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Review on physiological and molecular mechanisms for enhancing salt tolerance in turfgrass

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Abstract

Salt stress is a significant abiotic stress factor that hampers the growth and turf quality of turfgrass. This review examines the physiological and molecular mechanisms by which turfgrass responds to salt stress and explores strategies to enhance its salt tolerance. In high-salinity environments, turfgrass exhibits reduced quality, color, coverage, seed germination rate, root growth, and overall yield. Key physiological and molecular responses, including seed germination, morphological changes, ion balance, osmotic regulation, antioxidant systems, and gene expression are systematically discussed. Turfgrass adapts to salt stress by regulating ion transporter genes (e.g., NHX1 and HKT), synthesizing osmotic regulators (e.g., proline), and boosting antioxidant enzyme activity. The review highlights significant variations in salt tolerance across turfgrass species and varieties, reflecting their diverse genetic backgrounds. To enhance salt tolerance, three primary strategies are discussed: genetic engineering, conventional breeding, and scientific cultivation management. Genetic engineering focuses on modifying specific genes (e.g., NHX1, HKT, antioxidant enzymes), while conventional breeding emphasizes selection, hybridization, and marker-assisted approaches. Scientific cultivation management optimizes growth conditions through proper fertilization, irrigation, soil improvement, and cultivation techniques. Advancements in molecular biology and breeding technologies, particularly gene editing tools like CRISPR/Cas9, are expected to yield more salt-tolerant turfgrass varieties. The integration of genetic engineering, conventional breeding, and cultivation management will provide a strong foundation for the sustainable application of turfgrass in ecological restoration and landscaping.

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Introduction

Salt stress is one of the major abiotic stresses affecting plant growth and productivity, with a particularly significant impact on turfgrass[1]. High-salinity environments severely restrict plant growth and development, leading to reductions in various growth parameters of turfgrass, including quality, color, coverage, seed germination rate, root growth, and yield[1,2]. Turfgrass has significant potential for use in saline-alkaline soil management, as it can rapidly increase vegetation coverage on exposed soil surfaces, reduce surface water evaporation, and decrease the accumulation of soluble salts on the soil surface[3,4]. However, under salt stress conditions, turfgrass growth is significantly hindered, highlighting the urgent need for in-depth research and exploration of mechanisms and methods to cope with salt stress[5,6].

This review systematically explores the molecular and physiological response mechanisms of turfgrass under salt stress conditions. By analyzing changes in seed germination, growth and development, ion balance, osmotic regulation, antioxidant systems, and gene expression regulation in turfgrass under salt stress, it reveals the mechanisms of salt tolerance[1,2]. Furthermore, this study also discusses effective strategies for enhancing turfgrass salt tolerance, including genetic engineering, conventional breeding, and cultivation management, to provide a scientific basis and technical support for the management and application of turfgrass in saline-alkaline soils[3,7].

Physiological effects of salt stress on turfgrass

Seed gemination

Seed germination is a critical initial phase in the life cycle of plants, particularly susceptible to salt stress. The germination process includes four stages: water absorption and swelling, germination initiation, sprout extension, and seedling morphogenesis. This phase represents the most vulnerable period of plant growth, making it highly sensitive to environmental stresses, especially salt stress.

Turfgrass seeds exhibit significant differences in salt tolerance during germination, influenced by their genetic characteristics and maturity. Studies have shown that increased salinity delays the germination time of turfgrass seeds, reduces germination speed, and lowers overall germination rates[2]. For example, tall fescue (*Festuca arundinacea*) shows a higher germination rate at low to moderate salt concentrations, while creeping bentgrass (*Agrostis stolonifera*) and red fescue (*Festuca rubra*) barely germinate at high salt concentrations[1,3]. Furthermore, different types of salts have varying effects on turfgrass seed germination, with Na_2CO_3 exhibiting the most significant inhibitory effect, while the effects of NaCl and Na_2SO_4 are relatively weaker[8].

Under single salt treatments, different turfgrass cultivars in a turfgrass species also show significant differences in salt tolerance. For instance, some tall fescue cultivars maintain a high

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germination rate even at higher salt concentrations, demonstrating strong salt tolerance[9]. Overall, research on the salt tolerance of cool-season turfgrass seeds is more extensive, while warm-season turfgrasses have received relatively less attention, primarily due to their different usage patterns in turf establishment.

