at $p < 0.05$; ns: non-significant. Means within columns followed by different lowercase letters differ significantly according to Duncan's multiple range test ($p < 0.05$)

Under non-saline conditions (SAL−), all species maintained TC values at or above the acceptable threshold (≥6). Salinity (SAL+) reduced TC across species, with LP exhibiting the greatest decline. The effect of GB varied depending on species and salinity level.

Species, salinity, and GB exhibited significant main effects on turfgrass quality (TQ) in several evaluation weeks (Table 3). Species × salinity and species × GB interactions were significant in four of the five weeks, indicating that species responses varied depending on salinity level and GB application. The salinity × GB interaction was significant only in the first week, whereas the three-way interaction (species × salinity × GB) was not significant in any week.

Table 3. Effects of turfgrass species, salinity, and GB on turfgrass quality (TQ), measured weekly for five weeks after the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1})

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Avg.
Species (SP)						
F. rubra	5.90±0.80	6.90±1.20^{ab*‡}	5.80±1.40^{b}	5.80±1.60^{b}	5.40±0.80^{c}	6.00±1.20^{b}
L. perenne	6.30±1.20	4.20±0.90^{c}	4.60±0.80^{c}	4.40±1.10^{c}	4.20±1.50^{d}	4.70±1.50^{c}
F. arundinacea	6.40±0.50	7.00±0.90^{ab}	7.30±0.80^{a}	6.90±1.60^{a}	7.00±1.60^{a}	6.90±1.80^{a}
P. pratensis	6.60±1.40	7.80±1.90^{a}	6.20±1.10^{ab}	6.40±1.30^{a}	6.20±0.80^{b}	6.60±1.50^{a}
Salinity (SAL)						
SAL (+)	5.40±0.70^{b}	6.30±0.80	5.00±1.00^{b}	4.90±0.80^{b}	4.70±0.70^{b}	5.20±0.80^{b}
SAL (-)	7.30±1.10^{a}	6.60±1.20	7.00±1.50^{a}	6.8±1.20^{a}	6.70±1.30^{a}	6.90±1.30^{a}
Glycine betaine (GB)						
GB (+)	6.30±1.20	6.60±1.40	6.40±0.70^{a}	6.20±1.10^{a}	6.00±1.00^{a}	6.20±1.40^{a}
GB (-)	6.30±1.60	6.30±0.90	5.50±0.80^{b}	5.60±1.50^{b}	5.40±0.70^{b}	5.80±1.60^{b}
SP	ns	*	*	*	*	*
SAL	*	ns	*	*	*	*
GB	ns	ns	*	*	*	*
SP × SAL	*	ns	*	*	*	*
SP × GB	ns	*	*	*	*	*
SAL × GB	*	ns	ns	ns	ns	*
SP × SAL × GB	ns	ns	ns	ns	ns	ns

Data are presented as mean ± standard deviation (n = 3). Week 1–5 represent weekly measurements following the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1}). ANOVA was conducted using a three-way factorial design (Species (SP) × Salinity (SAL) × Glycine betaine (GB)). *: Significant at $p < 0.05$; ns: non-significant. Means within columns followed by different lowercase letters differ significantly according to Duncan's multiple range test ($p < 0.05$)

Overall, salinity treatment resulted in lower TQ values compared with non-saline conditions across most evaluation weeks. Among species, LP consistently exhibited the lowest overall TQ values and appeared more adversely affected under saline conditions, with ratings in some cases falling below the commonly accepted quality threshold (≥6). In contrast, FA and PP maintained comparatively higher TQ ratings throughout the experimental period, suggesting greater tolerance to salinity stress. FRR demonstrated intermediate responses depending on treatment combinations. The effects of GB on TQ were species- and time-dependent, as reflected by the significant species × GB interactions observed in multiple weeks.

A representative image of turfgrass species grown under different salinity and GB treatments is presented in Figure 2 to visually support the quality assessments.

