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Effects of iron tailings on the growth and physiological response of cool-season turfgrass

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Abstract

Over the past few decades, due to mining activity, a large amount of iron mine tailing sands have been deposited. Selection of appropriate plant species and studying their growth and physiological response under iron tailings are the key factors for successful phytoremediation of iron ore. For this reason, the growth and physiological responses of creeping bentgrass, tall fescue, and Kentucky bluegrass were evaluated by pot experiments with growth substrates supplemented with 0%, 25%, 50%, 75%, and 100% iron-tailed sand. With the increasing proportion of iron tailing sand, the plant height, root length, fresh weight, dry weight, and growth rate of the three turfgrass species decreased gradually. Similarly, the increase in iron ore reduced the chlorophyll content of the three turfgrass plants. The relative electrolyte leakage of creeping bentgrass and tall fescue showed a stable increase, usually higher than the control, while the relative conductivity of Kentucky bluegrass showed a gradual downward trend, but on the whole maintained a high level. The contents of malondialdehyde (MDA), soluble protein (SP), and hydrogen peroxide (H_2O_2) of the three cold season turfgrasses were generally higher than those of the control on the 20th and 60th day. Comprehensive analysis showed that the adaptability of tall fescue to iron tailings was better than the other two turfgrass. Therefore, tall fescue has potential application value in vegetation restoration of iron tailings and phytoremediation of iron polluted environments.

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Introduction

Tailings, also known as tailing sand, are fine granular powders left behind after the ore crushing and flotation concentrate processing, which is solid waste[1]. Among them, iron tailing sand are the tailings produced in the process of washing iron ore. Due to its high density and small particle size, tailing sand is prone to flow and collapse, which under certain conditions can lead to dam failures, resulting in loss of life and property[2]. It is also unsuitable for use as a building material because of its poor physical structure. Excessive iron (Fe) content and wind-driven dust, causes serious pollution of soil, water, and the atmosphere[3−5]. At present, the main treatment of iron tailing sand is still based on passive stockpiling and landfilling, which wastes a large amount of land resources. Therefore, plants are gradually being applied to the improvement and restoration of iron tailing sand for the treatment of iron tailing sand[6−8]. The selection of plants should not only consider their high resistance to heavy metal stress but also consider their tolerance to poor soils[9]. Therefore, the management of tailing ponds can be more effectively realized by screening the dominant grass species.

Relevant studies found that maize (*Zea mays*), and bean (*Phaseolus vulgaris*) cultivated in contaminated soils in the mining area have a certain enrichment capacity for heavy metals[10]. However, the selection of these plants for remediation of heavy metal pollution has some drawbacks, firstly, the biomass is too low, and secondly, the selection of crops can be spread through the food chain to jeopardize human health. The use of garden plant creeping bentgrass (*Agrostis stolonifera*), tall fescue (*Festuca arundinacea*), and Kentucky bluegrass (*Poa pratensis*) remediate heavy metal pollution, the product will not enter the food chain and the species provide biomass, regeneration capacity, dense root systems, fast growth, are easy to plant, have wide applicability and also have an ornamental value. It also has the dual role of remediation and environmental beautification[11]. It has been demonstrated that creeping bentgrass is tolerant to lead (Pb), tall fescue is tolerant to cadmium (Cd), and Kentucky bluegrass is tolerant to nickel (Ni) and Cd, and that the heavy metal content of the soil can be reduced by repeated mowing[12−14]. However, there are fewer studies on the Fe tolerance of creeping bentgrass, tall fescue, and Kentucky bluegrass and their remediation of iron tailing sands. Therefore, in this study, three cool-season turfgrasses, creeping bentgrass, tall fescue, and Kentucky bluegrass, were selected as materials, and the effects of iron tailing sand on three kinds of turfgrass biomass, relative electrical conductivity (EL), soluble protein (SP), malondialdehyde (MDA) content, hydrogen peroxide (H_2O_2), and other physiological indexes were investigated through potting experiments, to explore the tolerance ability of the turfgrasses to Fe and provide a basis for the research on the mechanism of resistance to iron tailing sand and screening of resistant grasses. The experiment aimed to explore the tolerance ability of turfgrass to iron (Fe), and to provide a theoretical basis for the research on the mechanism of turfgrass resistance to iron tailing sand and the screening of resistant grass species.

Materials and methods

Plant materials and cultivation substrates

The Kentucky bluegrass cultivar 'Green Summer', tall fescue cultivar 'Crown' and creeping bentgrass cultivar 'Penn-A4' were used for this study. Beneficiated iron tailing sand was collected from Laiyuan County, Baoding, Hebei Province, China. Nutrient substrate: peat (Pindstrup Plus Black: a substrate with medium fertilizer content, 100% dark peat) and vermiculite.

Experimental design and treatments

As shown in Table 1, iron tailing sand was proportionally mixed with the nutrient substrate (peat to vermiculite ratio 1:2) to make a cultivation substrate, where the volume fractions of iron tailing sand were 0% (control, CK), 25%, 50%, 75%, and 100%, respectively. The individual cultivation substrates were individually packed into plastic pots (diameter: 14 cm; depth: 12 cm) with three replications of each treatment for each grass species, totaling 45 pots, and 0.5 g of seeds of Kentucky bluegrass, tall fescue, and creeping bentgrass were sown in each pot, respectively. After sufficient watering, the nonwoven fabric was well covered and placed in a greenhouse at a temperature of 24 ± 5 °C, a humidity of 60%−70%, and a light period of 13−15 h for incubation. The first mowing was done to 5 cm and the clippings were collected for fresh and dry weight measurements. Relative electrical conductivity (EL), soluble protein (SP), malondialdehyde (MDA) content, and hydrogen peroxide (H_2O_2) were determined on days 20 and 60 after sowing. Meanwhile, the height of the seedlings was recorded periodically to calculate the growth rate and finally to determine the chlorophyll content.

Characterization of the substrates

The substrates were air-dried and sieved (4 mm mesh) and sent for physical and chemical characterization at Standard Testing Group Co., Ltd. Qingdao, China. The pH and conductivity of the tailings and the soil were determined in triplicate using a 1:2.5 (v/v) ratio of soil : distilled water[15]. Organic matter was determined using the oxidation in $K_2Cr_2O_7$ by the method of Walkey and Black[16].