Morphology and growth

In a saline environment, turfgrass must expend some of the energy that would otherwise be used for growth to maintain intracellular ion balance. This energy expenditure directly inhibits plant growth and may indirectly affect plant development by impacting photosynthesis. There are significant differences in the tolerance of different turfgrass species or cultivars to salt stress.

Studies have shown that salt stress affects the morphology and growth of turfgrasses such as bermudagrass (*Cynodon dactylon*), seashore paspalum (*Paspalum vaginatum*), perennial ryegrass (*Lolium perenne*), tall fescue, and zoysiagrass (*Zoysia spp*.) in various ways. Dudeck & Peacock[5] found that as salt concentration increased, the stem and leaf growth of bermudagrass and seashore paspalum slowed, but the growth of rhizomes accelerated. Hu et al.[10] observed that different genotypes of bermudagrass showed varying turf quality (TQ) under salt stress, with the salt-tolerant 'C43' exhibiting significantly higher TQ than 'C198' under high salt conditions. In ryegrass, turf quality, leaf water content, and photosynthetic stomatal conductance all significantly decreased under salt stress[11−13]. The salt tolerance of zoysiagrass varies by cultivar, with some maintaining good condition even in high-salt environments[14].

For tall fescue, seedling dry weight and tiller number decreased with increasing salt concentration, with the shoots being more affected than the roots[15]. Salt stress also significantly affects the leaf burn rate of turfgrass, with salt-tolerant species showing lower burn rates[16]. Overall, the salt tolerance of turfgrass varies significantly among cultivars, and the growth performance under salt stress differs across species. These differences provide valuable references for the selection and management of turfgrass under saline conditions.

Root growth

Under salt stress conditions, the root growth of turfgrass exhibits significant differences. Short-term salt stress experiments have shown that low salinity can promote root growth in *Cynodon dactylon*, *Paspalum vaginatum*, *Zoysia japonica*, and *Zoysia matrella*[17].

As salinity increases, the shoot growth rate of bermudagrass decreases, but the root growth rate increases, leading to a significant rise in the root-to-shoot ratio, especially in the salt-tolerant genotype 'C43'[10]. In contrast, the salt-sensitive genotype 'C198' shows significant root growth inhibition at high salt concentrations (400 mM), with root length and number both lower than control levels. The root growth of Seashore paspalum and *Zoysia* also follow a similar trend under salt stress. At low salt stress concentrations (below 200 mM), Seashore paspalum's root growth is initially promoted, but as salinity increases, particularly beyond 400 mM, root growth is significantly inhibited[18]. The root system of *Zoysia* begins to be significantly inhibited after the salt concentration exceeds 400 mM, with root length, projected area, surface area, and diameter all gradually decreasing as the duration and intensity of salt stress increase.

Overall, the salt tolerance of bermudagrass is closely related to the adaptability of its root system under salt stress, with an increase in the root-to-shoot ratio serving as an initial evaluation indicator. Seashore paspalum and *Zoysia* perform well in low salinity conditions but are limited under high salinity.

Physiological response mechanisms of turfgrass under salt stress

Ion content and distribution

Under salt stress, the ion balance in turfgrass is severely affected, and different species or varieties exhibit significant differences in their responses to salt stress. Research indicates that at low salt concentrations, the above-ground content of sodium (Na^+) and chloride (Cl^-) in *Paspalum vaginatum*, and *Zoysia matrella* significantly increases with rising salinity, while the levels of potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) gradually decrease. *Paspalum vaginatum* accumulates high levels of Na^+ and Cl^- even at lower salinity, whereas *Seashore paspalum* and *Zoysia matrella* maintain higher K^+ levels, demonstrating stronger salt tolerance[19,20]. In *Lolium perenne* and *Cynodon dactylon*, salt stress leads to increased Na^+ content and decreased K^+ content in both the above-ground parts and roots, significantly lowering the K^+/Na^+ ratio. The salt-tolerant genotype of *Cynodon dactylon* 'C43' maintains a relatively low Na^+/K^+ ratio under high salinity, indicating strong salt tolerance[6,11].

