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Effect of salt stress on the growth and development of six species of turf grasses in the eastern region of Morocco

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Abstract

The turfgrass plays a crucial role in landscaped ecosystems, including sports fields, golf courses, residential lawns, and parks. However, various turfgrass species face diverse abiotic stresses, including salinity as irrigation with fresh water becomes unavailable. These challenging environmental conditions can result in growth inhibition, damage to cellular structure, and metabolic disturbances. Therefore, the use of salt-tolerant turfgrass species or varieties proves to be one of the most effective methods to mitigate salinity issues. With this aim, a greenhouse experiment was conducted at the experimental station of the Faculty of Sciences in Oujda. The objective was to investigate the impact of saline stress on the morphological and physiological behavior of six grass species widely used in green spaces in Morocco, particularly in the eastern region irrigated with 3 concentrations of saline water. The results revealed that the species *A. stolonifera, F. arundinacea, and C. dactylon* exhibited some tolerance to salinity, especially at a dose of 3 g/l of NaCl, while the species *L. perenne* was found to be the most sensitive to saline stress, regardless of the applied dose. Concentrations of 6 g/l and 9 g/l of NaCl led to the decline of all studied species six months after application.

Keywords: Morphological behavior, Physiological behavior, Proline, Salinity, Salt-tolerant species, Soluble sugars, Turfgrass.

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Contribution of this paper to the literature
This study contributes to the existing literature by investigating the impact of saline stress on the morphological and physiological behavior of six grass species widely used in green spaces in Morocco, particularly in the eastern region irrigated with 3 concentrations of saline water.

1. Introduction

Salinity is a major problem worldwide, affecting around 900 million hectares, i.e. approximately 20% of land area and almost half of irrigated arable land [1]. Semi-arid and arid zones are particularly affected by a significant accumulation of salts in the soil [2]. In Morocco, soil salinisation is progressing in parallel with the expansion of irrigated areas, impacting around 500,000 hectares of arable land [3]. The harmful consequences of high salinity are felt throughout the life cycle of plants, from inhibition or delay of germination and growth to plant death [4, 5].

In a saline environment, plants must mobilise a series of physiological and biochemical mechanisms to cope with stress. These mechanisms include morphological and anatomical changes, adjustments in water relations and photosynthetic activity, alterations in hormone profiles, redistribution of toxic ions, as well as biochemical adaptations such as the activation of antioxidant metabolism responses [4, 6-9].

Excess salts in water can cause damage to turf due to salinity and sodicity [10, 11]. According to Qian and Mecham [12] long-term irrigation with saline water, over a period of 4 to 33 years, led to an increase in soil electrical conductivity (EC) and sodium absorption rate, as well as elevated pH, sodium, boron and phosphorus levels on golf fairways. Turf degradation has also been observed on courses that have been subjected to prolonged irrigation with effluents [12].

Many researchers have studied the use of low-quality water for turf irrigation, focusing mainly on natural soils or sand-based artificial substrates [13-15]. Leaching plays a crucial role in reducing or preventing salt stress in turf irrigated with salt water [16]. This process involves applying irrigation water volumes greater than the actual water requirements, calculated on the basis of evapotranspiration replenishment, in order to promote continuous vertical movement of salts [17].

Choosing turfgrass species suitable for irrigation with saline water is essential to optimise maintenance costs while preserving turf quality [18]. In general, warm-season grasses have higher salt tolerance than cool-season grasses [19]. However, cool-season grasses are more appreciated compared to warm-season ones because these last species express winter dormancy which has as consequence the loss of the green colour during that period. T*all fescue (Festuca arundinacea (Schreb). syn., Schedonorus arundinaceus (Schreb.) Dumort*) is the most cultivated cool-season grass in the Mediterranean region thanks to its tolerance to the most common abiotic stresses characterizing this climate such as high summer temperatures combined with low or no precipitations, besides the resistance to salinity and the more technical advantage of resisting trampling [20]. Literature shows that tall fescue survives with levels of soil salinity up *to 10 dS* m^{-1}, making this species moderately tolerant [21-23].