Measurement of plant height and biomass

The natural height of the above-ground portion of the plant was measured (the height from the ground to the very top of the main stem) with a straightedge with an accuracy of 1 mm, and the height of the plant was recorded regularly. After six months, the plant was removed from the pot to measure its root length. At the first mowing, Kentucky bluegrass, tall fescue, and creeping bentgrass were mowed to a height of 5 cm. The shoot clippings of three turfgrass species were collected and bagged separately for recording, placed sequentially on the instrument for fresh weight (FW) determination, weighed, and placed in the oven with the temperature raised to 100~105 °C for 10 min, then dried at 70~80 °C to a constant weight.

Physiological parameter measurement

Chlorophyll content was determined according to the procedure described by Sun et al[17]. Fresh leaf tissues (50 mg) were sliced and incubated in 7 mL acetone in the dark for 48 h. Absorbance was measured at wavelengths of 649 and 665 nm by a spectrophotometer, and the chlorophyll content was calculated using the formula of Smethurst & Shabala[18].

The relative conductivity (Electrolyte leakage, EL) was determined following the method of Sun et al.[17].

Soluble Protein (SP) was determined using Bradford's method[19].

Malondialdehyde (MDA) was measured according to the procedure described by Li et al.[20]. 0.1 g samples were ground in a prechilled mortar and homogenized in 2 mL of 5 % (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 g for 10 min. 1.5 mL of the supernatant was mixed with 2-mL aliquot of TBA [0.67% (w/v)] and incubated at 100 °C for 15 min. The mixture was quickly cooled on ice and centrifuged at 5,000 g for 10 min at 4 °C. The supernatant was measured at 450, 532, and 600 nm on a spectrophotometer.

Hydrogen peroxide (H_2O_2) was determined using the procedure described by Sun et al.[21]. 0.25 g fresh leaf samples were homogenized on ice with 2 mL 0.1% (w/v) trichloroacetic acid (TCA). After centrifugation at 15,000 g for 15 min, 1 mL 10 mM sodium phosphate buffer (pH 7.0) and 2 mL 1 M potassium iodide (KI) were added into 1 mL of supernatant and mixed well. The absorbance was measured at 390 nm and H_2O_2 content was calculated using a standard curve.

Table 1. Potting experiment treatments.

Treatment	CK (A0)	A1	A2	A3	A4
Iron tailing ore	0%	25%	50%	75%	100%
Nutritional mechanisms	100%	75%	50%	25%	0%

Statistical analysis

All data were processed and analyzed using Excel 2022 and SPSS 27.0. Analysis of variance (ANOVA) and multiple comparisons were performed using one-way ANOVA and LSD method (α = 0.05), and GraphPad prism 9 was used for graphing.

Results

Physico-chemical properties of iron tailing sand and substrates

Iron tailing sand chemical composition analyses are shown in Table 2. Amongst the metals estimated in the iron tailing sand, the concentration of Fe was highest (72.97 $g \cdot kg^{-1}$), followed by Al, Mn, Zn, In, Ti, Co, Cu, and Sr. Changes in PH, EC, and organic matter under nutrient substrate treatments with different volumes of iron tailing sand added (Table 3). The pH of the iron tailing sand was more alkaline than that of the nutrient substrate, with the iron tailing sand having a pH of 10.15 and the nutrient substrate having a pH of 6.05. The pH, conductivity, and organic matter content gradually decreased with increasing volume of iron tailing sand.

Plant height and root length

Plant height of creeping bentgrass, tall fescue, and Kentucky bluegrass showed a significant decrease with increasing iron tailing sand additions (Fig. 1a–o). Among them, creeping bentgrass was significantly reduced by 58.06%, 67.74%, and 81.72% in plant height under A2, A3, and A4 treatments compared to CK (p < 0.05). Compared with CK, the plant height of tall fescue treated with A1, A2, A3, and A4 was significantly decreased by 15.66%, 31.31%, 35.66%, and 44.35% (p < 0.05). The plant height of Kentucky bluegrass treated with A1, A2, A3, and A4 was significantly decreased by 9.77%, 62.85%, 69.21%, and 80.89% compared with CK (p < 0.05). Under A1 treatment, the plant height of tall fescue was significantly higher than that of Kentucky bluegrass (p < 0.05). Under A2, A3, and A4 treatments, tall fescue was significantly higher than grass and creeping bentgrass (p < 0.05) (Fig. 1p).

For root length (Fig. 1q), creeping bentgrass was significantly elevated by 10.38% (p < 0.05) under A1 treatment compared to CK. In addition, creeping bentgrass, tall fescue, and Kentucky bluegrass all showed significant (p < 0.05) reduction in root length under A2, A3, and A4 treatments compared to CK, with creeping bentgrass significantly reduced by 86.13%, 91.46%, and 92.68% (p < 0.05), tall fescue by 29.82%, 48.62%, and 62.24% (p < 0.05), and Kentucky bluegrass was significantly reduced by 80.58%, 88.92%, and 91.67% (p < 0.05). Under A1 treatment, there was a significant trend of

Table 2. Chemical composition analysis of iron tailing sand.

Ingredient	Fe	Al	Mn	Zn	Cu	Ti	In	Sr	Co
Content ($g \cdot kg^{-1}$)	72.97 ± 12.19	9.13 ± 1.03	5.00 ± 0.32	1.10 ± 0.23	0.26 ± 0.02	0.44 ± 0.05	0.48 ± 0.03	0.11 ± 0.03	0.30 ± 0.02

creeping bentgrass > Kentucky bluegrass > tall fescue ($p < 0.05$). Under A2, A3, and A4 treatments, the root length of tall fescue was significantly higher than that of Kentucky bluegrass and creeping bentgrass ($p < 0.05$).

Table 3. Physico-chemical properties of iron tailing sand with different treatments (n = 3, mean ± SE).

Treatment	pH value	Electric conductivity EC (mS·m^{-1})	Organic matter (g·kg^{-1})
CK	6.05 ± 0	17.4 ± 0.78	380 ± 12.35
A1	7.84 ± 0.16	12.07 ± 0.03	85 ± 0.17
A2	9.25 ± 0.08	10.23 ± 0.29	34.53 ± 0.09
A3	9.63 ± 0.01	10.2 ± 0.17	24.57 ± 0.12
A4	10.15 ± 0.03	9.27 ± 0.35	19.17 ± 0.41

CK: Pure nutritional substrate, A1: 75% nutritional substrate with 25% iron tailing sand, A2: 50% nutritional substrate with 50% iron tailing sand, A3: 25% nutritional substrate with 75% iron tailing sand, A4: Pure iron tailing sand.

