Impact of Nitrogen and Glycine Betaine on Winter and Spring Performance of Zoysiagrass

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Zoysiagrass (*Zoysia japonica* Steud.) is a widely planted warm-season turfgrass; however, its long winter dormancy limits broader adoption, especially in transitional and subtropical regions. Glycine betaine (GB) is known to mitigate cold stress in controlled environments, but its potential to improve chilling tolerance of zoysiagrass as measured by winter color retention under field conditions remains unclear. This study investigated the effects of fall-applied GB and nitrogen (N) on winter color and spring green-up of zoysiagrass under field conditions in a Mediterranean climate in Türkiye. Conducted from 2018 to 2020 in Antalya, the experiment tested different application levels of N (0, 2.5, and 5 g/m²) and GB (0, 0.8, and 1.6 g/m²). N applications significantly improved key parameters, such as winter color retention, turf quality, chlorophyll content, and spring green-up. The highest N (5 g/m²) extended the green period from 7 to 10 months by delaying dormancy. In contrast, GB treatments had no significant effects. Although GB showed limited effectiveness, its evaluation under field conditions provides valuable insight into its practical relevance for warm-season turf management. The limited effect may be related to application amount and application timing. Further research with a broader range of GB application rates may help uncover its full potential under chilling stress conditions.

DOI: 10.15376/biores.20.2.4549-4567

Keywords: Fall color retention; Dormancy; Spring green-up; Zoysia japonica

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INTRODUCTION

Achieving high-quality, low-maintenance turf in arid and semiarid regions, such as the Mediterranean Basin, the southwestern United States, and parts of Central Asia, is a primary objective for turf managers. Warm-season turfgrasses, notably *Zoysia japonica* (zoysiagrass or Japanese lawngrass), offer superior turf quality and enhanced water conservation compared to cool-season species (Beard 1989). Zoysiagrass is particularly valued for its drought tolerance, low maintenance requirements, and adaptability to various climates (Patton *et al*. 2017). The optimal temperature for zoysiagrass growth is between 27 and 35 °C (Beard 1973; Li *et al.* 2018). Zoysiagrass grows well on a wide range of well-drained soils, including sands, loams, and clays, with a pH tolerance ranging from strongly acidic (pH 5 or lower) to mildly alkaline (up to pH 8) (Loch 2013). It also requires full sun exposure for optimal growth (Rahayu *et al.* 2020). Consequently, in climates not favorable to the growth of cool-season turfgrasses, such as subtropical climates, the species is an excellent option, offering a lush green lawn from mid-spring to mid-fall during its active growth.

Zoysiagrass is the most cold-tolerant warm-season turfgrass (Dunn *et al*. 1999). However, after chilly temperatures and subsequent fall frosts, it goes into dormancy and remains as such until spring, similar to other warm-season turfgrasses (Razmjoo *et al.* 1994). Dormancy causes the leaves and stems to completely lose their pigment, giving them a brownish-white appearance, which many people find undesirable in comparison with cool-season turfgrasses that maintain their green color in winter. This dormancy challenges turf managers, as winter dormancy and low-temperature susceptibility limit the wider adoption of warm-season turfgrasses, including zoysiagrass (Magni *et al.* 2017; Zuo *et al.* 2019) both in transitional and subtropical climates.

Because color retention at low temperatures is crucial for expanding the use of zoysiagrass into colder climates, improving its winter color retention and accelerating its spring green-up are key research priorities (Guo *et al.* 2012). Various management strategies have been investigated to extend the green period of zoysiagrass by reducing or delaying winter dormancy, including mixing zoysiagrass with cool-season grasses, the use of fertilizers, plant growth regulators, and biostimulants (Gusta *et al.* 1980; Dunn *et al.* 1993; Zhang and Xiao 1997; Dunn *et al.* 1999; Li *et al.* 2003; Shahba *et al.* 2003, Patton and Reicher 2007; Xuan *et al.* 2008; Çakır *et al.* 2024). For example, mixing zoysiagrass with a relatively high temperature tolerant cool-season species such as *Festuca arundinacea* can provide year-round green areas (Li and Hunt 1997). The green period has been prolonged by mixtures of zoysiagrass with *Festuca* sp. or *Poa pratensis*, but preserving species balance is still difficult (Hunt and Cai 1993). To provide a temporary, green, and growing playable surface, cool-season grasses are also overseeded into zoysiagrass in the fall (Hurley *et al.* 1989). In addition to being costly, this practice often results in relatively low success, as the zoysiagrass has difficulty recovering in the spring when overseeded. Additionally, carboxin, a systemic fungicide, has been shown to delay cold-induced discoloration in *Z. japonica* and *Cynodon dactylon* (Sachs *et al*. 1971). Foliar calcium treatments improved chilling and freezing resistance in *C. dactylon* (Shi *et al.* 2014). Furthermore, Schmidt and Chalmers (1993) found an improvement in turf color due to iron application.