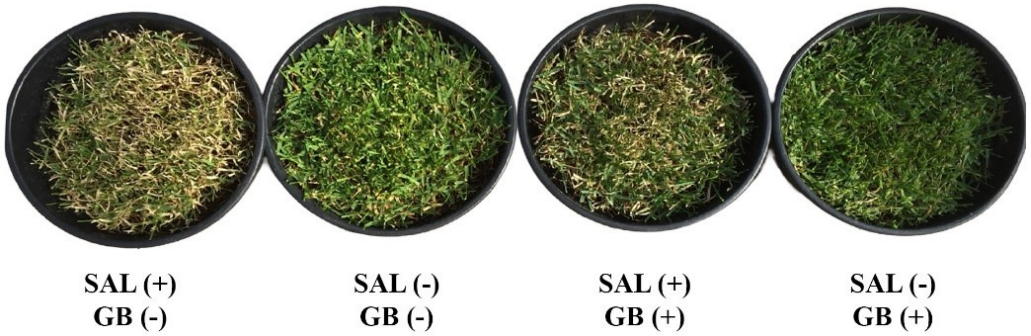



Figure 2. Representative appearance of turfgrass species in pots under different salinity (SAL) and glycine betaine (GB) treatments, illustrating visual differences in turfgrass color and quality

Species significantly affected CDW in all evaluation weeks and in the overall average (Table 4). Salinity had a significant effect only in Week 3. GB significantly influenced CDW in Weeks 2, 3, and 4, as well as in the overall average. Significant species × salinity interactions were detected in Weeks 2 and 3 and in the overall average. Species × GB and salinity × GB interactions were significant in Weeks 2-4 and in the average values. The three-way interaction (species × salinity × GB) was significant in Weeks 2 and 3.

Table 4. Effects of turfgrass species, salinity, and GB on clipping dry weight (CDW), measured weekly for five weeks after the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1})

Parameter	Week 1	Week 2	Week 3	Week 4	Avg.
Species (SP)					
F. rubra	0.40±0.06^{ab*‡}	0.67±0.07^{a}	0.47±0.07^{b}	0.38±0.06^{b}	0.48±0.03^{ab}
L. perenne	0.24±0.03^{c}	0.32±0.06^{b}	0.34±0.09^{c}	0.28±0.01^{c}	0.30±0.08^{c}
F. arundinacea	0.32±0.09^{b}	0.57±0.21^{a}	0.51±0.04^{ab}	0.30±0.01^{bc}	0.43±0.06^{b}
P. pratensis	0.51±0.01^{a}	0.61±0.09^{a}	0.56±0.07^{a}	0.51±0.08^{a}	0.55±0.02^{a}
Salinity (SAL)					
SAL (+)	0.35±0.03	0.55±0.04	0.49±0.08^{a}	0.35±0.07	0.44±0.04
SAL (-)	0.38±0.06	0.54±0.02	0.45±0.07^{b}	0.38±0.07	0.44±0.06
Glycine betaine (GB)					
GB (+)	0.37±0.06	0.63±0.05^{a}	0.57±0.05^{a}	0.45±0.04^{a}	0.51±0.03^{a}
GB (-)	0.36±0.04	0.45±0.09^{b}	0.37±0.02^{b}	0.28±0.06^{b}	0.37±0.04^{b}
SP	*	*	*	*	*
SAL	ns	ns	*	ns	ns
GB	ns	*	*	*	*
SP × SAL	ns	*	*	ns	*
SP × GB	ns	*	*	*	*
SAL × GB	ns	*	*	*	*
SP × SAL × GB	ns	*	*	ns	ns

Data are presented as mean ± standard deviation (n = 3). Week 1–5 represent weekly measurements following the final GB application (Week 1 = first week after the last GB treatment; salinity maintained at 6 dS m^{-1}). ANOVA was conducted using a three-way factorial design (Species (SP) × Salinity (SAL) × Glycine betaine (GB)). *: Significant at $p < 0.05$; ns: non-significant. Means within columns followed by different lowercase letters differ significantly according to Duncan's multiple range test ($p < 0.05$)

Across species, PP generally exhibited the highest CDW values, whereas LP showed the lowest overall performance. The response of CDW to salinity and GB application varied among species, as indicated by the significant interaction effects.

Species, salinity, and GB had significant main effects on RDW (Table 5). A significant species × salinity interaction was detected, whereas species × GB, salinity × GB, and the three-way interaction were not significant.