Growth rate, fresh and dry weight

The plant height of each turfgrass was uniformly mowed to 5 cm, and the growth rate of the three turfgrasses are shown in Fig. 2a–f. Creeping bentgrass and Kentucky bluegrass plants died under treatments A2, A3, and A4. At day 50, differences in plant height of tall fescue under A1, A2, and A3 treatments compared to CK treatment were 7.16 cm, 18.36 cm, and 20.16 cm (Fig. 2g–i). Compared with the three turfgrasses, tall fescue had the fastest growth rate, creeping bentgrass was the next fastest, and Kentucky bluegrass was the worst. Tall fescue could continue to grow under A2 and A3 treatments.

The fresh and dry weights of creeping bentgrass, tall fescue, and Kentucky bluegrass showed a gradual decrease with the increase of

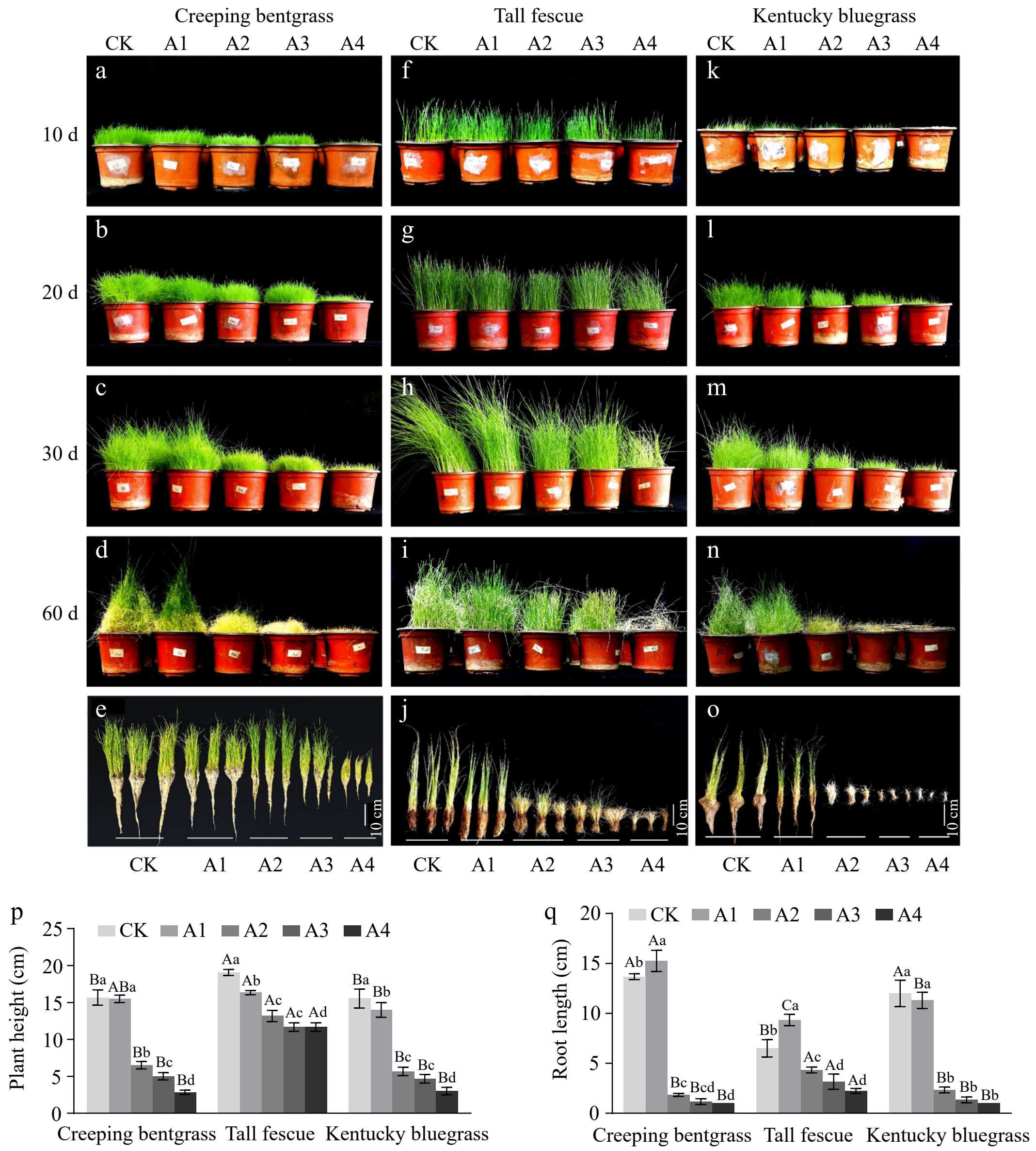

Fig. 1 Effect of adding iron tailing sand on plant height and root length of three cool-season turfgrass species. Plant height of creeping bentgrass after (a) 10, (b) 20, (c) 30, and (d) 60 d of sowing. Plant height of tall fescue after (f) 10, (g) 20, (h) 30, and (i) 60 d of sowing. Plant height after (k) 10, (l) 20, (m) 30, and (n) 60 d of sowing of Kentucky bluegrass. Root length of (e) creeping bentgrass two months after sowing, (o) Kentucky bluegrass, and (j) tall fescue six months after sowing. The (p) plant height, and (q) root length of three types of cool-season turfgrass in nutritional substrate treatments with different proportions of iron tailing sand. Data are presented as means of three technical replicates, and error bars represent SD. Different lowercase letters indicate significant differences between the same grass species and different iron tailing sand treatments ($p < 0.05$), different uppercase letters indicate significant differences between different grass species and the same iron tailing sand treatment ($p < 0.05$).

iron tailing sand addition. In terms of fresh weight, creeping bentgrass showed a significant reduction in root length by 45.22% and 76.41% ($p < 0.05$) under A1 and A2 treatments compared to CK, and plants died under A3 and A4 treatments. Tall fescue fresh weight was significantly reduced by 58.42% and 65.53% ($p < 0.05$) under A2 and A3 treatments compared to CK, and plants died under A4 treatment. The fresh weight of Kentucky bluegrass was significantly reduced by 51.7% ($p < 0.05$) under the A1 treatment compared to CK, and plant death was observed under the A2, A3, and A4 treatments. Tall fescue was significantly ($p < 0.05$) higher than creeping bentgrass and Kentucky bluegrass under A1 treatment. The fresh weight of tall fescue was higher than creeping bentgrass under A2 treatment ($p < 0.05$). Under the A3 and A4 treatments, creeping bentgrass and Kentucky bluegrass died, except for tall fescue (Fig. 2j).

