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Abiotic Stress Response in Native Grasses and Their Suitability as Garden Plants under Global Climate Change

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Abstract: Native garden plants significantly contribute to the conservation of biodiversity and ecosystem functions in urban environments. This study aimed to identify the physiochemical differences among native grasses subjected to drought and salinity stress and to assess their potential as garden plants adaptable to outdoor conditions and global climate change. Physiological parameters, such as photosynthetic rate, water use efficiency, and chlorophyll and carotenoid contents, decreased in *Melica grandiflora* and *Carex forficula* with increasing drought or salinity. In contrast, *Carex boottiana* maintained high water use efficiency and stable chlorophyll and carotenoid levels even under severe drought and salinity conditions. Biochemical analyses showed that *C. boottiana* had lower levels of malondialdehyde and reactive oxygen species (including $O_2^{\cdot-}$ and H_2O_2) than those in the other species under stress conditions. Principal component analysis revealed clear differences in tolerance levels among the grass species. *C. boottiana* demonstrated high adaptability to both drought and salinity stress, indicating its potential as a sustainable and resilient garden material for urban landscapes facing severe climatic challenges.

Keywords: drought; garden material; native grass; physiochemical attributes; salinity

1. Introduction

The global population is rapidly urbanizing; therefore, the protection of ecosystem services and biodiversity in urban areas has become increasingly vital. Vegetation plays an essential role in these environments by providing breeding habitats and altering microclimates [1]. Consequently, gardens are considered significant contributors to biological diversity [2–4]. In particular, native plants can positively impact urban biodiversity conservation as they are well suited to the local climate and soil conditions where they naturally thrive [5,6]. However, unlike most naturally occurring communities, urban gardens typically consist of diverse mixtures of planted and volunteer species, with a substantial proportion of non-native species [1,7,8]. Invasive non-native species are now considered significant threats to global biodiversity [9], and garden plants account for > 40% of the widely recognized invasive species [10]. With the enforcement of the Nagoya Protocol, it is increasingly urgent to reinforce biological sovereignty and reduce dependence on foreign biological resources as well as the associated royalty costs for imported ornamental plants. Among 4,000 native plant species in the Republic of Korea, approximately 600 have notable developmental potential, and very few are currently utilized [11]. Despite the emphasis on biological sovereignty, the expansion of arboretums and gardens in urban areas faces challenges. For example, the utilization of native flora remains limited owing to insufficient information regarding their adaptability to local environments.

Ornamental grasses have become increasingly popular as key landscaping elements, as modern gardens are specifically tasked with addressing climate change, biodiversity loss, and urban ecosystem services. In the Republic of Korea, interest in ornamental grasses is rapidly increasing along with a growing variety of available species [12]. These grasses play a crucial role in landscaping as they are aesthetically appealing and offer significant ecosystem services. They enhance soil health through their extensive root systems, which improve soil structure and prevent erosion [13]. Notably, many ornamental grass varieties used in other countries originated in East Asia before being introduced to Europe and North America, where selective breeding occurred before their return to the Republic of Korea [14]. However, imported grasses often face challenges adapting to unique Korean climatic conditions. Additionally, domestic native species are underutilized, largely owing to insufficient information regarding their habitat preferences and ecological characteristics [15,16]. The Korean native plant *Melica grandiflora* is a perennial grass that is native to the temperate regions of Asia and is primarily found in mountainous meadows and at the edges of forests [17,18]. *Carex forficula* is a perennial herbaceous plant that is primarily native to wetlands and lowland riparian areas in East Asia [19]. Similarly, *Carex boottiana*, which is found in comparable habitats, is tolerant to various soil conditions and plays a significant role in nutrient cycling. These three native grass species play crucial roles in preventing soil erosion and maintaining the health of the ecosystem [20].

Abiotic stressors, such as drought, salinity, and cold, are significant environmental factors that negatively affect plant growth, survival, and performance [21–23]. Remarkably, rapid changes in global climate and various projections indicate increases in aridity and salinity in semiarid regions in the near future [24]. As water evaporates from the soil, its salt concentration increases, thereby leading to drought and salinity [15]. Although plant tolerance to drought or salt has been reported, garden plants have received relatively little attention in this regard [23]. Drought stress leads to plant dehydration, stomatal closure, and restricted gas exchange, subsequently inhibiting metabolism and photosynthetic rates and ultimately resulting in plant death. However, the capacity of plants to endure stressful environments depends on the species, phase of growth, and duration and intensity of water deficit [25,26]. In the initial phase of salinity stress, plants undergo water stress, which restricts leaf expansion. The osmotic impact of salinity stress can inhibit cell development and division and cause stomatal closure [27,28]. Under prolonged salinity conditions, plants undergo ionic stress, which may result in premature senescence of mature leaves, thereby reducing the photosynthetic area required for continued growth [29]. Similarly, salt stress affects various crucial processes, such as growth and development, carbon fixation, and protein synthesis, leading to growth inhibition. However, lethal concentrations, growth reduction rates, and salt tolerance levels vary among plant species [30].

Identifying drought or salinity tolerance in plants is essential for promoting the development of urban gardens and conserving biodiversity through the use of native species. Therefore, the present study investigated the selection of outdoor garden plant species by studying the adaptability of three Korean native grasses to various drought or salinity stress conditions for providing basic information, such as appropriate irrigation intervals and lethal salt concentrations in soil, to assess whether these three native grasses are suitable as garden plants in outdoor spaces.