Table 5. Effects of turfgrass species, salinity, and GB on root dry weight (RDW), measured at the end of the experimental period

SAL	GB	Species				GB mean	SAL mean
		F. rubra	*L. perenne*	*F. arundinacea*	*P. pratensis*		
SAL (+)	GB (+)	2.90 ± 0.61^{Ac}	5.40 ± 0.94^{Aa}	4.60 ± 0.28^{Bab}	3.10 ± 0.35^{Abc}	4.00^{B}	4.40^{B}
	GB (-)	3.90 ± 0.15^{Abc}	5.00 ± 1.08^{Ab}	7.40 ± 0.56^{Aa}	2.80 ± 0.28^{Ac}	4.70^{A}	
	Species mean	3.40 ± 0.67^{b}	5.20 ± 0.93^{a}	6.00 ± 1.60^{a}	2.90 ± 0.35^{b}	-	
SAL (-)	GB (+)	$3.60\pm0.02^{A‡b*†}$	6.40 ± 0.48^{Aa}	4.40 ± 0.47^{Bb}	3.80 ± 0.88^{Bb}	4.50^{B}	4.90^{A}
	GB (-)	4.30 ± 1.03^{Aa}	5.70 ± 0.98^{Aa}	5.70 ± 1.10^{Aa}	5.70 ± 0.93^{Aa}	5.30^{A}	
	Species mean	3.90 ± 0.74^{b}	6.00 ± 0.80^{a}	5.00 ± 1.07^{ab}	4.80 ± 1.31^{b}	-	
Overall average of SP		3.70 ± 0.73^{b}	5.60 ± 0.94^{a}	5.50 ± 1.38^{a}	3.80 ± 1.32^{b}	-	
Overall average of GB		-					
GB (+)	4.3^{B}	-					
GB (-)	5.0^{A}	-					
SP		*	-				
SAL		*	-				
GB		*	-				
SP × SAL		*	-				
SP × GB		ns	-				
SAL × GB		ns	-				
SP × SAL × GB		ns	-				

Data are presented as mean ± standard deviation (n = 3). RDW is expressed as g pot^{-1} and was measured once at the end of the experimental period. ANOVA was conducted using a three-way factorial design (Species (SP) × Salinity (SAL) × Glycine betaine (GB)). *: Significant at $p < 0.05$; ns: non-significant. Uppercase letters indicate significant differences within columns (vertical comparison), whereas lowercase letters indicate significant differences within rows (horizontal comparison) according to Duncan's multiple range test ($p < 0.05$)

Across treatments, LP and FA exhibited the highest overall RDW values, whereas FRR and PP showed comparatively lower root biomass. The significant species × salinity interaction indicates that species responded differently to salinity. In particular, FA maintained relatively high RDW values under saline conditions, whereas other species showed variable reductions depending on treatment combinations. No significant species × GB interaction was observed, suggesting that GB effects on RDW were relatively consistent across species.

Species had a significant main effect on SDW (Table 6). Although salinity and GB did not show significant main effects independently, significant species × salinity and species × GB interactions were detected, indicating species-specific responses to salinity and GB application. The salinity × GB and three-way interactions were not significant.

Table 6. Effects of turfgrass species, salinity, and GB on shoot dry weight (SDW), measured at the end of the experimental period

SAL	GB	Species				GB mean	SAL mean
		F. rubra	*L. perenne*	*F. arundinacea*	*P. pratensis*		
SAL (+)	GB (+)	$3.7\pm0.58^{A‡b*†}$	4.5 ± 0.25^{Aab}	4.6 ± 0.24^{Aab}	4.8 ± 0.13^{Aa}	4.4	4.3
	GB (-)	3.9 ± 0.15^{Ab}	3.7 ± 0.74^{Ab}	4.5 ± 0.39^{Aab}	4.8 ± 0.30^{Aa}	4.2	
	Species mean	3.8 ± 0.39^{b}	4.0 ± 0.62^{b}	4.5 ± 0.29^{ab}	4.8 ± 0.21^{a}	-	
SAL (-)	GB (+)	3.9 ± 0.43^{Aa}	4.6 ± 0.38^{Aa}	4.2 ± 0.08^{Aa}	4.7 ± 0.12^{Aa}	4.4	4.2
	GB (-)	4.2 ± 0.34^{Aab}	3.8 ± 0.50^{Ab}	3.5 ± 0.36^{Bb}	4.9 ± 0.48^{Aa}	4.1	
	Species mean	4.0 ± 0.28^{b}	4.2 ± 0.57^{b}	3.9 ± 0.45^{b}	4.8 ± 0.32^{a}	-	
Overall average of SP		3.9 ± 0.39^{b}	4.1 ± 0.58^{b}	4.2 ± 0.50^{b}	4.8 ± 0.26^{a}	-	
Overall average of GB		-					
GB (+)	4.4	-					
GB (-)	4.1	-					
SP		*	-				
SAL		ns	-				
GB		ns	-				
SP × SAL		*	-				
SP × GB		*	-				
SAL × GB		ns	-				
SP × SAL × GB		ns	-				