For dry weight, creeping bentgrass and Kentucky bluegrass died under treatments A2, A3, and A4. Tall fescue was significantly reduced by 60.24%, and 66.58% ($p < 0.05$) under A2, and A3

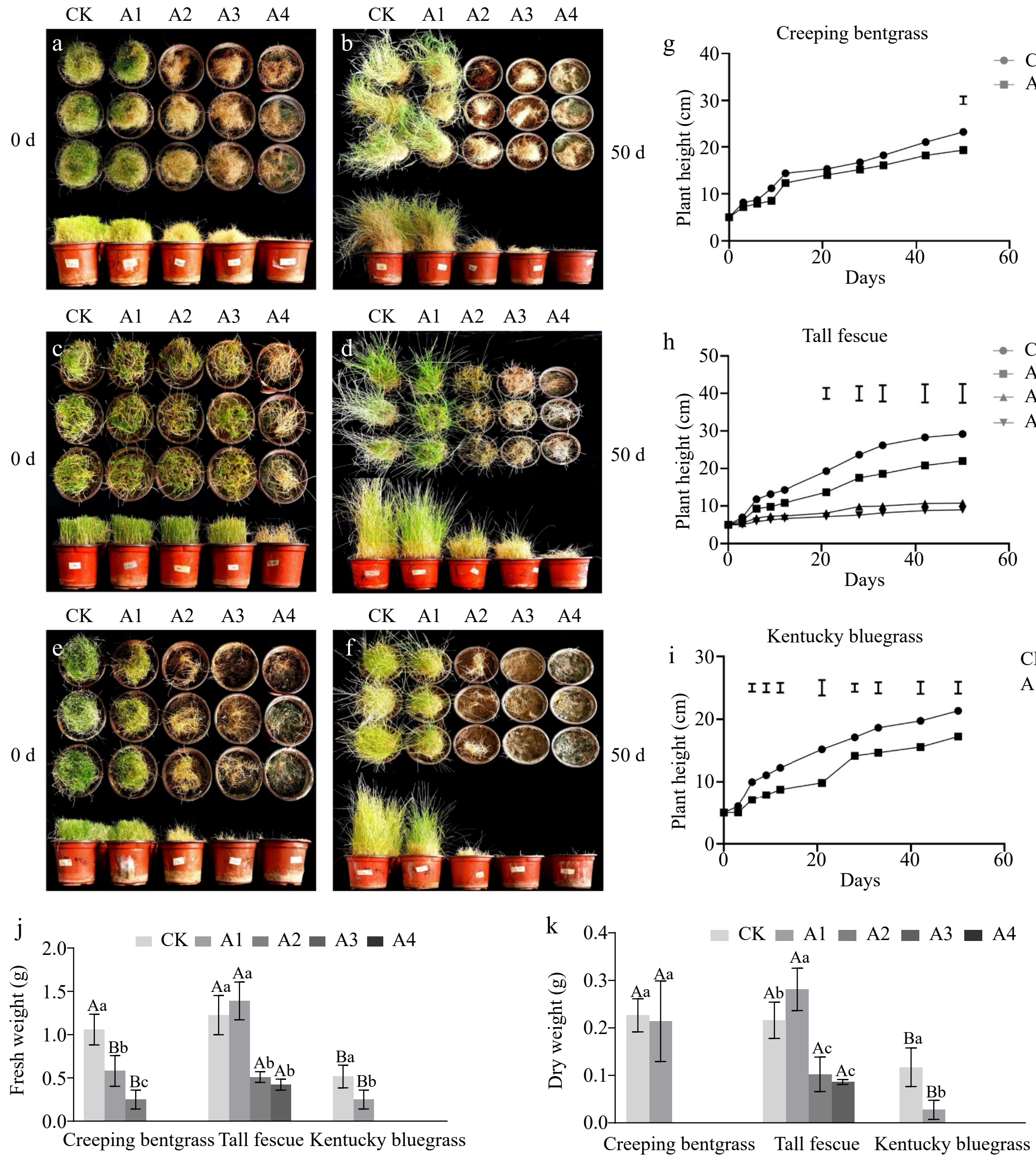

Fig. 2 Effect of nutrient substrates with different proportions of iron tailing sand on the growth rate of three cool-season turfgrasses. Fully developed (a) creeping bentgrass, (c) tall fescue, and (e) Kentucky bluegrass grown in pots were mowed to the same height of 5 cm. Fifty days growth of (b) creeping bentgrass, (d) tall fescue, and (f) Kentucky bluegrass after the first mowing. Growth rate of (g) creeping bentgrass, (h) tall fescue, and (i) Kentucky bluegrass in the natural state from the first mowing to 50 d later. The (j) fresh weight, and (k) dry weight of three types of cool-season turfgrass in nutritional substrate treatments with different proportions of iron tailing sand. A2 (50% nutrient substrate with 50% tailing sand), A3 (25% nutrient substrate with 75% iron tailing sand), A4 (pure iron tailing sand) for creeping bentgrass; A4 of tall fescue (pure iron tailing sand); A2 (50% nutrient substrate with 50% iron tailing sand), A3 (25% nutrient substrate with 75% iron tailing sand), and A4 (pure iron tailing sand) of Kentucky bluegrass were dead at the time of the first pruning and were less than 5 cm tall. Data are presented as means of three technical replicates, and error bars represent SD. Vertical bars on the top indicate least significant difference values ($p < 0.05$) for treatment comparison. Different lowercase letters indicate significant differences between the same grass species and different iron tailing sand treatments ($p < 0.05$), different uppercase letters indicate significant differences between different grass species and the same iron tailing sand treatment ($p < 0.05$).