2. Materials and Methods

2.1. Plant Materials and Experimental Design for Drought and Salinity Stress

The experiments were conducted between June and August 2023 in the Sejong National Arboretum greenhouse in the Republic of Korea. Three native grasses, *M. grandiflora* Koidz. (Poaceae), *C. forficula* Franch. & Sav, and *C. boottiana* hooks. & Arn. (Cyperaceae), with high ornamental values and excellent potential for use in gardens were included in this study [12] (Figure 1). The grasses were planted in 19 cm Pixel Garden plastic pots filled with eden soil (a mixture of peat moss, cocopeat, vermiculite, zeolite, and pearlite; pH 5.5–6.5, Electrical Conductivity (EC) < 1 ds/m). Air temperature and humidity in the greenhouse were monitored for 30 days using a data logger (Spectrum Watchdog 1650 Micro Station Data Logger; Spectrum Technologies, Inc, USA). Environmental conditions were affected by weather conditions; however, temperature was maintained between 15–28 °C (Figure S1). Plants were watered daily to field moisture capacity for two weeks prior to starting the treatments. Plants were divided into five replicates in each group and subjected to five treatments, each of drought and salinity stresses, for 30 days (June–July 2023).

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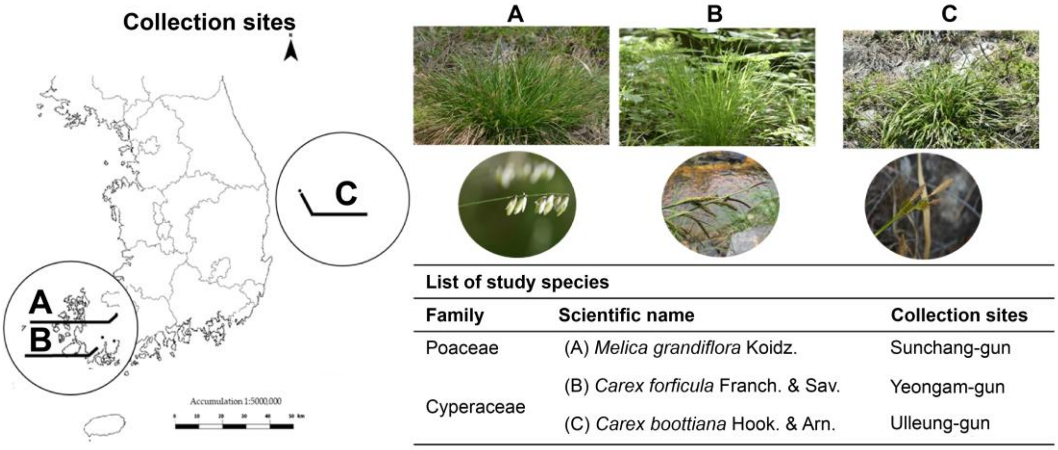
Drought stress was applied using an automatic irrigation system (self-made), and the irrigation cycle was as follows: well-watered condition (WW, once every 72 h), moderate drought stress (MD, once every 336 h), and severe drought stress (SD, no irrigation). Drought-treated plants were irrigated with 500 mL water in all treatments by measuring the water content in the pot. In general, glycophytes die at salinity (NaCl) concentrations of 100–200 mM, whereas halophytes survive at salinity concentrations of 300–500 mM [30–32]. Salinity stress was induced using NaCl as follows: untreated condition (UT), moderate salinity stress (MS, 250 mM NaCl), and severe salinity stress (SS, 500 mM NaCl). Salinity-treated plants were irrigated with 500 mL saline or distilled water at every 3-day interval (Table 1).



List of study species

Family	Scientific name	Collection sites
Poaceae	(A) *Melica grandiflora* Koidz.	Sunchang-gun
Cyperaceae	(B) *Carex forficula* Franch. & Sav.	Yeongam-gun
	(C) *Carex boottiana* Hook. & Arn.	Ulleung-gun

Figure 1. List of Korean collection sites for the three native species.

Table 1. Experimental design for the three treatments of drought and salinity stresses.

Experimental design			
Drought stress		Salinity stress	
Well-watered (irrigation once every 72 h)	WW	Untreated (no NaCl treatment)	**UT**
Moderate drought treated (irrigation once every 336 h)	MD	Moderate salinity-treated (250 mM NaCl)	**MS**
Severe drought treated (no irrigation for 30 days)	SD	Severe salinity-treated (500 mM NaCl)	**SS**

2.2. Photosynthetic Parameters

Photosynthetic measurements were conducted on fully expanded leaves from 9:00 am to 4:00 pm using an LI-6800 portable photosynthesis system (LICOR-6800; Li-Cor Inc., USA). The CO_2 concentration was maintained at 400 μmol·mol^{-1}, with a flow rate of 600 μmol·s^{-1}. The cuvette was maintained at a temperature and relative humidity range of 25 °C and 50–60%, respectively. Light was supplied by red-blue LEDs mounted on the LI-6800 cuvettes, providing a constant artificial irradiation of 1,000 μmol·m^{-2}·s^{-1}. The same leaves were used for measurements of various photosynthetic parameters, such as net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r); leaf-level instantaneous water use efficiency (WUE, μmol CO_2 per mmol H_2O) was calculated as the ratio of photosynthetic rate (P_n) to transpiration rate (T_r), as described in a previous study [33].

2.3. Measurements of Chlorophyll Parameters

After the experiment, leaf samples weighing 0.1 g were collected. The leaves were submerged in 10 mL glass vials containing 80% acetone and stored in the dark at 4 °C for 7 d to ensure complete pigment extraction. The chlorophyll contents (Chla, Chlb, Chl_T, carotenoid, Chla/b, and Car/Chl_T) were calculated using equations according to a previous study [34]. Absorbance values at

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wavelengths of 663, 645, and 470 nm were then measured with a spectrophotometer (Multiskan Skyhigh, Thermo Fisher Scientific, USA).