Nitrogen (N) is a critical factor influencing turfgrass performance, affecting quality aspects, such as density, color, recovery, and resilience, against environmental stresses (Kopec *et al.* 2007). While some earlier studies raised concerns regarding late-season (fall) fertilization of warm-season turfgrasses (Beard 1973), some recent research suggests that late-season N applications can extend green color retention in the fall and promote spring green-up without leading to winter injury (Reeves *et al.* 1970; Richardson 2002; Rimi *et al.* 2013). For instance, Goatley *et al.* (1994) and Richardson (2002) found that late N applications improved fall color retention and spring regrowth in *C. dactylon* without negative effects. Munshaw *et al.* (2006) and Goldsby and Keeley (2009) also reported enhanced fall color and spring quality in *Cynodon* spp. with late-season N. Similarly, *Zoysia matrella* 'Zeon' showed delayed discoloration and good recovery with late N applications (Volterrani *et al.* 2010). However, fall N treatments delayed spring green-up in some zoysiagrass cultivars (Dunn *et al.* 1993) and reduced cold tolerance in *Z. matrella* (Pompeiano *et al.* 2011). Nevertheless, the effects of late-season N applications on cold tolerance and spring recovery in zoysiagrass remain inconclusive.

Glycine betaine (GB), an osmoprotectant synthesized by plants in response to abiotic stresses, such as cold, contributes to the maintenance of water status in challenging conditions including cold, drought, and salinity stress (Rajashekar *et al.* 1999; Ashraf and Foolad 2007). This N-rich compound accumulates in the chloroplast and helps protect the thylakoid membrane, allowing photosynthesis to continue under stress (Ashraf and Foolad 2007). Exogenous applications of GB have been shown to enhance chilling and freezing tolerance in various plants, including *Medicago sativa*, *Arabidopsis thaliana*, *Lycopersicon esculentum,* and *Zea mays* (Farooq *et al.* 2006; Park *et al.* 2006; Chen and Murata 2008).

Despite its well-established impact on improving turfgrasses' resistance to drought and salinity (Zhao *et al.* 1992; Xing and Rajashekar 2001; Hu *et al.* 2012; Yang *et al.* 2012; Liu *et al.* 2017), little is known about its effects on the performance of warm-season turfgrasses under chilling conditions. Keough (2016) reported that GB improved the freezing tolerance of *C. dactylon* in controlled environments. Given GB's ability to stabilize proteins, enzymes, and cellular membranes under stress, it may also help mitigate cold-induced dormancy and enhance winter color retention in zoysiagrass. Although promising results have been observed under controlled settings, the field-level efficacy of GB under moderate but non-freezing winter stress remains unclear. This study aimed to address this knowledge gap by evaluating the combined effects of fall-applied GB and N on winter performance of *Zoysia japonica* 'El Toro' under Mediterranean field conditions. The Mediterranean climate, characterized by mild, wet winters and hot, dry summers, presents unique challenges for warm-season turfgrasses. While air temperatures rarely fall below freezing, extended periods of chilling are sufficient to induce dormancy and reduce aesthetic quality. Therefore, Antalya is an ideal location to investigate effectiveness of GB in zoysiagrass management if the goal is focused on fall color retention improvement under chilling temperatures. Through testing different application rates and combinations of N and GB, this study investigates their potential to extend green color retention during winter and promote faster spring green-up in zoysiagrass.

EXPERIMENTAL

Materials and Methods

The field experiment was conducted at the Akdeniz University Agricultural Research Farm, located between 36°53′59″ north latitude and 30°38′15″ east longitude, at an elevation of 33 m above sea level in Antalya, Türkiye. The study was carried out in two consecutive growing seasons, from 2018 to 2019. A mature sward of *Zoysia japonica* 'El-Toro', growing on a silty clay loam (typical Xerochrept/Paralithic Xerortent), was used as the plant material. The physical and chemical characteristics of the soil were determined according to Jackson (1962). The experimental soil was classified as clay in texture (45% clay, 40% sand, 15% silt). It had a pH of 8.17 (slightly alkaline) and was non-saline, with an EC of 0.24 dS m^{-1}. Organic matter content was moderate (2.64%), and lime ($CaCO_3$) content was 16.07%. Total nitrogen was 456 ppm, available phosphorus was low (3 ppm), and exchangeable potassium was high (4,949 ppm). The climate in the area is classified as typical Mediterranean, with average temperatures ranging from 7.6 to 34.3 °C during the study period. Climate data collected from a weather station near the study area are presented in Table 1.

The field treatment plots were organized in a trial designed as a randomized complete block with three replications. Each plot measured 1.5 m × 1 m separated by 0.5 m alleys. The treatments included three different application levels of N (0, 2.5, or 5 g/m^2) and GB (0, 0.8, or 1.6 g/m^2). N was applied as ammonium sulfate [$(NH_4)_2SO_4$; N: 21% and S: 24%] at the specified amounts. This product is commonly used in warm-season grass management in Antalya.