In *Festuca arundinacea*, Na^+ and Cl^- content increases with salinity, while K^+ content decreases, and the absorption and distribution of calcium and magnesium are also affected. However, under adequate Ca^{2+} supply, salt can promote Ca^{2+} uptake, indicating a certain level of self-regulation[21,22]. Additionally, there is a significant difference in ion distribution between *Puccinellia* and *Festuca arundinacea* under salt stress. *Puccinellia* effectively reduces the entry of harmful ions into leaf tissues by selective absorption at the roots and redistribution in the stems, thereby mitigating ion toxicity, whereas *Festuca arundinacea* accumulates more Na^+ and Cl^- in leaf tissues, resulting in relatively weaker salt tolerance[23]. These studies reveal the ion regulation mechanisms under salt stress and the differences in salt tolerance among different turfgrass species.

Metabolites

Salt stress significantly affects the content of organic substances in turfgrass, particularly soluble sugars, proline, and soluble proteins, which play critical roles in maintaining cellular osmotic potential and resisting salt damage. Studies have found that moderate salt stress significantly increases the content of soluble sugars and proline in *Zoysia japonica*. As the stress duration lengthens, these levels initially rise and then decrease[24]. Similarly, the fructose and glucose content in perennial ryegrass varieties 'Overdrive' and 'PI 538976' also increased significantly under salt stress, with the latter accumulating higher sugar levels under high salt conditions[25].

Under 400 mM sodium chloride conditions, proline content in *Cynodon dactylon* significantly increased, while in *Stenotaphrum secundatum*, *Paspalum vaginatum*, and *Zoysia spp.*, proline content only showed a slight increase. Glycine betaine accumulated most in *Cynodon dactylon* and *Zoysia matrella*[19], while *Paspalum vaginatum* exhibited the strongest proline accumulation capacity[26]. The soluble protein content in *Paspalum* *vaginatum* increased with salt concentration, peaking after 21 d at 200 and 300 mmol/L, suggesting that moderate salt stress may promote protein synthesis to some extent[27]. These findings indicate significant differences in the organic matter response of different turfgrass species to salt stress, shedding light on their varied salt tolerance mechanisms.

Salt stress affects the source-sink relationship, which alters the distribution of nutrients and energy between growth and storage tissues. Different turfgrass species exhibit varying metabolic responses to salt stress, primarily involving changes in metabolites such as sugars, amino acids, and organic acids.

Research by Hu et al.[28] found that salt stress increased organic acid content in the leaves of *Cynodon dactylon* while the roots were rich in soluble sugars and nitrogenous metabolites, helping maintain root function and adapt to the stress environment. In perennial ryegrass, salt stress significantly increased the levels of sugars and organic acids in the leaves, indicating that plants adjust metabolite levels to cope with salt stress[29]. *Poa pratensis* adapts to salt stress mainly through the accumulation of amino acids and sugars, with metabolites like glucose, fructose, and sucrose significantly increasing, while levels of some organic acids and fatty acids also change[30]. In creeping bentgrass, the salt-tolerant cultivar 'NTAS' exhibited higher levels of soluble sugars, proline, and glycine betaine under salt stress, suggesting that the accumulation of these metabolites might be key factors in its salt tolerance[31].

Overall, the significant changes in metabolites under salt stress in turfgrass include sugars, sugar alcohols, amino acids, and organic acids. The accumulation of these substances helps enhance osmotic regulation, stabilize cell membranes, and reduce damage caused by stress. Although different grass species exhibit similar metabolic pathways under salt stress, there are variations in the types and levels of metabolites, reflecting the diversity in their salt tolerance abilities.

Reactive oxygen species and antioxidant enzymes

Salt stress not only increases the production rate of superoxide anions and the content of malondialdehyde (MDA) in turfgrass, leading to oxidative stress and enhanced cell membrane permeability, but also activates the plant's antioxidant defense system to maintain the balance of reactive oxygen species (ROS) within the plant. This defense system includes enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as non-enzymatic antioxidants like ascorbic acid and glutathione.