2.4. Determination of Malondialdehyde Content for Estimating Membrane Damage

Following the experiment, 0.1 g of fresh leaf tissue was homogenized in 0.5 mL of 0.2% (w/v) trichloroacetic acid (TCA) and centrifuged at 1,500 × *g* for 10 min at 4 °C. The resulting supernatant was then combined with a 1.5-mL reaction mixture containing 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95 °C for 20 min and subsequently cooled on ice. Absorbance was measured at 532 nm and 600 nm using a microplate reader (Multiskan Skyhigh, Thermo Fisher Scientific). The malondialdehyde (MDA) content was calculated using an extinction coefficient of $\varepsilon = 155\ \mathrm{mM^{-1}\cdot cm^{-1}}$, as described previously [35].

2.5. Determination of $O_2^{\cdot-}$ and H_2O_2 Concentration

O_2^- content was measured according to a modified version of a previously described method [36]. The concentration of O_2^- was quantified based on its ability to reduce nitro blue tetrazolium (NBT). Leaf samples (0.1 g) were homogenized in 1.5 mL of 10 mM potassium phosphate buffer (pH 7.8) containing 0.05% NBT and 10 mM NaN_3. After a 20-min incubation at room temperature (20–25 °C), a 1-mL aliquot of the reaction mixture was heated in a water bath at 85 °C for 15 min and then cooled rapidly. The O_2^- content was expressed as the absorbance at 580 nm per gram of fresh weight ($A580\cdot g^{-1}$ FW). The H_2O_2 content was determined as described previously [37]. Leaf samples (0.1 g), frozen in liquid nitrogen, were extracted with 1 mL of 0.1% TCA and centrifuged at 12,000 × *g* for 15 min. The resulting supernatant (0.5 mL) was mixed with 0.5 mL of 100 mM potassium phosphate buffer (pH 7.8) and 1 mL of 1 M KI. After incubating the mixture in the dark for 1 h, the absorbance at 390 nm was measured using a microplate reader (Multiskan Skyhigh, Thermo Fisher Scientific, USA), and the H_2O_2 content was calculated from a standard curve.

2.6. Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA), with statistical significance determined at $p < 0.05$. To assess differences among groups, a one-way Analysis of Variance (ANOVA) was employed, followed by Tukey's HSD test for chlorophyll and biochemical parameters and Scheffe’s test for photosynthetic parameters, accounting for the varying number of repetitions. An independent *t*-test was used to examine significant differences in photosynthetic parameters between *M. grandiflora* and *C. forficula* under different treatments, including well-watered (WW), moderate drought (MD), untreated (UT), and mild salinity (MS). Additionally, principal component analysis (PCA) was performed to identify key factors influencing drought and salinity stress and to further understand plant tolerance to the conditions. The data are expressed as mean ± standard deviation.

3. Results

3.1. Changes in Growth and Visible Injuries

The average temperature during drought and salinity treatment was 25.8 ℃. The relative humidity was high (78%) owing to continuous rainfall during the experimental period (Figure S1). Differences in growth changes and visible injuries were noticeable depending on drought or salinity treatments in the three native grasses (Figure 2). For *C. boottiana*, the structure of the canopy, by altering its length and width, showed little change under SD compared to that under WW. No visible necrotic areas were noticed on the leaves. In contrast, the other two species exhibited reduced growth rates and bronzing of >90% leaves under SD. In particular, *M. grandiflora* was the first to show noticeable leaf necrosis and browning under SD and died 24 days after the start of the experiment (DAE, data not shown). Under salt stress, there was a marked reduction in growth and visible damage (necrosis) in the two species, with the exception of *C. boottiana*. However, visible foliar damage to *M. grandiflora* was more pronounced under drought stress than under salinity stress. Conversely, *C. forficula* exhibited more visible foliar damage caused by salinity stress than by drought stress.