Data are presented as mean ± standard deviation (n = 3). SDW is expressed as g pot^{-1} and was measured once at the end of the experimental period. ANOVA was conducted using a three-way factorial design (Species (SP) × Salinity (SAL) × Glycine betaine (GB)). *: Significant at $p < 0.05$; ns: non-significant. Uppercase letters indicate significant differences within columns (vertical comparison), whereas lowercase letters indicate significant differences within rows (horizontal comparison) according to Duncan's multiple range test ($p < 0.05$)

PP consistently exhibited the highest SDW values across treatments. The significant species × salinity interaction suggests that species responded differently to salinity, with LP and FRR showing slight reductions under saline conditions, whereas PP maintained relatively stable SDW values. FA exhibited moderate variation depending on treatment combinations. The significant species × GB interaction further indicates that GB effects differed among species.

Correlation analysis revealed a strong positive correlation between TQ and TC ($r = 0.824$, $p < 0.01$) (Table 7). CDW showed a significant negative correlation with RDW ($r = -0.518$, $p < 0.01$).

Table 7. Correlation coefficients between observed parameters

Parameters	TC	TQ	CDW	RDW	SDW
CC	0.539**	0.677**	0.295*	ns	ns
TC	1	0.824**	0.407**	ns	ns
TQ	-	1	0.403**	ns	ns
CDW	-		1	-0.518**	0.436**
RDW	-			1	ns
SDW	-				1

Pearson correlation coefficients among the measured variables (n = 48). Values marked with ** and * indicate significance at $p < 0.01$ and $p < 0.05$, respectively; ns: non-significant. CC: chlorophyll content; TC: turfgrass color; TQ: turfgrass quality; CDW: clipping dry weight; RDW: root dry weight; SDW: shoot dry weight

Discussion

Strategies to cope with salinity are crucial in turf management practices, as salinity can significantly impact plant health and performance. The CDW, RDW, and SDW are commonly used indicators to evaluate the effects of salt stress on turfgrass. Additionally, monitoring CC and collecting data on TQ and TC is necessary to determine whether the turfgrass retains its visual appeal and utility under stress conditions. By assessing these parameters, the study identified the effects of salinity and GB on four cool-season turfgrass species.

Salinity-induced reductions in chlorophyll content observed in the present study are consistent with previous reports indicating that salt stress disrupts chlorophyll biosynthesis and accelerates pigment degradation (Maxwell and Johnson, 2000; Liu *et al*., 2023). The approximately 10% decline in CC under saline conditions indicates that even moderate salinity (6 dS m^{-1}) can adversely affect photosynthetic performance in cool-season turfgrasses.

GB application was associated with increased CC across treatments, in agreement with earlier studies demonstrating enhanced chlorophyll stability and improved membrane protection following exogenous GB application (Hu *et al*., 2012; Yang *et al*., 2012). Although physiological mechanisms were not directly evaluated in the present study, previous research suggests that GB may support osmotic adjustment and stabilization of cellular structures under saline stress (Ashraf and Foolad, 2007).

Species-dependent differences were evident. FA maintained consistently higher CC values than the other species, which may reflect comparatively greater tolerance to saline conditions. In contrast, LP exhibited greater sensitivity. Such variation likely reflects genotypic differences in stress tolerance mechanisms, as widely reported in turfgrass species (Dajic, 2006).

Visual parameters such as TC and TQ are key indicators of turf performance under stress. The significant species × salinity and species × GB interactions indicate that visual responses were treatment-specific rather than uniform across species. LP exhibited the greatest decline in TC and TQ under saline conditions, occasionally falling below the acceptable threshold (≥6), whereas FA and PP maintained comparatively stable visual ratings. These findings align with previous reports documenting interspecific variability in salinity tolerance among cool-season turfgrasses (Dean *et al*., 1996; Alshammary *et al*., 2004).

The effect of GB on visual parameters varied among species. In certain treatment combinations, GB appeared to partially mitigate salinity-induced reductions in color and quality, whereas in others the response was limited. Similar species-dependent responses to GB under saline conditions have been documented in LP and PP (Hu *et al*., 2012; Yang *et al*., 2012; Scalia *et al*., 2014), indicating that GB effectiveness may depend on genotype and stress severity.