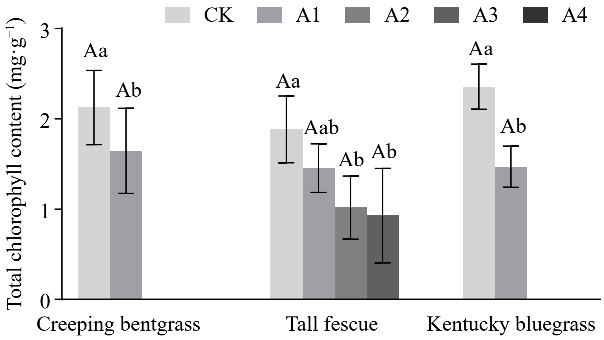

Fig. 3 Chlorophyll content of three types of cool-season turfgrass in nutritional substrate treatments with different proportions of iron tailing sand. Different lowercase letters indicate significant differences between the same grass species and different iron tailing sand treatments (p < 0.05), different uppercase letters indicate significant differences between different grass species and the same iron tailing sand treatment (p < 0.05).

treatments compared to CK and significantly increased by 23.13% under A1 treatment compared to CK. The plants died under A4 treatment. Compared with CK, the plants under A1 treatment significantly decreased by 76.32% (p < 0.05), and the plants under A2, A3, and A4 treatment died. Kentucky bluegrass was significantly reduced by 76.32% (p < 0.05) under A1 treatment as compared to CK and plant death was observed under A2, A3, and A4 treatments. Tall fescue and creeping bentgrass were significantly (p < 0.05) higher than Kentucky bluegrass under A1 treatment (Fig. 2k).

Chlorophyll content

As shown in Fig. 3, iron tailings reduced chlorophyll content of three turfgrass species. Creeping bentgrass and Kentucky bluegrass showed a significant reduction of 22.45% and 37.59% (p < 0.05), respectively, in chlorophyll content compared to the CK under treatment A1. Tall fescue was significantly reduced by 46.06% and 50.76% (p < 0.05) under A2 and A3 treatments compared to CK. The differences among the three turfgrasses were not significant under the A1 treatment. The chlorophyll content of tall fescue was higher than that of creeping bentgrass and Kentucky bluegrass under A2 and A3 treatments.

Cell membrane stability

At 20 d after sowing (Fig. 4a, c), MDA content and electrolyte leakage (EL) were significantly higher (p < 0.05) than CK in all three turfgrass species; except for tall fescue EL under A1 treatment. Creeping bentgrass and tall fescue showed a gradual increase with the increase of iron tailing sand addition, EL and MDA content of creeping bentgrass and tall fescue reached the highest under A4 treatment, the increase of EL was 69.5%, 82.75%, and the increase of MDA was 67.1%, 49.65%, respectively. EL of Kentucky bluegrass was highest under A1 treatment with an increase of 69.72% and MDA content was highest under A3 treatment with an increase of 66.99%.

At 60 d after sowing (Fig. 4b, d), MDA content was significantly higher (p < 0.05) than CK under all treatments among the three turfgrass species. EL relative conductivity was significantly higher (p < 0.05) than CK under all treatments, except for Kentucky bluegrass, which did not show any significant difference under the A3 treatment, and A4, which was significantly lower (p < 0.05) than CK under the A4 treatment. The EL of creeping bentgrass and tall fescue increased gradually with the increase of iron tailing sand, and the EL of creeping bentgrass and tall fescue reached the highest in A4 treatment, with increases of 52.92% and 78.27%, respectively; the EL of Kentucky bluegrass reached the highest in A1 treatment, with the increase of 42.83%. The MDA content of creeping bentgrass, tall fescue, and Kentucky bluegeass reached the highest under A2 treatment with an increase of 41.52%, A4 treatment with an increase of 49.02%, and A2 treatment with an increase of 51.47%, respectively.

Hydrogen peroxide content

At 20 d after sowing (Fig. 5a), the H_2O_2 content of the three turfgrass species was significantly higher (p < 0.05) than that of CK under all treatments except for tall fescue under treatment A1. The H_2O_2 content of creeping bentgrass reached the highest under A2 treatment with an increase of 30.31%, that of tall fescue reached the

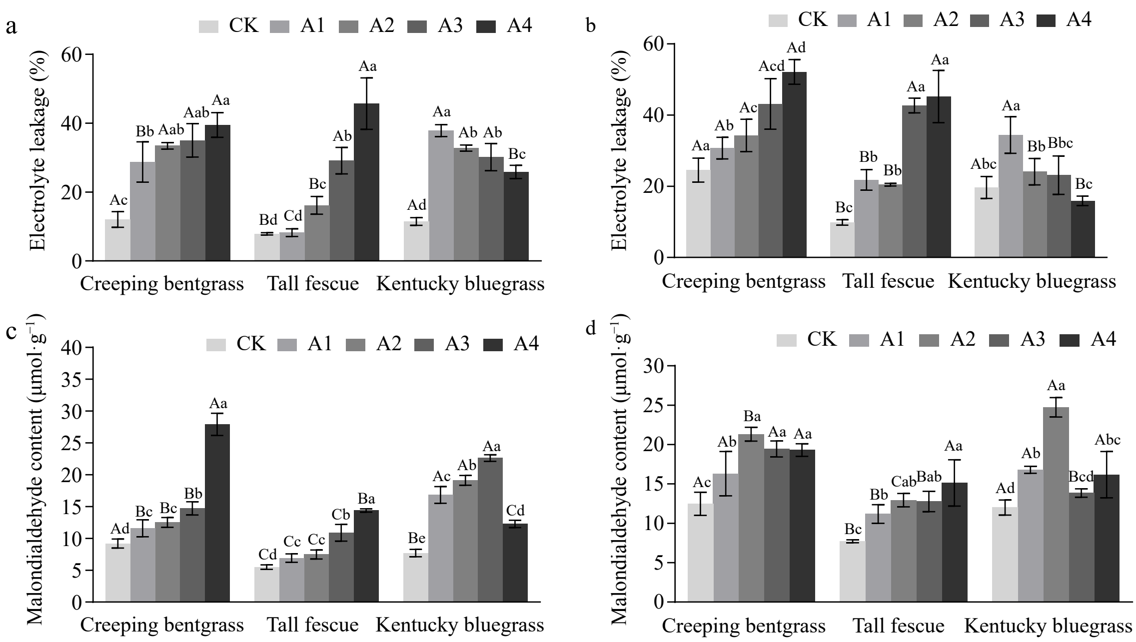

Fig. 4 Electrolyte leakage at (a) 20 and (b) 60 d and malondialdehyde content at (c) 20 and (d) 60 d in three cool-season turfgrasses treated with nutrient substrates supplemented with different proportions of iron tailing sand. Different lowercase letters indicate significant differences between the same grass species and different iron tailing sand treatments (p < 0.05), different uppercase letters indicate significant differences between different grass species and the same iron tailing sand treatment (p < 0.05).

highest under the A2 treatment with an increase of 19.48%, and that of Kentucky bluegrass reached the highest under the A1 treatment with an increase of 30.34%.