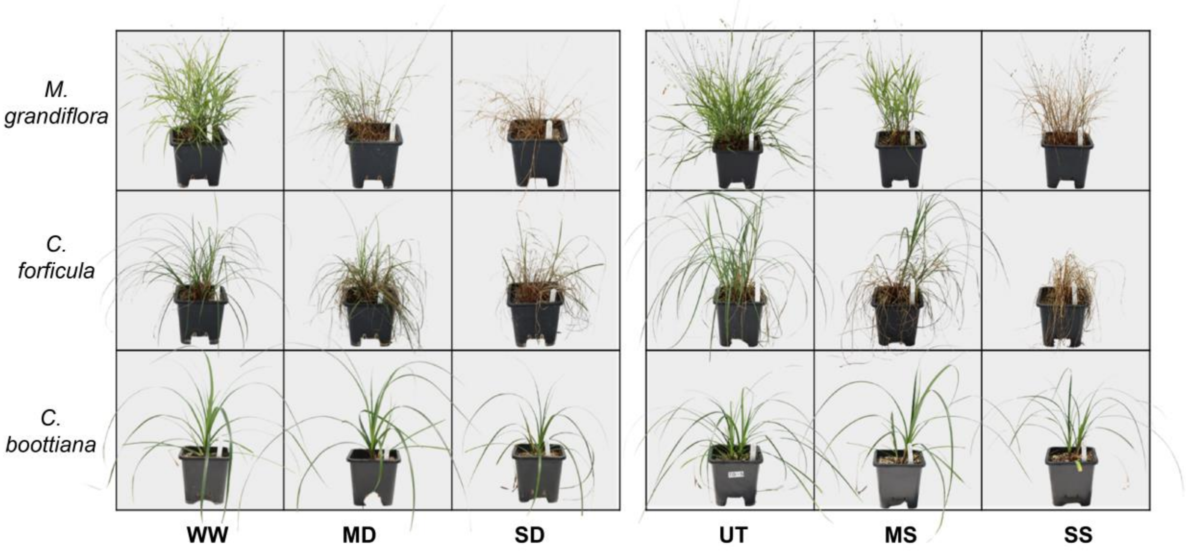

Figure 2. Images showing changes in growth and visible injuries of the three native grasses following 30 days of drought [well-watered condition (WW), irrigation once every 72 h; moderate drought stress (MD), irrigation once every 336 h; severe drought stress (SD), no irrigation for 30 days] and salinity [UT, untreated condition; moderate salinity stress (MS), 250 mM NaCl; severe salinity stress (SS), 500 mM NaCl] stresses.

3.2. Photosynthetic Parameters

The P_n of all three native grass species significantly decreased with increased drought stress. In particular, *C. boottiana* showed the largest decline (9.1-fold) under SD compared to that under WW (Figure 3a). The photosynthetic efficiencies of *M. grandiflora* and *C. forficula* could not be measured owing to withering and necrosis of leaves under SD. The g_s and T_r indicated similar trends in the three native grasses (Figure 3b, c). The g_s and T_r of *M. grandiflora* did not significantly differ under WW and MD treatments. *C. forficula* and *C. boottiana* showed decreases in g_s and T_r with increased drought stress (MD and SD). WUE differed depending on the species and drought treatment. WUE of *M. grandiflora* decreased more under MD than under WW; however, those of the other two species did not show significant differences. *C. boottiana* had a higher WUE under SD than under WW and MD; however, the difference was not significant (Figure 3d).

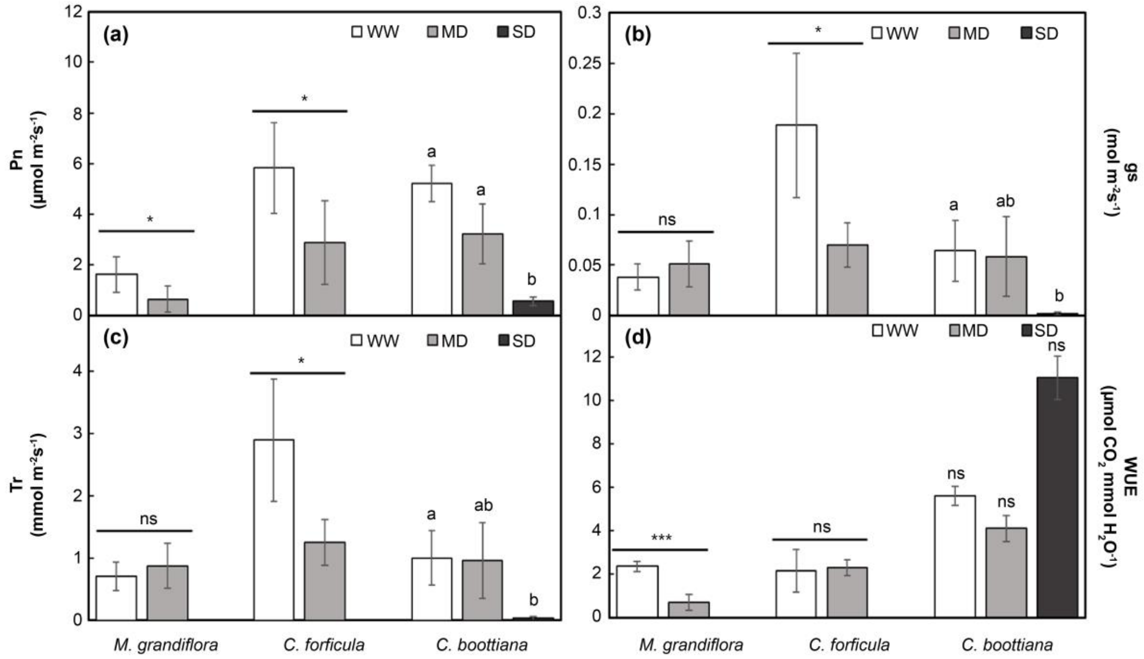

Figure 3. (a) Photosynthetic rate (P_n), (b) stomatal conductance (g_s), (c) transpiration rate (T_r), and (d) water use efficiency (WUE) of the three native grasses under drought. The values for *C. boottiana* were analyzed by Scheffe's test (as lowercase letters), and those for *M. grandiflora* and *C. forficula* were analyzed using *t*-test (as asterisks) for significant differences under drought treatments ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$; ns, not significant). Bars in each graph indicate mean ± standard deviation (n = 5). There is no significant difference between bars marked with the same letters ($p > 0.05$).

The photosynthetic efficiencies of *M. grandiflora* and *C. forficula* could not be measured owing to withering and necrosis of leaves under SS. The P_n of the three species significantly decreased with increased salinity; however, that of *M. grandiflora* was not significantly different under UT and MS (Figure 4a). The g_s and T_r values of *M. grandiflora* and *C. forficula* were not significantly different under UT and MS. In contrast, the g_s and T_r of *C. boottiana* rapidly decreased under salinity stress (MS and SS) (Figure 4b, c). A significant difference in WUE was noticed among the three grasses (Figure 4d). *M. grandiflora* had a lower WUE under MS than under UT; however, the difference was not significant. *C. forficula* and *C. boottiana* showed significant increases of 6.6-fold (under MS) and 3.6-fold (under SS), respectively, compared to that under UT.