Salinity stress influenced biomass production differently among species. The significant species × salinity interaction for CDW indicates that clipping biomass responses to salinity varied among species, with LP and FRR tending to show greater sensitivity compared with FA and PP. This interspecific variability is consistent with previous findings in cool-season turfgrasses exposed to saline irrigation (Alshammary *et al*., 2004; Saud *et al*., 2013).

Root dry weight responses were particularly informative. The significant species × salinity interaction indicates contrasting root responses among species. FA maintained relatively high RDW values under saline conditions, whereas other species tended to show lower root biomass depending on treatment combinations. Sustained or enhanced root development under saline conditions has been associated with improved stress adaptation and greater salt tolerance (Marcum and Kopec, 1997). The relatively stable or elevated RDW observed in FA may therefore reflect a comparatively more effective adaptive response under moderate salinity.

Shoot dry weight responses were likewise species-dependent. Although salinity did not produce a significant main effect on SDW, interaction analysis revealed differential responses among species. LP tended to show slightly lower SDW under saline conditions, whereas PP maintained relatively stable shoot biomass. These findings are consistent with previous reports indicating that shoot growth sensitivity to salinity varies

among turfgrass species (Munns and Tester, 2008). Similarly, Çakır and Sever Mutlu (2025) reported that GB application did not significantly improve shoot dry weight in *Zoysia japonica,* a warm season turfgrass, under saline conditions, suggesting that GB-induced biomass responses may not be consistently expressed across turfgrass species. Such variability further supports the present findings that the influence of GB on shoot biomass is species-dependent rather than uniformly effective.

The negative correlation observed between CDW and RDW suggests a potential shift in biomass allocation under stress conditions. Trade-offs between aboveground and belowground growth have been described as adaptive responses to environmental constraints (Poorter *et al*., 2012), potentially reflecting altered carbon partitioning under saline stress.

Although GB application was associated with improvements in certain parameters, its effectiveness was not uniform across species or traits. The absence of consistent three-way interaction effects indicates that GB-mediated responses were conditional rather than universally expressed. In FA and PP, GB was associated with partial mitigation of biomass reductions under salinity, whereas LP showed limited responsiveness. Similar variability has been reported in other turfgrass species, where GB effects depended on genotype, salinity intensity, and environmental conditions (Hu *et al*., 2012; Oddo *et al*., 2019).

Overall, the present findings suggest that while GB may contribute to improved performance under moderate salinity, its application should be considered within a species-specific management framework. Genotype selection remains a primary determinant of turfgrass performance under saline irrigation, with GB serving as a supplementary management strategy rather than a universally effective solution.

Conclusion

This study demonstrated that turfgrass responses to saline irrigation were strongly species-dependent and largely governed by significant species × salinity and species × GB interactions. Salinity stress negatively affected visual and physiological parameters (TC, TQ, and CC) and altered biomass production (SDW and RDW), although the magnitude and pattern of these responses varied among species. LP exhibited the highest sensitivity to salinity, particularly in shoot and clipping biomass, whereas FA and PP maintained comparatively greater visual quality and growth stability under saline conditions. The relatively stable root performance observed in FA may indicate a higher tolerance to salt stress.

Although glycine betaine (GB) did not show a universally significant main effect, its influence appeared to be species-specific and conditional, with partial mitigation of salinity effects observed in certain treatment combinations. The negative correlation between root dry weight and clipping dry weight suggests that salinity may induce shifts in biomass allocation, reflecting different tolerance strategies among species. Overall, the findings highlight the importance of species selection for sustainable turfgrass management under saline irrigation conditions, while GB application may provide a supplementary benefit depending on species characteristics and environmental context. Further studies should explore the physiological mechanisms underlying these species-specific responses.

Authors' Contributions

Conceptualization: CS, MÇ; Data curation: CS, MÇ; Formal analysis: MÇ; Funding acquisition: CS; Investigation: CS; Methodology: CS, MÇ; Project administration: CS, MÇ; Resources: CS; Software: CS, MÇ; Supervision: CS, MÇ; Validation: CS, MÇ; Visualization: CS, MÇ; Roles/Writing - original draft: CS, MÇ; and Writing - review & editing: CS, MÇ.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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