Table 1. Air Temperatures and Precipitation in Antalya (2018 to 2020)

Months	Mean Air Temperature (°C)		Precipitation (mm)
	Min.	Max.	Mean
Oct. 2018	17.2	26.9	7.9
Nov. 2018	13.4	22.4	32.8
Dec. 2018	9.2	16.8	31.4
Jan. 2019	7.6	14.4	282.7
Feb. 2019	8.7	16.8	62.4
Mar. 2019	10.3	18.6	51.0
Apr. 2019	12.6	20.3	52.3
May 2019	17.3	24.5	10.6
June 2019	21.8	29.3	34.5
July 2019	24.7	32.5	0.0
Aug. 2019	25.7	34.3	0.4
Sept. 2019	22.3	30.4	2.1
Oct. 2019	19.2	28.3	17.5
Nov. 2019	14.6	23.1	179.6
Dec. 2019	9.7	17.8	287.2
Jan. 2020	7.9	16.1	158.7
Feb. 2020	8.6	16.1	79.6
Mar. 2020	10.8	18.7	51.1
Apr. 2020	13.2	20.7	14.9
May 2020	17.8	25.7	60.9
June 2020	19.8	27.2	3.6

The N application levels were determined based on the methods of Oh *et al.* (2015) and Miller *et al.* (2016). The GB application levels were selected based on previous studies, particularly Keough (2016), who applied GB at concentrations of 30 to 100 mM (approximately 3.5 to 11.7 g/L) in controlled experiments to enhance cold tolerance in warm- and cool-season turfgrasses. In this study, 0.8 and 1.6 g of GB were applied per square meter using 0.2 L of spray solution, resulting in concentrations of 4 and 8 g/L, respectively—comparable to those previously reported in the literature. Throughout this study, the following nomenclature was used: N_0, N_1, and N_2 stand for the absence of N application, a low level (2.5 g/m^2), and a high level (5 g/m$_2$), respectively. Likewise, GB_0, GB_1, and GB_2 denote the lack of GB application, a low amount (0.8 g/m^2), and a high amount (1.6 g/m^2), respectively. N was applied manually to the plots in weighted amounts, and approximately 10 mm of irrigation was provided following each N fertilization. The GB was uniformly applied using a backpack sprayer equipped with a single flat nozzle to ensure even coverage. The foliar spray of GB was applied at the specified rates on the day after the N application. Both N and GB were applied in two perpendicular orientations to improve application consistency and promote uniform, ideal grass absorption. The initial treatment was applied on September 28, 2018, followed by a second treatment four weeks later on October 28, 2018, and a third treatment on November 28, 2018. After the initial applications, mowing was performed regularly as needed using a walk-behind rotary mower (*Husqvarna LC140S*) set to a height of 50 mm. Clippings were collected and removed after each mowing operation. Irrigation was consistently provided throughout the study to maintain adequate soil moisture levels and prevent wilting. No chemical treatments were applied during the experiment, and weed management was carried out mechanically. In 2019, the study was repeated with identical conditions and timeframe.

Biweekly evaluations of turfgrass quality, color, turf index, and chlorophyll content were conducted shortly after the third treatment application in November and continued until June. Turfgrass quality, integrating factors, such as color, uniformity, and density, were visually evaluated on a scale from 1 (representing the poorest quality: dead, fully dormant, or brown turf) to 9 (the highest quality: fully green and healthy turf), with a score of 6 considered acceptable turf quality (NTEP 2018). Turf color, which reflects the overall appearance of the plots, was rated visually on a 1 to 9 scale, with 1 indicating straw-brown and 9 representing dark green color (NTEP 2018).

The turf index, also referred to as the green color index, was measured using a handheld NDVI (Normalized Difference Vegetation Index) color meter (TCM 500 NDVI, Spectrum Technologies Inc., Aurora, IL, USA). This device captures light reflectance from the turf in both the red (600 nm) and near-infrared (850 nm-NIR) spectral bands. The NDVI meter was placed directly on the turf, and measurements were taken by pressing the device, yielding an index ranging from 1.0 to 9.0. Higher index values correspond to greener and actively growing turf of high quality. Chlorophyll content was quantified using a handheld chlorophyll meter (Field Scout CM 1000, Spectrum Technologies, Inc., Plainfield, IL, USA), which calculates relative chlorophyll content by measuring the reflection of red and far-red light. Measurements were taken at a height of 80 cm above the turf surface, with the resulting chlorophyll index being unitless and ranging from 0 to 999. To ensure accuracy, both turf index and chlorophyll content values for each plot were averaged from ten individual readings per plot.

Winter dormancy is an important trait used to evaluate a genotype’s ability to retain green color in response to decreasing temperatures or frost events (Sever Mutlu *et al.* 2020). Dormancy was assessed weekly during each winter season starting in December when the plants began entering dormancy and continuing through to March when spring green-up commenced. The evaluations were based on a visual scale ranging from 0% (no dormancy; green vegetation covering the entire plot) to 100% (full dormancy; no green vegetation). Spring green-up or recovery from winter dormancy is generally a good indicator of a genotype’s cold hardiness (Patton and Reicher 2007; Kauffman 2010). Spring green-up was rated on a visual scale from 0% to 100%, with 100% indicating complete green coverage across the entire plot and 0% indicating no visible green vegetation. These assessments were conducted weekly from March to May until all plots achieved full green-up.