At 60 d after sowing (Fig. 5b), H_2O_2 was significantly higher ($p < 0.05$) than that of CK under each treatment among the three turfgrass species, except that there was no significant difference in Kentucky bluegrass under the A3 and A4 treatments. The H_2O_2 content of creeping bentgrass and Kentucky bluegrass reached the highest under A1 treatment, with increases of 47.12% and 26.1%. The H_2O_2 content of tall fescue was highest under the A2 treatment with an increase of 41.35%.

Soluble protein content

At 20 d after sowing (Fig. 6a), soluble proteins were significantly higher ($p < 0.05$) than those of CK under each treatment among the three turfgrass species, except for tall fescue under treatment A1, which showed no significant difference. Tall fescue showed a gradual increase with the increase in the amount of iron tailing sand added. The soluble protein content of creeping bentgrass, tall fescue, and Kentucky bluegrass reached the highest under treatment A3 with an increase of 13.44%, treatment A4 with an increase of 24.2%, and treatment A2 with an increase of 23.83%, respectively. Creeping bentgrass was significantly lower ($p < 0.05$) than tall fescue, and Kentucky bluegrass CK under A1 treatment. Under A2 and A3 treatments, soluble protein showed a significant trend ($p < 0.05$) of tall fescue > Kentucky bluegrass > creeping bentgrass. Kentucky bluegrass was significantly higher ($p < 0.05$) than creeping bentgrass and tall fescue under the A4 treatment.

At 60 d after sowing (Fig. 6b), the soluble protein content under each treatment was significantly higher ($p < 0.05$) than that of CK for all three turfgrasses. The soluble protein content of creeping bentgrass, tall fescue, and Kentucky bluegrass reached the highest under A2 treatment with an increase of 23.35%, A3 treatment with an increase of 33.58%, and A2 treatment with an increase of 22.65%, respectively. Kentucky bluegrass was significantly lower ($p < 0.05$) than creeping bentgrass and tall fescue under the A1 treatment. Under A2 treatment, the soluble protein content showed a significant trend ($p < 0.05$) of tall fescue > creeping bentgrass > Kentucky bluegrass. Under A3 and A4 treatments, tall fescue was significantly higher than creeping bentgrass and Kentucky bluegrass ($p < 0.05$).

Correlation matrix and principal component analysis of cool-season turfgrass indicators

There was a positive correlation between plant height, fresh weight, and dry weight. Negative correlations were found between plant height, fresh weight, and dry weight and EL, SP, MDA, H_2O_2, and cultivation substrate PH, and positive correlations were found between cultivation substrate organic matter and electrical conductivity. There was a positive correlation between EL, SP, MDA, H_2O_2, and cultivated substrate pH, and a negative correlation with cultivated substrate organic matter and electrical conductivity. Cultivation substrate pH was negatively correlated with cultivation substrate organic matter and electrical conductivity. Cultivation substrate organic matter was positively correlated with cultivation substrate conductivity (Fig. 7a–c).

Principal component analysis (PCA) of the three cool-season turfgrasses showed significant aggregation among the same treatments, indicating little within-group variability. The two principal components of Kentucky bluegrass were 70.8% PC1 and 16.8% PC2. Treatments A2 and A3 were closer to each other with less variability, while treatments A0, A1, and A4 were farther apart with significant differences. The two principal components of tall fescue were 75.2% PC1 and 12.6% PC2. The distance and variability between the A1, A2, A3, and A4 treatments gradually increased with increasing sludge composition from the A0 control. The two principal components of creeping bentgrass were 73.6% PC1 and 16.2% PC2. The distance between A0 and all other treatments was significant. Differences between A3 and A4 were not significant at closer distances, and

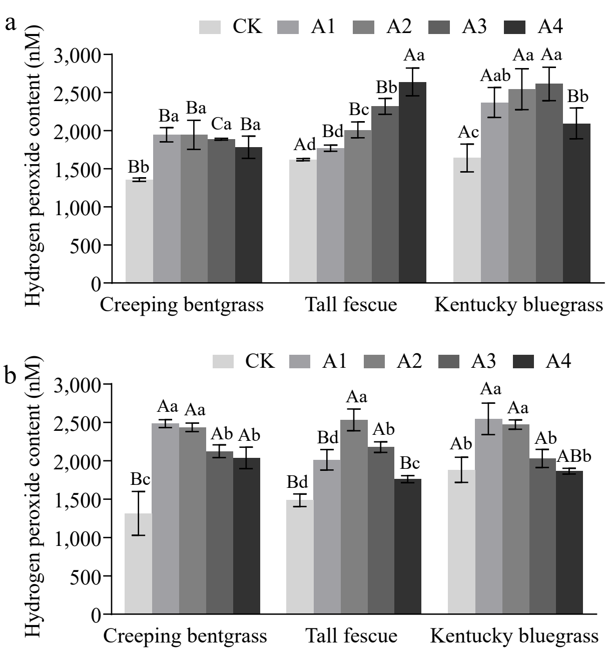

Fig. 5 H_2O_2 content of three types of cool-season turfgrass after (a) 20 and (b) 60 d of treatment with nutritional substrates amended with different proportions of iron tailing sand. Different lowercase letters indicate significant differences between the same grass species and different iron tailing sand treatments ($p < 0.05$), different uppercase letters indicate significant differences between different grass species and the same iron tailing sand treatment ($p < 0.05$).