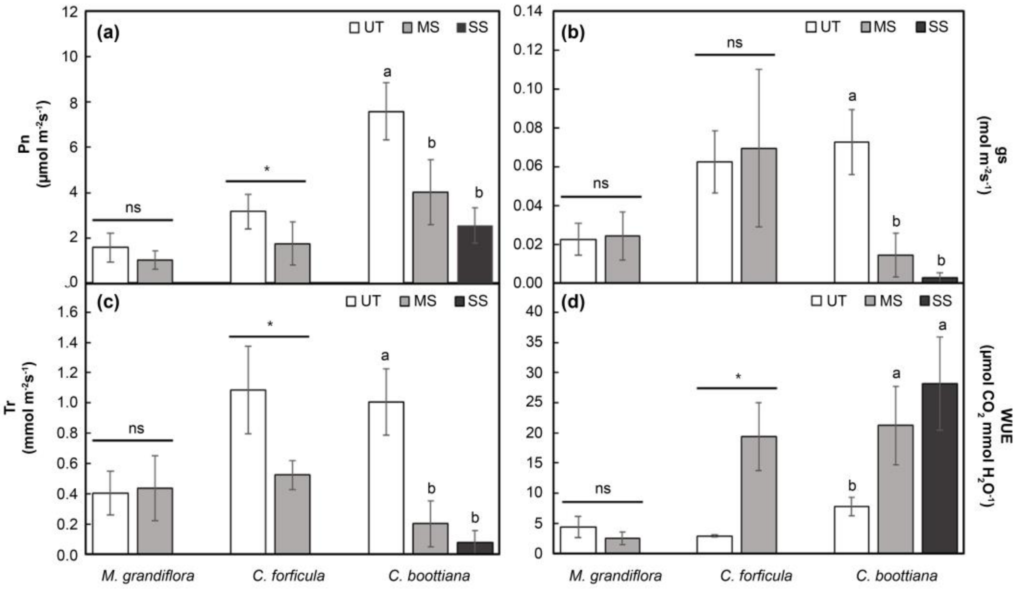

Figure 4. (a) P_n, (b) g_s, (c) T_r, and (d) WUE in three native grasses under salinity stress. The data of *C. boottiana* were analyzed by Scheffe's test (as lowercase letters), and those of *M. grandiflora* and *C. forficula* were analyzed using *t*-test (as asterisks) to determine significant differences under different salinity treatments (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant). Bars in each graphic indicate mean ± standard deviation (n = 5). There is no significant difference between bars marked with the same letters ($p > 0.05$).

3.3. Chlorophyll Parameters

The growth of plants was associated with substantial reductions in chlorophyll and carotenoid contents and Chl a/b ratios in fully expanded leaves of native grasses under abiotic stress (Tables 2 and 3). The Chl$_T$ and carotenoid contents of *M. grandiflora* and *C. forficula* under SD were notably lower by 86.7% and 61.5 %, respectively than those under WW. In contrast, photosynthetic pigments (chlorophyll and carotenoids) of *C. boottiana* showed no pronounced differences under different drought conditions. In all species, the Chl a/b ratio gradually decreased as drought stress increased. Among them, *C. boottiana* showed the lowest decrease of 9.1%, whereas *M. grandiflora* showed the highest decrease of 67.6% under SD compared to that under WW. The Car/Chl$_T$ ratio was highest for all three species under SD and was more strongly influenced by carotenoids than by chlorophyll (Table 2). The Chl$_T$ and carotenoid levels gradually decreased in *M. grandiflora* and *C. forficula* with increasing salt concentrations. *C. boottiana* exhibited the highest Chl$_T$ and carotenoid levels among the three species under SD, and did not show pronounced differences in Chl$_T$ and carotenoid levels under different salt concentrations. The Chl a/b ratios of *M. grandiflora* and *C. forficula* significantly decreased with increasing salt concentrations. The Car/Chl$_T$ ratios of *M. grandiflora* and *C. boottiana* under salt treatment had stronger effects on carotenoids than on chlorophyll and were approximately 28% and 4.4% higher, respectively, under SS than under UT. In contrast, *C. forficula* did not show a defined trend under those treatments (Table 3).

Table 2. Chl$_T$ and carotenoid contents, and Chla/b and Car/Chl$_T$ ratios in three native grasses under drought stress.

Chlorophyll (mg·g^{-1} FW)	Drought level	*M. grandiflora*	*C. forficula*	*C. boottiana*
Chl$_T$	WW^1	4.01±0.90 a	3.20±0.28 a	2.94±0.27 b
	MD^2	0.73±0.14 b	1.85±0.16 b	4.51±0.12 a
	SD^3	0.53±0.04 b	1.23±0.04 c	3.22±0.24 b
Carotenoid	WW	8.13±0.84 a	6.48±0.50 a	5.81±0.48 c
	MD	1.63±0.28 b	3.93±0.24 b	8.85±0.19 a
	SD	1.26±0.06 b	2.70±0.10 c	7.86±0.47 b
Chl a/b ratio	WW	2.38±0.04 a	2.05±0.04 a	2.52±0.07 a
	MD	1.02±0.11 b	1.83±0.04 b	2.31±0.06 b
	SD	0.77±0.03 c	1.10±0.04 c	2.29±0.05 b
Car/Chl$_T$ ratio	WW	2.03±0.02 c	1.58±0.88 c	1.54±0.86 b
	MD	2.24±0.06 b	1.66±0.93 b	1.96±0.02 b
	SD	2.39±0.06 a	2.19±0.02 a	2.44±0.05 a

The data were analyzed using Tukey's HSD test and are expressed as mean ± standard deviation (n = 5). Means with the same letters are not significantly different ($p < 0.05$).