In cool-season turfgrass, salt stress significantly increases the electrical conductivity and MDA content in ryegrass leaves, with the salt-sensitive cultivar 'DP1' being more affected[10]. As salt stress continues, the activity of antioxidant enzymes such as SOD, POD, and APX initially increases and then decreases, with salt-tolerant cultivars showing stronger regulatory capacity in enzyme activity. In warm-season turfgrass, studies have found that the sensitivity of antioxidant enzymes in *Zoysia matrella* to salt stress follows the order SOD > APX > POD > GR > CAT, indicating that SOD is the most sensitive to high-salt environments[32]. The Cynodon dactylon cultivar 'C43' exhibits stronger antioxidant capacity under high salt stress by enhancing the activities of enzymes like SOD and CAT, while the salt-sensitive cultivar 'C198' shows a decrease in the activity of certain antioxidant enzymes[10].

Overall, turfgrass responds to salt stress by regulating the antioxidant enzyme system. This comprehensive antioxidant strategy helps maintain physiological stability and reduce cellular damage. The significant differences in the regulation of antioxidant enzyme systems among different cultivars and genotypes reveal the diverse mechanisms of salt tolerance in various turfgrass species.

Molecular mechanisms of salt stress response in turfgrass

Omics analysis

Salt stress significantly affects plant physiological metabolism and enhances salt tolerance by regulating gene expression and protein synthesis. Transcriptomic and proteomic studies have revealed the complex mechanisms plants use to respond to salt stress. In studies on bermudagrass, Hu et al.[29] identified numerous salt-tolerance-related genes through transcriptome analysis, particularly in the salt-tolerant variety 'C43'. Several transcription factors were specifically expressed, and these genes were involved in key functions such as stress response, lignin synthesis, and cell wall regulation. The expression of these genes promoted root elongation, helping the plant adapt to salt stress. Similarly, in Kentucky bluegrass (*Poa pratensis*), salt-tolerant varieties showed enhanced adaptation to salt stress by reducing sodium ion absorption and regulating the expression of water transport genes.

Proteomic studies have also revealed the mechanisms underlying plant salt tolerance. Ye et al.[33] found that bermudagrass (*Cynodon dactylon*) exhibited significant protein changes under salt stress, mainly in metabolic pathways such as photosynthesis, glycolysis, and antioxidant responses. In particular, the activity of antioxidant enzymes such as catalase (CAT) and glutathione reductase (GR) increased, indicating that the plant responds to stress by activating its antioxidant defense system. In studies on seashore paspalum and seashore crowngrass, Liu et al.[34] discovered that proteins related to ROS detoxification and energy metabolism were significantly upregulated under salt stress, which is closely linked to their strong salt tolerance.

Overall, transcriptomic and proteomic studies have revealed that plants adapt to salt stress by regulating genes and proteins, enhancing metabolic pathways, and activating antioxidant defense systems. These findings provide important theoretical insights for breeding salt-tolerant crops.

Genome-wide association analysis (GWAS)

To identify candidate genes associated with key agronomic traits, Wang et al.[35] conducted a GWAS analysis related to salt tolerance based on genome-wide SNPs and phenotypic data from 102 bermudagrass accessions. In the study, 102 tetraploid bermudagrass germplasm resources were treated with NaCl under hydroponic conditions. The salinity concentration was gradually increased from 1.0% to 2.0%, with 0.5% increments, and each concentration was maintained for 7 d. At the end of the experiment, agronomic traits such as electrolyte leakage ratio, relative growth ratio, and leaf width were measured.

Subsequently, the GWAS analysis was conducted based on the genome-wide SNPs and phenotypic data from the 102 bermudagrass accessions. Among 3,626,522 SNPs, 82 candidate genes associated with six agronomic traits were identified, including growth height ratio, relative water content ratio, relative electrical conductivity ratio, turf quality ratio, rewater survival rate, and leaf width. RNA-seq analysis revealed that nine of these genes exhibited significantly different expression levels under salt stress compared to the control group, indicating their potential breeding value.

Among these nine genes, ethylene-responsive transcription factor (RAP2-2), the cyclic nucleotide-gated ion channel 1 (CNG channels), and the probable leucine-rich repeat receptor-like protein kinase (F14D7.1) are known to be associated with salt stress response. The homologs of these genes in rice and Arabidopsis have been shown to confer salt stress resistance. The study found that RAP2-2 and CNG channel genes were significantly downregulated in salt-treated bermudagrass, while F14D7.1 was significantly upregulated under salt stress. Therefore, RAP2-2, CNG channel, and F14D7.1 are potential candidate genes for enhancing salt tolerance in bermudagrass.