Turf density was evaluated by manually counting the number of shoots from three plugs (each with a diameter of 108 mm, covering an area of 91.61 cm²) that were randomly extracted from different sections of each treatment plot using a cup cutter in June when the plots reached 100% green cover. These same plugs were subsequently used to determine shoot and root dry weights. After thoroughly rinsing the plugs to remove soil residues, the shoots were separated from the roots, which were then clipped. Both were then placed in labeled paper bags and oven-dried at 72 °C for 72 h to accurately assess the dry weights of the shoots and roots. Flower density, which refers to the number of flowers present per unit area, was evaluated in June. A visual scoring scale ranging from 1 to 9 was utilized, where a score of 1.0 indicates minimal flowering, and a score of 9.0 signifies maximum flowering (Çakır 2020).

All observations collected on a biweekly basis, such as turf quality, color, grass index, and chlorophyll content, were averaged on a per-plot basis throughout the season (winter or spring) to streamline data presentation. Given the climate conditions of the region, November, December, January, and February are classified as winter months, while

March, April, and May are classified as spring months. Statistical analysis was performed using analysis of variance (ANOVA) with SPSS software (IBM, Armonk, NY, USA), and mean comparisons were conducted using Duncan's multiple range test at a significance level of 0.05. Additionally, Hartley's F_{max} test (Hartley 1950) was employed to assess the homogeneity of variances between the first-year and second-year trials of the experiments.

RESULTS AND DISCUSSION

The F_{max} test confirmed homogeneous variance between the two experimental years, allowing for data pooling. Table 2 summarizes the significance levels of the main effects (N and GB) and their interaction (N × GB) for all evaluated traits. N had a significant effect on all parameters except for the grass index and shoot/root dry weights, while GB had no significant effect on any parameter. The interaction between N and GB was also found to be non-significant for all observed parameters. Therefore, data were pooled across GB treatments, and only the main effect of N was further analyzed, presented, and discussed in detail (Table 3).

Table 2. Probabilities for Treatment Effects and their Interactions on Turfgrass Quality, Color, Chlorophyll Content, NDVI, Dormancy, Spring Green-Up, Flower Density, Shoot Density, Root and Shoot Dry Weight

Source of Variation		N	GB	N × GB
df		2	2	4
Quality	Winter	<0.001	0.426	0.545
	Spring	<0.001	0.624	0.914
Color	Winter	<0.001	0.812	0.892
	Spring	<0.001	0.553	0.682
Chlorophyll Content	Winter	<0.001	0.727	0.998
	Spring	<0.001	0.617	0.996
Grass Index	Winter	0.181	0.964	0.995
	Spring	0.440	0.977	0.995
Dormancy	November	<0.001	0.583	0.949
	December	<0.001	0.378	0.824
	January	<0.001	0.513	0.902
	February	<0.001	0.840	0.651
Spring Green-Up	March	<0.001	0.787	0.834
	April	<0.001	0.605	0.841
	May	<0.001	0.908	0.999
Flower Density	June	<0.001	0.441	0.604
Shoot Density	June	<0.001	0.350	0.634
Root Dry Weight	June	0.896	0.884	0.913
Shoot Dry Weight	June	0.060	0.453	0.973

Table 3 presents the impact of various N rates on several observed parameters of turfgrass during different seasonal phases.

Table 3. Effect of Different Rates of N on Winter and Spring Turfgrass Performance

Observed Parameters		N_0 0 g/m^2	N_1 2.5 g/m^2	N_2 5 g/m^2
Quality	Winter	3.3[c*‡]	4.3[b]	5.1[a]
	Spring	4.4[c]	5.8[b]	6.7[a]
Color	Winter	3.24[c]	4.38[b]	5.54[a]
	Spring	4.18[c]	5.84[b]	6.81[a]
Chlorophyll Content	Winter	100[c]	117[b]	135[a]
	Spring	116[c]	138[b]	163[a]
Grass Index	Winter	4.53	4.80	4.99
	Spring	4.85	5.05	5.22
Dormancy	November	26.6[c]	11.6[b]	4.3[a]
	December	46.7[b]	28.8[a]	22.3[a]
	January	76.4[c]	51.7[b]	36.7[a]
	February	84.1[c]	76.3[b]	65.4[a]
Spring Green-Up	March	42.3[c]	64.7[b]	78.1[a]
	April	60.6[c]	79.7[b]	90.3[a]
	May	73.8[b]	85.3[ab]	95.3[a]
Flower Density	June	3.93[b]	5.49[a]	6.12[a]
Shoot Density	June	79.0[b]	94.6[a]	9.6[a]
Root Dry Weight	June	7.52	7.52	8.06
Shoot Dry Weight	June	2.01	2.76	2.63

*Significant at the 0.05 probability level.
‡Lowercase letters given vertically (along the column) indicate the comparison of averages.
Note: Because the effect of GB was not significant, data were pooled across GB treatments and only the main effect of N was analyzed.