Table 3. Chl$_T$ and carotenoid contents, and Chla/b and Car/Chl$_T$ ratios in three native grasses under salinity stress.

Chlorophyll (mg·g^{-1} FW)	Salt level	*M. grandiflora*	*C. forficula*	*C. boottiana*
Chl$_T$	UT^1	2.80±0.05 a	3.37±0.24 a	7.19±0.10 ns
	MS^2	1.42±0.09 b	1.47±0.28 b	7.02±0.11 ns

	SS[3]	0.42±0.02 c	0.79±0.04 c	6.92±0.23 ns
Carotenoid	UT	5.61±0.38 a	6.40±0.52 a	17.75±0.09 a
	MS	3.11±0.16 b	3.20±0.59 b	17.53±0.13 b
	SS	1.09±0.03 c	1.53±0.10 c	17.82±0.09 a
Chl a/b ratio	UT	2.28±0.05 a	2.38±0.06 a	0.73±0.06 ns
	MS	1.83±0.07 b	1.31±0.06 b	0.81±0.06 ns
	SS	1.06±0.05 c	1.16±0.06 c	0.88±0.15 ns
Car/Chl$_T$ ratio	UT	2.00±0.02 c	1.89±0.03 b	2.47±0.03 b
	MS	2.19±0.03 b	2.17±0.01 a	2.50±0.02 ab
	SS	2.56±0.05 a	1.94±0.06 b	2.58±0.07 a

The data were analyzed using Tukey's HSD test and are expressed as mean ± standard deviation (n = 5). Means with the same letters are not significantly different ($p < 0.05$).

3.4. MDA and Reactive Oxygen Species ($O_2^{\cdot-}$, H_2O_2)

Phytotoxicity in native grasses under abiotic stress was quantified by evaluating MDA contents, an indicator of lipid peroxide ions, in leaves. As seen in Figures 5a and 6a, MDA contents of *M. grandiflora* and *C. forficula* significantly increased with increasing drought and salinity stresses. *M. grandiflora* showed the most rapid increase (2.7-fold) under SD compared to that under WW, and *C. forficula* showed the most acute increase (2.9-fold) under SS compared to that under UT. However, *C. boottiana* showed little difference in MDA levels under abiotic stress, which increased 2.0-fold under SD compared to that under WW; MDA levels in *C. boottiana* increased under MS compared to that under UT and slightly decreased under SS. $O_2^{\cdot-}$ and H_2O_2, indicators to evaluate oxidative stress in plants, also showed a similar trend of change (Figure 5b, c and 6b, c). The $O_2^{\cdot-}$ and H_2O_2 contents of *M. grandiflora* significantly increased under drought and salinity stress by 2.1-fold and 3.9-fold, respectively. *C. forficula* showed highly enhanced $O_2^{\cdot-}$ and H_2O_2 contents under drought stress; however, they were slightly changed under salt stress. In addition, *C. boottiana* showed differences in H_2O_2 contents (highest levels under SD and SS) under drought and salinity stresses. However, $O_2^{\cdot-}$ levels in *C. boottiana* under drought and salinity stresses were not significantly different.

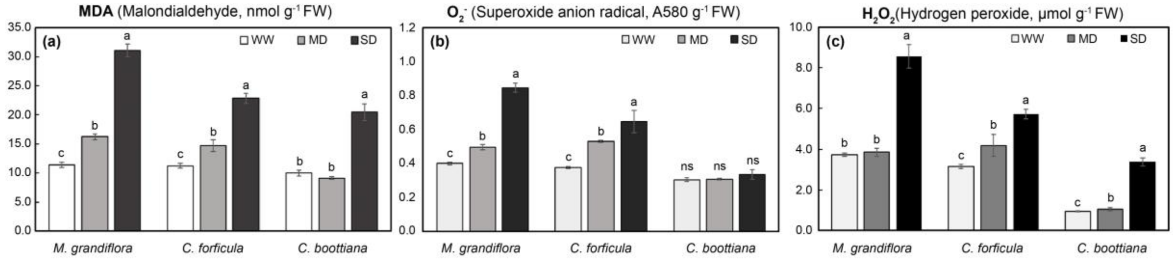

Figure 5. (a) Malondialdehyde (MDA), (b) $O_2^{\cdot-}$, and (c) H_2O_2 contents in three native grasses under drought stress. Significant differences, based on Tukey's HSD test, are denoted by lowercase letters. The data are presented as mean ± standard deviation (n = 4). Bars marked with the same letters are not significantly different ($p < 0.05$).

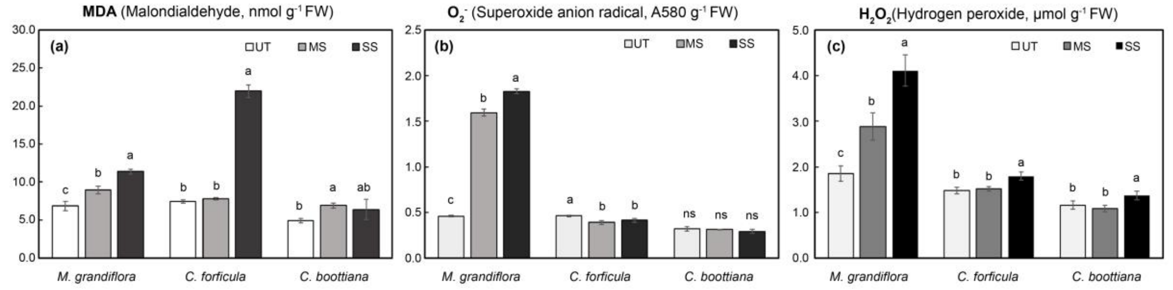

Figure 6. (a) MDA, (b) O_2^-, and (c) H_2O_2 contents in three native grasses under salinity stress. Significant differences, based on Tukey's HSD test, are denoted by lowercase letters. The data are presented as mean ± standard deviation (n = 4). Bars marked with the same letters are not significantly different ($p < 0.05$).