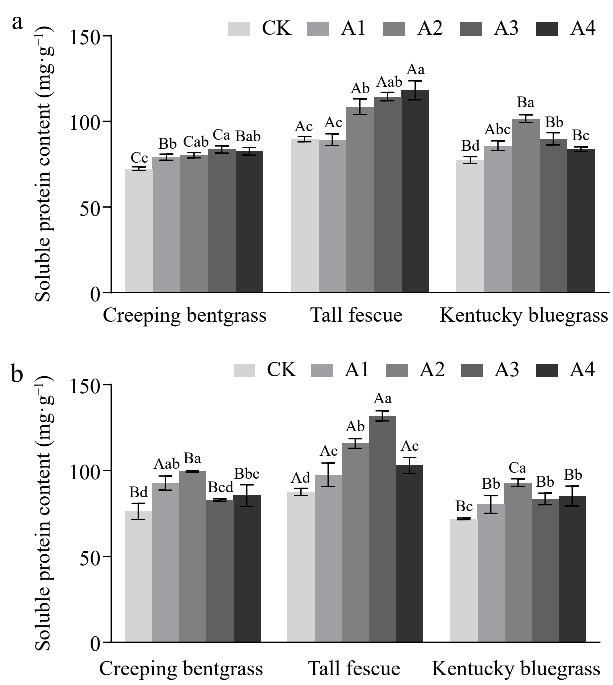

Fig. 6 Soluble protein content of three types of cool-season turfgrass in substrates treated with iron tailing sand at different ratios after (a) 20 and (b) 60 d. Different lowercase letters indicate significant differences between the same grass species and different iron tailing sand treatments ($p < 0.05$), different uppercase letters indicate significant differences between different grass species and the same iron tailing sand treatment ($p < 0.05$).

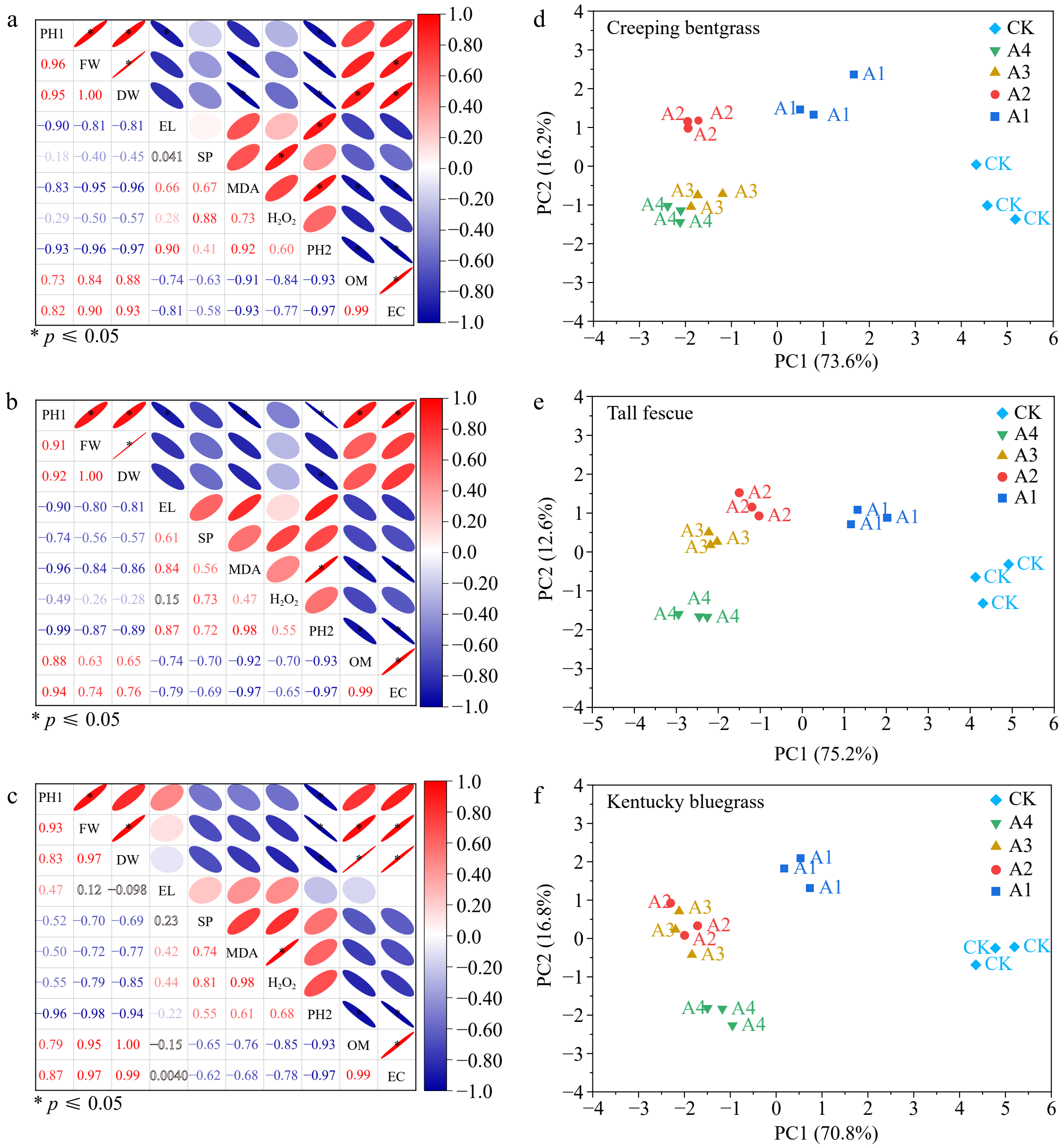

Fig. 7 Correlation matrix and principal component analysis of cool-season turfgrasses in nutrient substrates added with iron tailings sand. Correlation analysis of morpho-physiological, physico-chemical properties of substrates attributes of (a) creeping bentgrass, (b) tall fescue, and (c) Kentucky bluegrass under iron tailings treatment. PH1: plant height; FW: fresh weight; DW: dry weight; EL: Electrolyte leakage; SP: soluble protein; MDA: malondialdehyde; H_2O_2: hydrogen peroxide; PH2: cultivation substrate PH; OM: cultivation substrate organic matter; EC: cultivation substrate electric conductivity. Principal component analysis biplot between morpho-physiological, physico-chemical properties of substrates of (d) creeping bentgrass, (e) tall fescue, and (f) Kentucky bluegrass under different iron tailings treatments.

differences between A1 and A2 treatments were significant (Fig. 7d–f).