As the winter progressed, the quality declined below the acceptable level due to growth reduction and loss of green color in response to suboptimal temperatures. However, winter quality scores showed significant improvements with increased N levels (Table 3). The N_2 application enhanced turfgrass quality 55% over control plots. Spring quality was also enhanced with increasing N rates. Only plots treated with N_2 achieved above the acceptable turf quality score of 6.0 in spring. These findings align with those of Çakır *et al.* (2024), suggesting that N may enhance resilience during adverse conditions. The positive effects of N_2 treatment on turfgrass appearance in winter and spring are likely due to enhanced metabolic activity and nutrient uptake, leading to better density and color. Supporting the current study, late-season N applications enhanced warm-season turfgrass quality including *C. dactylon* both in winter and spring (Munshaw *et al.* 2006; Rimi *et al.* 2013) and *Paspalum vaginatum* in spring. Okeyo *et al.* (2011) noted a positive correlation between low-temperature tolerance and green color retention in ten hybrid progenies derived from interspecific crosses between *Z. matrella* and *Z. japonica*. Additionally, Serena *et al.* (2017) observed that raising annual N application from 10 to 20 g N/m² improved winter turf quality in *P. vaginatum* "Sea Spray" due to denser, darker green turf while increasing it from 20 to 30 g N/m² in *C. dactylon* "Princess 77" did not yield similar benefits. These variations may be attributed to the specific N requirements for optimal growth of different grass species.

As fall progresses, color becomes a critical component of warm-season turfgrass quality because it defines the general appearance and influences users' preference for grass. During this period, the physiological processes cause loss of color due to the degradation of chlorophylls, the primary pigment in photosynthesis. Therefore, chlorophyll content in turfgrass is a crucial indicator of overall health and photosynthetic capacity, directly affecting growth, color, and quality. Results showed that both winter and spring chlorophyll content values significantly increased with N applications. Compared to the N_0 application, the chlorophyll content increased 17% and 35% in the winter and 18% and 40% in the spring with the N_1 and N_2 treatments, respectively. Similarly, an increase in chlorophyll content as a result of fall N application was also reported by other researchers in *C. dactylon* and *Z. japonica* (Pompeiano *et al.* 2013; Çakır *et al.* 2024). In accordance with the results of chlorophyll content, color ratings increased in response to N applications, and plots under the N_2 treatment retained their green color better than N_1 and N_0 treatments. Correlation analyses result further confirmed significant positive relationships between color and chlorophyll content in winter (P < 0.001; *r* = 0.76) and spring (P < 0.001; *r* = 0.92) (Table 1A, Appendix). The enhancement of color with N application might be associated with both the new synthesis of and preservation of existing chlorophyll molecules against degradation. As N is essential for the production of chlorophyll (Mendoza-Tafolla *et al.* 2019), the N level of the plant directly affects its synthesis in chloroplasts (Marschner 1995; Wen *et al.* 2019). As N levels rise, turfgrass has a great capacity for chlorophyll synthesis, resulting in darker green color, enhanced photosynthetic efficiency and growth, and improved vigor (Jagschitz and Skogley 1965; Makino *et al.* 2022). Similar to the current study's findings, White and Schmidt (1990) observed that *C. dactylon* "Midiron" treated with 4.8 g N/m² per month retained fall color better than those receiving 2.4 g N/m². Trenholm and Unruh (2005) also found that higher N levels significantly improved fall color retention in *Stenotaphrum secundatum* "Floratam". Additionally, Rimi *et al.* (2013) stated that fall-applied N prolonged fall color retention in four *Cynodon* spp. cultivars: Princess-77, Riviera, SWI 1014, and Yukon. Enhanced winter color retention due to fall N application was also reported with cool-season turfgrass species including *Agrostis palustris* (Powell *et al.* 1967) and *Poa pratensis* (Wilkinson and Duff 1972). There was also a high positive correlation between winter color and spring color (P < 0.001; *r* = 0.93). Supporting the current findings, Okeyo *et al.* (2011) found a positive correlation between fall color and spring color.

The grass index, derived from NDVI, has been proposed as an objective alternative for assessing turf quality. It provides a more reliable assessment compared to traditional subjective visual estimation (Keskin *et al.* 2008; Bremer *et al.* 2011). Results indicated that the grass index values also increased with the increase in the N rate. Although these increases suggest a potential benefit of N in promoting turf growth in winter and regeneration after dormancy in spring, the differences were not statistically significant. Correlation analysis further revealed significant positive relationships between the turf index and winter quality (P < 0.001; *r* = 0.46) and spring quality (P < 0.001; *r* = -0.51) (Table 1A, Appendix).

There were significant differences in winter dormancy rates between N treatments, and as N levels increased, the rate of dormancy decreased. This pattern was also reflected in the field appearance during mid-winter, where higher N plots retained green color and untreated plots exhibited advanced dormancy (Fig. 1). The dormancy rate for N_0 in November was 26.6%; for N_1 and N_2, it was 11.6% and 4.3%, respectively, indicating that fall-season N treatments increase grass resistance to chilling stress. In January, the dormancy rate for N_0 was 76.4%, while it was only 36.7% for N_2, highlighting the contribution of N deficiency to increased stress.