3.5. Principal Component Analysis

To better understand the interaction between drought or salt stress and the three native grass species, PCA was conducted (Figure 7). In the PCA, PC1 accounted for 61.6% and 46.4% of the total variance in drought and salinity stress, respectively, whereas PC2 explained 21.7% of drought stress and 25.3% of salinity stress. The biplot analysis revealed the correlations between the stress indices calculated for all traits. Under drought stress, positive correlations were observed among T_r, g_s, P_n, Chl_T, carotenoids, Chl a/b, and WUE, whereas negative correlations were observed among MDA, O_2^-, H_2O_2, and Car/Chl_T (Figure 7a). For salinity stress, positive correlations were noted with P_n, Chl_T, and carotenoids, whereas negative correlations were observed with MDA, O_2^-, H_2O_2, and Chl a/b (Figure 7b). Additionally, the scatterplot classified the grasses into tolerant or sensitive groups based on their response to drought or salinity stress (Figure 7c, d). As a result of comparing the three species in drought or salinity treatments, differences in the degree of tolerance and sensitivity to stress were distinguished clearly.

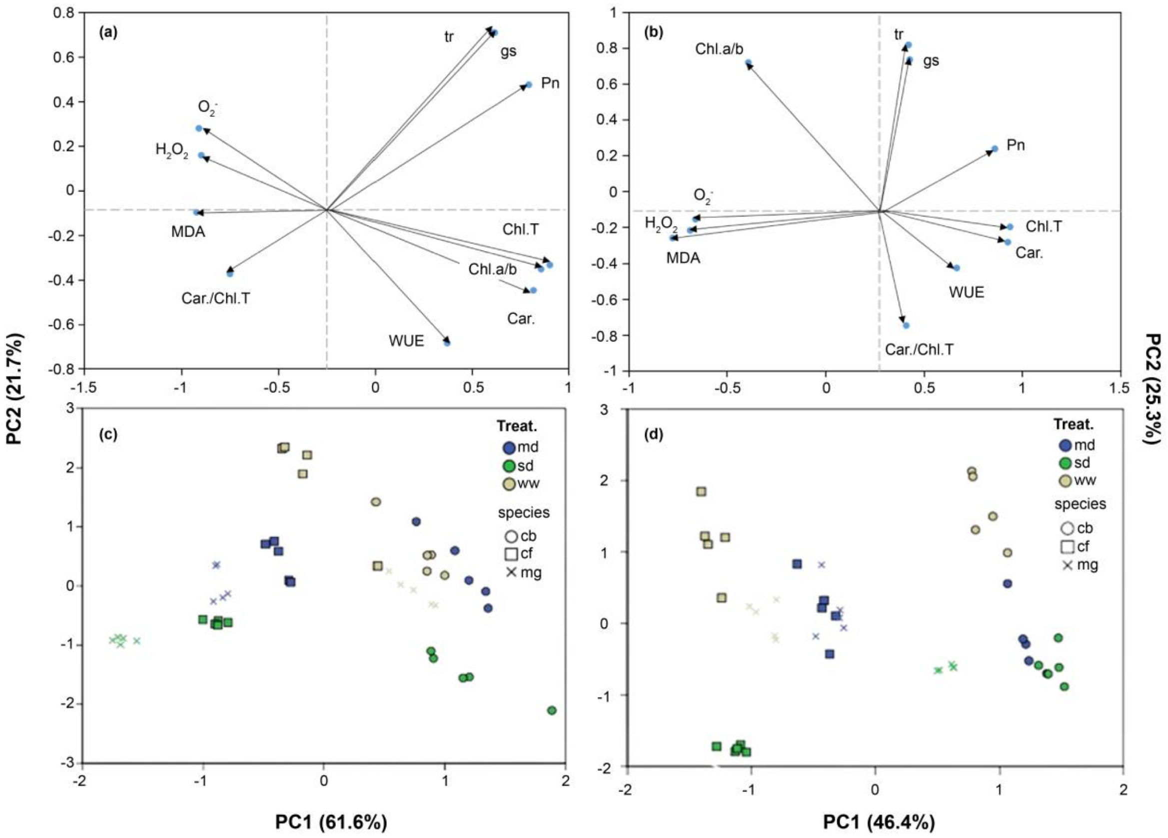

Figure 7. Principal component analysis (PCA) of physiochemical parameters in three native grasses with distinct degrees of tolerance to drought (a, c) and salinity (b, d) stresses. (a, b) PCA-associated loading plots. (c, d) PCA-associated scatter plots.

4. Discussion

Drought and salinity are two key abiotic stressors that negatively impact plant growth and development [38–40]. As a result, plants initiate various physiological and biochemical responses to mitigate the effects of the stresses. The responses involve alterations in morphology, photosynthesis, and distribution of harmful ionic species, in addition to biochemical adjustments, for example, in antioxidant or metabolic activities [30,41-45]. In the present study, all three grass species exhibited osmotic stress under drought conditions due to the accumulation of intracellular salts and ions caused by dehydration. In contrast, salinity stress triggered ionic stress, characterized by the buildup of toxic ions in the intercellular space, resulting from osmotic stress. Consequently, the grasses showed more severe morphological damage under saline conditions than under drought stress.