Discussion

Heavy metals are a class of potentially hazardous pollutants in the soil environment, that are difficult to decompose, are long-term, cumulative, and irreversible[22]. In this study, the substrates containing iron tailings showed significantly higher pH values than those without. The conductivity, and organic matter content of the cultivation substrate significant decreased with the increase in the volume of iron tailing sand. Long-term use of substrates containing large amounts of iron tailing sand may lead to soil acidification, affecting plant absorption of mineral elements, and even harming the plant root system, which then results in the restriction of seed germination and plant growth[23−25]. Low organic matter content which is the common property of many tailings means that fewer nutrients are available for plant growth[24,26,27]. Meanwhile, Fe toxicity leads to root apoplasty, which can decrease uptake of other essential nutrients, further inhibiting plant growth and development[28]. This investigation revealed that in nutrient substrates augmented with varying concentrations of iron tailing sand, the growth attributes—including height, root length, fresh and dry biomass, and overall growth rate—of three cool-season turfgrasses were inferior to those observed in control conditions (CK). Moreover, a positive correlation was observed between the escalating proportions of iron tailings content and temporal exposure, with the former exacerbating the growth suppression of the turfgrasses. In soils contaminated with heavy metals, plant growth is generally encumbered, leading to mortality at heightened concentrations of such contaminants, which echoes the conclusions of previous research[29,30]. Comparative analysis among the trio of turfgrasses indicated that tall fescue demonstrated superior growth and higher tolerance under various treatment conditions, outperforming Kentucky bluegrass and creeping bentgrass. Notably, the parameters of root length, fresh mass, and dry mass for tall fescue surpassed control levels under the regime consisting of 25% iron tailing sand inclusion, which suggests the addition of low-levels of iron tailing sand in the substrate had a positive effect on tall fescue growth and development.

Chlorophyll, serving as the principal photosynthetic pigment in plants, is a critical indicator of the photosynthetic system's integrity[31]. Fe toxicity affects physiological traits by reducing gas exchange and chlorophyll levels, deactivating the PSII reaction center, decreasing saturated fatty acids, and increasing unsaturated fatty acids in chloroplasts[32]. Esteves et al. reported[26] that the chlorophyll content of maize, millet, and sorghum reduced in the treatment of iron mine tailings. In the current study, a systematic decline in the chlorophyll content was observed across the three cool-season turfgrass species as the proportion of iron tailing sand in the growth medium increased. Specifically, chlorophyll levels were nullified in creeping bentgrass and Kentucky bluegrass for substrate treatments containing 50%, 75%, and 100% iron tailing sand, as well as in tall fescue at the 100% concentration. This outcome suggests extensive damage to the chloroplast membrane, culminating in a complete cessation of the photosynthesis process[33]. Compared with the other two grass species, tall fescue retained higher chlorophyll levels than Kentucky bluegrass and creeping bentgrass under varied treatment conditions. This disparity may be attributable to distinct iron tolerance mechanisms operative within the different grass species.

High levels of Fe in the plant could potentially induce oxidative stress[34]. Fe stress may cause an overproduction of ROS, potentially resulting in membrane damage and lipid peroxidation. Increased levels of MDA and EL are generally considered indicative of lipid peroxidation and membrane damage[20,35]. A previous study showed that higher MDA levels were observed in two tropical grasses Paspalum densum and Echinochloa crusgalli cultivated under excess iron conditions[36]. In the present study, EL in creeping bentgrass and tall fescue tended to rise concurrently with the augmentation of iron tailing sand, suggesting heightened membrane permeability impairment. However, Kentucky bluegrass exhibited lower relative conductivity in treatments compared to the control (CK), potentially attributable to the minimal impact of iron tailing sand on membrane leakage in this species. Our results showed that in the presence of increased iron tailing sand, MDA concentrations in the leaves of the three turfgrasses typically exhibited an upward trend in comparison to the control. Specifically, Kentucky bluegrass revealed an initial increase followed by a decline in MDA content on day 20. Moreover, tall fescue maintained lower MDA levels under various treatments than Kentucky bluegrass and creeping bentgrass, indicative of reduced membrane peroxidation. This suggests that tall fescue possesses a more robust antioxidant capacity, exhibiting greater stability under stress conditions. When plants absorb and accumulate too much free iron, it can alter the cell's redox balance, creating a pro-oxidant environment and oxidative stress. Therefore, an iron surplus could lead to heightened ROS production[34,37,38]. Besides causing cell collapse and tissue degradation, ROS also oxidize sugars, proteins, nucleic acids, and lipids, disrupt electron transport, and inhibit or activate enzymes[32]. Our results showed that H_2O_2 levels in tall fescue rose on the 20th day, while other species either decreased or initially increased then decreased after adding iron tailing sand. This may be due to higher iron tailing sand levels causing significant cell membrane damage and loss of cellular content. By the 20th day, H_2O_2 concentrations were highest in Kentucky bluegrass treatments, followed by tall fescue, and lowest in creeping bentgrass. Conversely, by the 60th day, the highest H_2O_2 levels were observed in creeping bentgrass treatments, with tall fescue exhibiting intermediate values, and Kentucky bluegrass showing the lowest concentrations. These findings suggest that Kentucky bluegrass may rely more heavily on H_2O_2 generation as an early response mechanism to environmental stress, whereas creeping bentgrass appears to enhance its resilience by increasing H_2O_2 levels at later stages. Concurrently, tall fescue may possess a more efficient antioxidant system that regulates both the production and neutralization of hydrogen peroxide, thereby protecting cells from oxidative damage at various stages, in comparison to the other two grass species. Osmolytes such as soluble proteins are small organic compounds produced by plants in response to abiotic stresses including salinity, drought, and heavy metals[39]. Our investigation revealed that with the increment of iron tailing sand incorporation, the soluble protein levels in creeping bentgrass and Kentucky bluegrass initially increased, followed by a decline by day 20. This dynamic may arise from damage to leaf cellular structures, impairing regular protein synthesis and accelerating degradation of proteins, thus sabotaging soluble protein function. By day 60, all three turfgrass species showed similar trends in protein levels, likely due to the combined effects of higher iron tailing sand content and prolonged stress affecting protein integrity. In rice, the Pokkali genotype maintained membrane stability and total soluble protein in shoots due to Fe toxicity, confirming its ability to tolerate excess Fe[40]. Notably, tall fescue maintained higher soluble protein levels under various treatments compared to Kentucky bluegrass and creeping bentgrass, signifying its superlative stress tolerance among the grasses studied. In summary, the performance of tall fescue plants provides evidence of their higher tolerance to cultivation in iron mine tailings than the other two turfgrass species.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Sun X; experiments conducting: Chen R, Zhu Y, Han W, Chen L, Guo X, Jin X, Li H, Jin M; data analysis: Chen R, Li L, Li C; manuscript writing: Chen R, Sun X, Chen Y. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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