Exploration of salt tolerance genes

With advancements in functional genomics, significant breakthroughs have been made in understanding the molecular mechanisms underlying salt tolerance in turfgrasses and forage grasses. Using forward and reverse genetics, researchers have identified and cloned several key genes related to salt stress, which play crucial roles in osmotic regulation, ion transport, and signal transduction.

In terms of osmotic regulation, studies have shown that choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) are essential genes for improving salt tolerance in Kentucky bluegrass[36]. Introducing these genes into turfgrasses has significantly enhanced their salt tolerance. Another key gene, pyrroline-5-carboxylate synthetase (P5CS), expressed in *Leymus chinensis*, has been shown to increase proline content, thereby enhancing the plant's resistance to salt stress[37].

Ion balance genes play a critical role in improving salt tolerance. The plasma membrane Na^+/H^+ antiporter (SOS1) and the vacuolar membrane Na^+/H^+ antiporter (NHX) help maintain normal physiological functions in plants under high salt conditions by regulating ion concentrations inside and outside the cells[38]. Studies have demonstrated that transgenic plants overexpressing these genes exhibit stronger growth and less cellular damage under salt stress[39].

In signal transduction, the abscisic acid (ABA) pathway and dehydration-responsive element binding protein (DREB) transcription factors are crucial in regulating the expression of salt-responsive genes. For instance, the expression of the FaDREB1 gene significantly enhances plant resistance to salt stress by promoting the accumulation of osmotic regulators, and reducing cell membrane damage[40]. Additionally, transcription factor families like AP2/ERF and WRKY play key roles in the response of turfgrasses to salt stress, further enhancing salt tolerance by regulating the expression of multiple salt-responsive genes[29].

However, the low efficiency of genetic transformation in turfgrasses like bermudagrass has hindered gene function studies and the improvement of salt-tolerant varieties. Recently, researchers have developed more efficient Agrobacterium-mediated transformation systems, significantly improving the transformation efficiency of bermudagrass, providing new technical support for cloning and expressing salt tolerance-related genes[41]. This advancement opens new avenues for the molecular improvement of salt tolerance in turfgrasses, further advancing the understanding and application of salt tolerance mechanisms.

Salt tolerance enhancement and breeding in turfgrass

Molecular markers associated with salt-tolerance

Studies have shown that there are significant differences in salt tolerance among various turfgrass species and germplasm. Overall, the strongest salt-tolerant turfgrasses are mainly found among warm-season species, such as alkali grass (Puccinellia spp.), seashore paspalum (*Paspalum vaginatum*), St. Augustinegrass (*Stenotaphrum secundatum*), and zoysiagrass (*Zoysia spp.*)[42]. In contrast, fewer cool-season turfgrass species exhibit strong salt tolerance. The salt tolerance of bermudagrass (*Cynodon spp.*) also varies significantly among different germplasms. Research has found that under high salt concentrations, only a few common bermudagrass varieties can survive, while *Cynodon transvaalensis* and *Cynodon dactylon* × *C. transvaalensis* hybrids exhibit poor salt tolerance under salt stress[43]. These highly salt-tolerant common bermudagrass varieties are mainly distributed in coastal areas, suggesting that their salt tolerance may be closely related to their growth environment.

Among cool-season turfgrasses, tall fescue (*Festuca arundinacea)* exhibits relatively strong salt tolerance, with a salt tolerance index significantly higher than that of Kentucky bluegrass (*Poa pratensis*) and perennial ryegrass (*Lolium perenne*)[44]. Cluster analysis revealed that tall fescue varieties are consistent in terms of salt tolerance across indicators such as biomass, green leaf number, and photochemical efficiency, indicating strong and stable salt tolerance. Zoysiagrass (*Zoysia spp.*) exhibits rich genetic variation in salt tolerance. A study evaluating the salt tolerance of 206 zoysiagrass germplasms found significant differences in indicators such as leaf chlorosis rate and clipping dry weight, categorizing them into five distinct salt tolerance categories[45]. Among these, the highly salt-tolerant types maintained good growth under high salt stress, providing important references for the improvement of salt-tolerant zoysiagrass varieties.