Photosynthesis, a critical and complex physiological process, is impacted heavily by abiotic stresses such as drought and salinity [42]. Such stresses directly inhibit photosynthesis, leading to declines in morphological and physiological traits, leaf water potential, sap flow, and stomatal conductance [38,45–48]. In the present study, the P_n values of all grasses decreased steadily as drought and salinity levels increased. Notably, *C. boottiana* exhibited lower g_s and T_r but showed enhanced WUE under both SD and SS conditions. Typically, plants that are tolerant to drought or salt stress improve their leaf water status by reducing transpiration through decreased g_s, which minimizes water loss, enhances P_n, and ultimately boosts WUE [45,49–51].

Reduction in chlorophyll content under abiotic stress is commonly recognized as an indicator of oxidative stress [52], which triggers pigment photooxidation and chlorophyll degradation [53]. As such, chlorophyll content serves as a key indicator of a plant's physiological state [54]. In response to drought or salinity stress, *M. grandiflora* and *C. forficula* exhibited a decline in total chlorophyll (Chl_T) content. However, *C. boottiana* maintained the highest levels of Chl_T and carotenoids under MD, with no significant changes observed under salinity stress. Carotenoids, which play a secondary role in light harvesting, assist chlorophyll in absorbing solar energy when excess light cannot be managed by chlorophyll alone. Additionally, carotenoids help prevent the formation of reactive oxygen species (ROS) [55]. In drought- or salt-tolerant plants, carotenoid levels rise under stress, indicating enhanced photoprotection [56].

MDA is formed predominantly through the peroxidation of polyunsaturated fatty acids by ROS, and it is applied extensively as an indicator for lipid peroxidation in plant cell membranes caused by oxidative stress. The phenomenon is acknowledged broadly as an indicator of stress-induced cellular damage in plants and is often associated with potassium (K^+) efflux from plant cells [57,58]. Thus, MDA can signal various acute responses to oxidative stress, reflecting damage severity. In the present study, all three grass species, excluding *C. boottiana* under severe salt stress (SS), exhibited elevated MDA, O_2^-, and H_2O_2 levels according to drought and salinity conditions. This aligns with previous studies that have reported an overall increase in lipid peroxidation alongside a decline in total chlorophyll and carotenoid content under prolonged stress [59]. The accumulation of MDA and ROS often correlates with a reduction in photosynthetic pigments during extended periods of stress [60]. In particular, *C. boottiana* showed no significant changes, or only slight variations, in MDA, O_2^-, and H_2O_2 levels between non-stress treatments (WW, UT) and stress treatments (MD, MS, and SS). This suggests that *C. boottiana* possesses a robust capacity to mitigate oxidative stress, maintaining stable levels of oxidative markers under stress conditions [61]. This stability likely indicates an efficient antioxidant defense system or reduced susceptibility to lipid peroxidation and ROS accumulation, which could explain its stress tolerance [62]. Moreover, plant cell membranes play a crucial role in the regulation of ion homeostasis and maintaining osmotic balance, both of which are essential for minimizing the impact of salinity stress [61]. As cell membranes are central to numerous physiological and biological processes, they are often key targets of environmental stress [63].

In general, tolerant plants show no major impact on growth or appearance. They are able to maintain gas exchange and water use at high levels while keeping oxidative stress low by effectively managing osmotic balance and nutrient levels [64,65]. Conversely, sensitive plants show low growth rates and decreased gas exchange ability due to intracellular nutrient imbalance, resulting in increased oxidative stress, ultimately leading to plant aging and death [66–68]. In the present study, there were different responses to drought or salinity stress in the three grass species, which were divided into sensitive and tolerant, based on the results of the analyses of factor results (Figure 7). Our findings also confirmed that the three species studied demonstrated a certain level of tolerance to drought and salinity stress; however, *M. grandiflora* and *C. forficula* were sensitive to severe abiotic stress. Notably, *C. boottiana* showed the highest adaptability to extreme drought and salinity conditions, exhibiting reduced gs and T_r alongside increased WUE and chlorophyll pigment contents under severe stress. In addition, MDA and ROS levels were not altered significantly under salinity stress. Drought- and salt-tolerant plants adopt strategies such as reducing stomatal conductance to minimize transpiration and water loss, thereby maintaining positive water balance under stress conditions. This adjustment also leads to improved photosynthesis rates (P_n) and overall WUE in tolerant plants [69–71]. Additionally, such plants activate their antioxidant defense systems to counteract excessive ROS production that results from drought and salinity stress. The balance between ROS generation and detoxification is crucial for preventing oxidative damage to cellular structures, such as proteins and lipids [45,72–74].

5. Conclusions

Increased tolerance to drought or salinity stress is a major aim in the genetic improvement of plants of environmental, horticultural, agricultural, and economic importance. This study highlights the importance of selecting native plant species with high adaptability to abiotic stresses for urban landscaping. *C. boottiana* has emerged as a promising candidate owing to its robust performance under adverse conditions, offering a sustainable solution for enhancing urban biodiversity and ecosystem services. These findings advocate for increased utilization of native species to minimize reliance on non-native plants, thereby supporting the goals of global biodiversity conservation. However, to better understand the adaptation mechanisms of *C. boottiana* to drought or salinity stress, further studies involving biochemical and ultra-morphological analyses are required.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Change in air temperature and relative humidity in the greenhouse during a month (June~July) of the study period. Data are represented as the daily average.

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