Overall, higher N application reduced dormancy under chilling temperatures and facilitated earlier, more vigorous spring growth, Çakır *et al.* (2024) similarly noted that increasing N rates decreased the dormancy rates in *Z. japonica*. Chilling temperatures increase the synthesis of ethylene, which is essential for regulating plant responses to biotic and abiotic stresses and plays a key role in regulating senescence (Wang 1989; Lin *et al.* 2009). It is well known that elevated ethylene levels cause chlorophyll breakdown, which leads to leaf senescence and increased dormancy (Aharoni and Lieberman 1979; Taiz and Zeiger 1998). Low-level N and/or N deficiency have been reported to play a positive role in ethylene biosynthesis in leaves as a consequence of stress (Lege *et al.* 1997; Zheng *et al.* 2013). The higher rate of dormancy and loss of green color in N_0 treatments therefore might be associated with an enhanced level of ethylene in response to low tissue level N in leaves. Ethylene biosynthesis is regulated by the activity of ACC synthase and ACC oxidase enzymes, which may be suppressed under high N availability (Xu *et al.* 2021). Iqbal *et al.* (2017) highlighted that reduced ethylene levels can delay leaf senescence in various plant species. Based on this, it may be inferred that suppressed ethylene biosynthesis under high N availability could contribute to delayed dormancy onset in zoysiagrass during mild chilling periods. In addition to ethylene, abscisic acid (ABA) is another hormone involved in dormancy induction under cold or stress conditions (Rohde and Bhalerao 2007). N levels can influence ABA biosynthesis, and high N availability has been associated with reduced ABA accumulation, which may delay dormancy onset and help maintain chlorophyll content (Wen *et al.* 2023). Moreover, N's effect on hormone balance is not uniform across species or even cultivars, as physiological responses may vary depending on inherent cold tolerance and growth characteristics (Kazan 2015). Further investigation into the hormonal crosstalk between ethylene, ABA, and N metabolism may help clarify the mechanisms underlying dormancy regulation in warm-season turfgrasses.

N application significantly enhanced the spring green-up rate of zoysiagrass throughout the evaluation period. In March, the spring green-up rates were 42.3% for N^0, 64.7% for N_1, and 78.1% for N_2, indicating that N accelerates regeneration from dormant buds in spring. Supporting the current results, Rimi *et al.* (2013) found that fall-applied N improved green-up in four *Cynodon* spp. cultivars. Similarly, fall N applications led to earlier spring green-up in *C. dactylon* (Schmidt and Chalmers 1993), without adversely affecting its cold tolerance (Munshaw *et al.* 2006).

Strong negative correlations ($P < 0.001$; r = -0.42 to -0.90) were observed between dormancy and spring green-up rates across all months measured, except one. This suggests that plots entering dormancy later in the fall tended to green-up earlier in the spring, thereby shortening the dormancy period. The duration of the period that 75 to 100% green grass cover could be maintained was extended from 7 to 10 months by applying 5 g N/m² monthly from September to November in the fall. Therefore, the N_2 treatment allowed zoysiagrass to maintain its green cover for an extended period by delaying winter dormancy and encouraging early spring green-up. Jia *et al.* (2014), stated that *Z. japonica* plots entering dormancy later also greened up later, leading to a longer dormancy period due to their slower green-up process in spring. Similarly, Severmutlu *et al.* (2011) reported that *C. dactylon* varieties retaining a better green color in the fall delayed spring green-up. Genetically and physiologically, later spring emergence might be associated with a delayed entry into dormancy in autumn, but N application seems to have changed this trend.