Salt-tolerant molecular markers

DNA molecular marker technology, unaffected by environmental factors and developmental stages can detect DNA-level differences across various tissues, making it widely applicable in fields like genome mapping, gene localization, and species classification. In recent years, the development of molecular marker technology, particularly in the selection and breeding of stress-resistant crops has significantly enhanced breeding efficiency.

Simple sequence repeat (SSR) marker technology, widely used for detecting polymorphisms in DNA fragments within the genome has been extensively applied in studies of salt tolerance in tall fescue. Amombo et al.[46] analyzed 114 tall fescue germplasm using 99 SSR markers and identified significant marker-trait associations, determining allele markers related to functional traits such as turf quality, leaf water content, and chlorophyll content. These markers were closely associated with the accumulation of Na^+ and Cl^- in tall fescue and effectively distinguished between salt-tolerant and salt-sensitive varieties, providing crucial insights for improving salt tolerance in tall fescue.

Sequence-related amplified polymorphism (SRAP) marker technology, known for its simplicity and cost-effectiveness in detecting polymorphisms in DNA fragments within the genome has been widely applied in plant genetic diversity analysis. Chen & Huang[47] utilized SRAP technology to screen out seven primers closely related to salt tolerance in Zoysia species, successfully amplifying 37 loci, of which 29.7% were salt-tolerance polymorphic loci. These markers offer strong support for the identification and breeding of salt-tolerant Zoysia species.

With the advancement of molecular biology research, the application of DNA molecular marker technology in analyzing salt tolerance in turfgrass is still relatively limited. However, its efficiency and stability indicate substantial potential for further application, likely driving the breeding process of salt-tolerant turfgrass in the future.

Application of exogenous substances

Plant hormones and growth regulators play a critical role in enhancing the salt tolerance of turfgrasses. Abscisic acid (ABA) significantly boosts plant salt tolerance by regulating stomatal closure, reducing transpiration, and promoting the expression of antioxidant enzymes[48]. Studies have shown that under salt stress conditions, ABA effectively maintains the physiological and metabolic stability of tall fescue by promoting root growth and increasing leaf water content, thereby significantly improving its salt tolerance.

Salicylic acid (SA), as a signaling molecule, activates the plant's defense mechanisms, enhancing salt resistance. Dong[49] found that exogenous SA treatment significantly improved the turf quality and growth performance of perennial ryegrass under salt stress, reduced cell membrane damage, and increased plant-relative water content and antioxidant enzyme activity, demonstrating a good effect in mitigating salt damage.

Paclobutrazol (PP333), by inhibiting vertical growth and enhancing antioxidant capacity, promotes lateral growth and root development of plants under salt stress. Chen & Huang[47] showed that different concentrations of PP333 significantly improved the growth performance of tall fescue, with the best results observed at 400 mg/L, enhancing its salt tolerance.

Additionally, osmotic regulators like trehalose and glycine betaine have significant effects on improving turfgrass salt tolerance. Studies indicate that exogenous trehalose can effectively mitigate the inhibitory effects of salt stress on plant growth and improve cellular ion balance by increasing the K^+/Na^+ ratio[50]. Glycine betaine, by enhancing antioxidant enzyme activity and maintaining cell membrane integrity, effectively alleviates oxidative damage in ryegrass seedlings under salt stress[25].

These studies highlight the crucial role of plant hormones, growth regulators, and osmotic regulators in enhancing turfgrass salt tolerance, providing scientific support for the breeding and management of salt-tolerant turfgrasses.

Conclusions

Salt stress impacts turfgrass through changes in seed germination, growth, ion balance, osmotic regulation, and antioxidant activity, with significant variation among species. Enhancing salt tolerance can be achieved through genetic engineering, conventional breeding, and optimized cultivation practices. Advances in molecular tools, such as gene editing, show great potential for developing more resilient turfgrass varieties. By integrating these strategies, sustainable turfgrass use in saline environments can be supported, benefiting ecological restoration and landscaping. Ongoing research will be crucial for further improvements in salt tolerance and turfgrass adaptability.

Author contributions

The authors confirm contribution to the paper as follows: literature collection and writing of the manuscript: Zhu Y; editing and revising manuscript: Fu Q; development of outlines and revision of the manuscript: Fu J. All authors, including Zhu C, Li Y, Yuan F, and Sun X, reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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