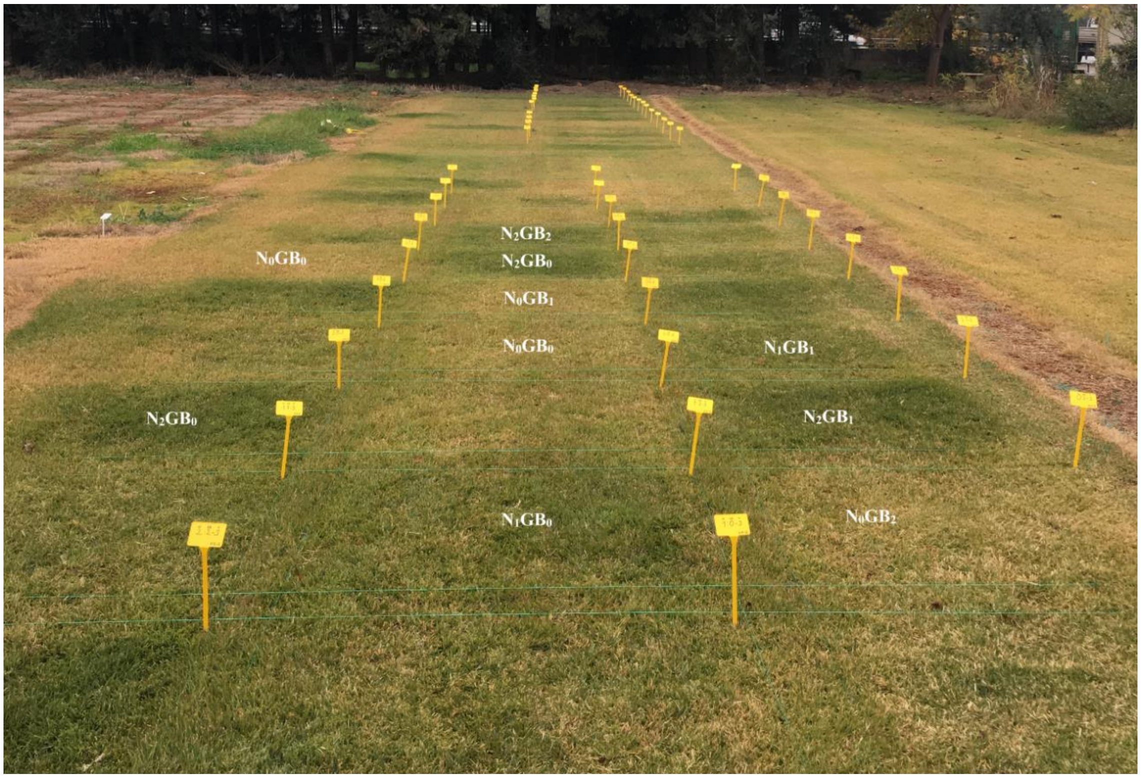

Fig. 1. Visual differences in turf performance among zoysiagrass plots under different N and GB treatments during mid-winter. Higher N plots maintained green color, while untreated plots showed advanced dormancy.

In comparison to untreated control plots, N_1 or N_2 treatments markedly increased flower density by 40% and 56%, respectively, in the summer. These results indicate that late-season N applications enhanced the reproductive capabilities of grass, promoting flowering and seed production. Mauad *et al.* (2013) similarly reported increased flower density in *Oryza sativa* with N application. The observed trend suggests that higher N levels enable turfgrass to maintain vegetative growth in winter and spring while also allocating resources toward flowering in late spring, reflecting a growth strategy that balances vegetative and reproductive development. However, high flower density is often undesirable for recreational or ornamental turf. To mitigate flowering, frequent mowing is recommended, as it encourages denser growth through enhanced tillering and helps maintain an aesthetically pleasing lawn. Therefore, careful management of N applications is crucial. Turf managers should align N application rates with specific goals—whether for aesthetics, functionality, or environmental sustainability—to optimize outcomes for their turfgrass systems.

The effect of N on shoot density was significant. The N_1 and N_2 treatments increased the shoot density 20% and 24%, respectively, over the untreated control. However, no significant difference was observed between N_1 and N_2. These findings also suggest that late-season N applications enhance vegetative growth in turfgrass by late-season N treatments. Oral and Açıkgöz (2007) reported a similar increase in shoot density for some cool-season turfgrasses, such as *Poa* sp., *Lolium* sp., and *Festuca* spp., with a 30 g N/m² annual application.

The root dry weight did not show significant differences among N treatments. Although N_1 and N_2 treatments increased shoot dry weight 37% and 31%, respectively, compared to the control, this increase was not significant. Alderman *et al.* (2011) reported that increased N rates in *C. dactylon* led to greater shoot growth, a finding supported by Guertal and Hicks (2009), Moeller *et al.* (2008), and Serena *et al.* (2017). Given that plant weight is a key indicator of health and performance, these results suggest that while specific N rates may not dramatically alter biomass accumulation in the short term, the cumulative effects over a growing season could lead to significant improvements in turf quality and performance. This underscores the importance of N as a crucial nutrient in turfgrass management, particularly for long-term sustainability and maintenance strategies.

The results showed that exogenous GB application in the fall did not improve the winter color retention of zoysiagrass. However, previous studies on other turfgrass species, such as *Cynodon dactylon* and *Agrostis stolonifera*, have reported improved cold tolerance, chlorophyll content, and turf quality following GB application under controlled conditions (Keough 2016). It was not possible to extensively compare and discuss the lack of responsiveness in zoysiagrass to fall-applied GB because no studies have investigated the effects of GB on the chilling tolerance of warm-season turfgrasses under field conditions. Kishitani *et al.* (1994) studied GB accumulation in *Hordeum vulgare* under cold stress, finding a 2- to 5-fold GB increase at 5 °C. They reported that GB levels correlated with green leaf survival after freezing (-5 °C), indicating a potential role in cold tolerance. Even though GB has been shown to improve cold tolerance in other plant species (Kishitani *et al.* 1994; Park *et al.* 2006; Chen and Murata 2008), the lack of significant effects of GB in *Z. japonica* found in this study highlights the need for more research to fully comprehend its function and underlying mechanisms under chilling temperatures. The lack of effectiveness of GB in this study may be attributed to several factors. First, the application timing may not have aligned optimally with the stress response window of zoysiagrass. This refers to the period when the plant is most physiologically responsive to stress and external protective agents such as GB. If GB is applied too early or too late, its uptake, translocation, or activation within plant tissues may be limited, thus reducing its effectiveness. Moreover, the absence of a significant N × GB interaction could be due to the dominant influence of N in promoting turf performance, potentially masking any additive or synergistic effects of GB. The absence of significant GB effects, either alone or in combination with N, could also be attributed to the application rate or source used. In the present study, the GB concentrations were similar to those reported by Keough (2016) in *Agrostis stolonifera* and *Cynodon dactylon*; however, no significant effect on winter color retention or spring green-up was observed in *Z. japonica*. This discrepancy suggests that the appropriate application rate of GB may differ across species and even among cultivars within the same species, highlighting the need for genotype-specific and field-based evaluation in turfgrass management practices. Moreover, soil and environmental conditions with highly variable parameters in field settings may have suppressed the physiological effect of GB at the doses used in this study. Therefore, further research is needed to explore the effects of a broader range of GB application rates on zoysiagrass.

It is important to note that the findings of this study are based on a single cultivar (*Zoysia japonica* 'El Toro') evaluated under Mediterranean climate conditions. Therefore, generalizing these results to other warm-season turfgrass species, cultivars, or different climatic zones should be done with caution. Further research is needed to validate these findings under diverse environmental and species-specific contexts.

CONCLUSIONS

1. The results showed that nitrogen (N) application significantly influenced key parameters such as winter color retention, turf quality, chlorophyll content, and spring green-up. The higher N rate (5 g/m²), applied monthly from September to November in the fall, allowed zoysiagrass to maintain its green cover for up to 10 months by delaying winter dormancy and promoting early spring green-up.
2. In contrast, the glycine betaine (GB) treatments did not result in any significant effects on the measured parameters. Although GB has been shown to alleviate cold stress under controlled environments in other species, it did not improve chilling tolerance or reduce dormancy of zoysiagrass grown in the field. Therefore, further research is needed to explore the effects of a higher range of GB application rates and sources on zoysiagrass under chilling stress conditions.
3. These findings contribute to clarifying the field relevance of GB in winter color retention, while quantifying the magnitude of N effects under a Mediterranean climate.

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Article submitted: November 30, 2024; Peer review completed: March 15, 2025; Revised version received: March 27, 2025; Accepted: April 1, 2025; Published: April 29, 2025.
DOI: 10.15376/biores.20.2.4549-4567
ERRATUM: May 23, 2025; Page 4552, 0. L has been updated to 0.2 L

APPENDIX
Supplementary Information

Table 1A. Correlation Coefficients Between Observed Parameters

	SQ	WC	SC	WCC	SCC	WGI	SGI	DN	DD	DJ	DF	SGM	SGA	SGMy	FD	TD	RDW	SDW
WQ	**,923**	**,976**	**,927**	**,762**	**,862**	*,320*	ns	**-,777**	**-,807**	**-,922**	**-,856**	**,851**	**,870**	**,510**	**,638**	**,467**	ns	ns
SQ	**1**	**,914**	**,975**	**,872**	**,943**	**,526**	**,466**	**-,864**	**-,781**	**-,843**	**-,777**	**,951**	**,948**	**,661**	**,743**	**,514**	ns	ns
WC		**1**	**,930**	**,763**	**,880**	*,317*	ns	**-,785**	**-,824**	**-,921**	**-,834**	**,827**	**,871**	**,574**	**,599**	**,460**	ns	ns
SC			**1**	**,833**	**,918**	**,459**	**,409**	**-,846**	**-,806**	**-,868**	**-,765**	**,918**	**,920**	**,642**	**,713**	**,507**	ns	ns
WCC				**1**	**,916**	**,779**	**,690**	**-,842**	**-,601**	**-,637**	**-,654**	**,903**	**,886**	**,700**	**,711**	**,370**	ns	*,384*
SCC					**1**	**,591**	**,545**	**-864**	**-,721**	**-,746**	**-,719**	**,906**	**,930**	**,709**	**,670**	**,467**	ns	ns
WGI						**1**	**,963**	**-,650**	ns	ns	ns	**,658**	**,614**	**,704**	**,577**	ns	ns	ns
SGI							**1**	**-,606**	ns	ns	ns	**,596**	**,555**	**,712**	**,523**	ns	ns	ns
DN								**1**	**,694**	**,650**	**,548**	**-,823**	**-,900**	**-,854**	**-,731**	**-,392**	ns	*-,444*
DD									**1**	**,846**	**,628**	**-,625**	**-,715**	**-,543**	**-,553**	**-,357**	ns	ns
DJ										**1**	**,821**	**-,742**	**-,766**	**-,421**	**-,523**	**-,454**	ns	ns
DF											**1**	**-,750**	**-,705**	**ns**	**-,509**	**-,516**	ns	ns
SGM												**1**	**,937**	**,614**	**,782**	**,478**	ns	ns
SGA													**1**	**,745**	**,734**	**,487**	ns	*,425*
SGMy														**1**	**,561**	ns	ns	ns
FD															**1**	*,296*	ns	ns
TD																**1**	ns	ns
RDW																	**1**	ns

Bold numbers: The correlation is significant at the 0.01 level;
Italic numbers: The correlation is significant at the 0.05 level;
ns: Non-significant
WQ: Winter quality, SQ: Spring quality, WC: Winter color, SC: Spring color, WCC: Winter chlorophyll content, SCC: Spring chlorophyll content, WGI: Winter grass index, SGI: Spring grass index, DN: Dormancy in November, DD: Dormancy in December, DJ: Dormancy in January, DF: Dormancy in February, SGM: Spring Green-up in March, SGA: Spring Green-up in April, SGMy: Spring Green-up in May, FD: Flower Density, TD: Turf Density, RFW: Root Fresh Weight, SFW: Shoot Fresh Weight, RDW: Root Dry Weight, SDW: Shoot Dry Weight