In Morocco, grasses are widely used, particularly on football pitches and golf courses, sometimes covering several hundred hectares for a single golf course. These surfaces require large quantities of irrigation water, which is often of poor quality in several regions where these sports and tourist facilities are located. The use of saline water to irrigate lawns is becoming a growing problem [24, 25]. The unavailability of fresh water is forcing lawn managers to explore alternative solutions, such as finding grass species that are more tolerant of salt.

The aim of this study is to analyse the impact of salt stress on the morphological and physiological responses of six grass species commonly used in green spaces in Morocco, particularly in the eastern region. The aim is to provide information on which species are most resistant to salinity.

2. Materials and Methods

2.1. Plant Material

The plant material used in this study consisted of seeds from six species of turfgrass, four of which are considered cool- season and two tropical. The selected species are as follows: *Festuca arundinacea*, *Lolium perenne*, *Poa pratensis*, *Agrostis stolonifera*, *Pennisetum clandestinum*, and *Cynodon dactylon*. Seedling doses are those recommended for each species [26].

The seeds were donated by the seed producing company "Les Gazons de France ", France and by "Golf Saidia Lacs", Saidia, Morocco.

2.2. Treatments Used

To assess the impact of saline stress on turfgrass, the grass, grown from germination and cultivation of the seeds of the studied species, was subjected, starting from January 2019 to three levels of salinity corresponding to three concentrations of NaCl in the irrigation water: 3, 6, and 9 g/l. The control was irrigated with tap water from the experimental station, with an electrical conductivity (EC) of 0.7 mS/cm. These treatments will be referred to subsequently as:

T0: control, irrigated with tap water.
T1: 3 g/l NaCl.
T2: 6 g/l NaCl.
T3: 9 g/l NaCl.

2.3. Installation and Conduct of the Experiment

This experiment was conducted in a greenhouse at the research experimental station of the Faculty of Sciences in Oujda, Morocco. Sowing was carried out in October 2018 in cylindrical pots with dimensions of 0.35 m deep by 0.22 m in diameter, filled with a substrate composed of peat and sand in a ratio of 2/3 peat to 1/3 sand by volume, preceded by a drainage layer of 1 cm. Light watering was performed once daily to keep the substrate moist with non-saline irrigation water. Starting from January 2019, turfgrass irrigation was carried out based on water requirements, calculated using the reference evapotranspiration (ET0) values for the city of Oujda [27] and using greenhouse water requirements calculated using the following equation [28]:

$$ETMs\ (mm/m^2) = 0.56 * ET_0 + 0.7 \qquad (1)$$

Where:

ETMs: Maximum evapotranspiration under greenhouse conditions.

ET_0: Reference evapotranspiration.

Table 1 shows the quantities of water supplied according to the reference evapotranspiration of the city of Oujda [27].

Table 1. Water quantities supplied for turfgrass species according to the imposed salinity stress based on the reference evapotranspiration of the city of Oujda.

Month	Daily ET_0 (mm/Day)	ETMs (mm/m²)	Water quantity (ml/pot)
Dec -18	1.9	1.8	136
Jan -19	1.8	1.7	132
Feb -19	2.8	2.3	175
Mar-19	3.5	2.7	205
Apr -19	4.0	2.9	226
May -19	5.0	3.5	270
Jun -19	6.0	4.1	313
Jul -19	7.1	4.7	360

Irrigations were carried out three times per week to facilitate the watering operation, and the turfgrass species were subjected to this stress for seven months. After establishment, the grass was mowed once a month to a height of 2 cm using a lawn shear. No fertilizers, herbicides, insecticides, or fungicides were applied throughout the duration of the experiment. The average, minimum, and maximum temperatures, as well as the humidity of the greenhouse, were measured daily, and the monthly averages are presented in the (Figure 1).

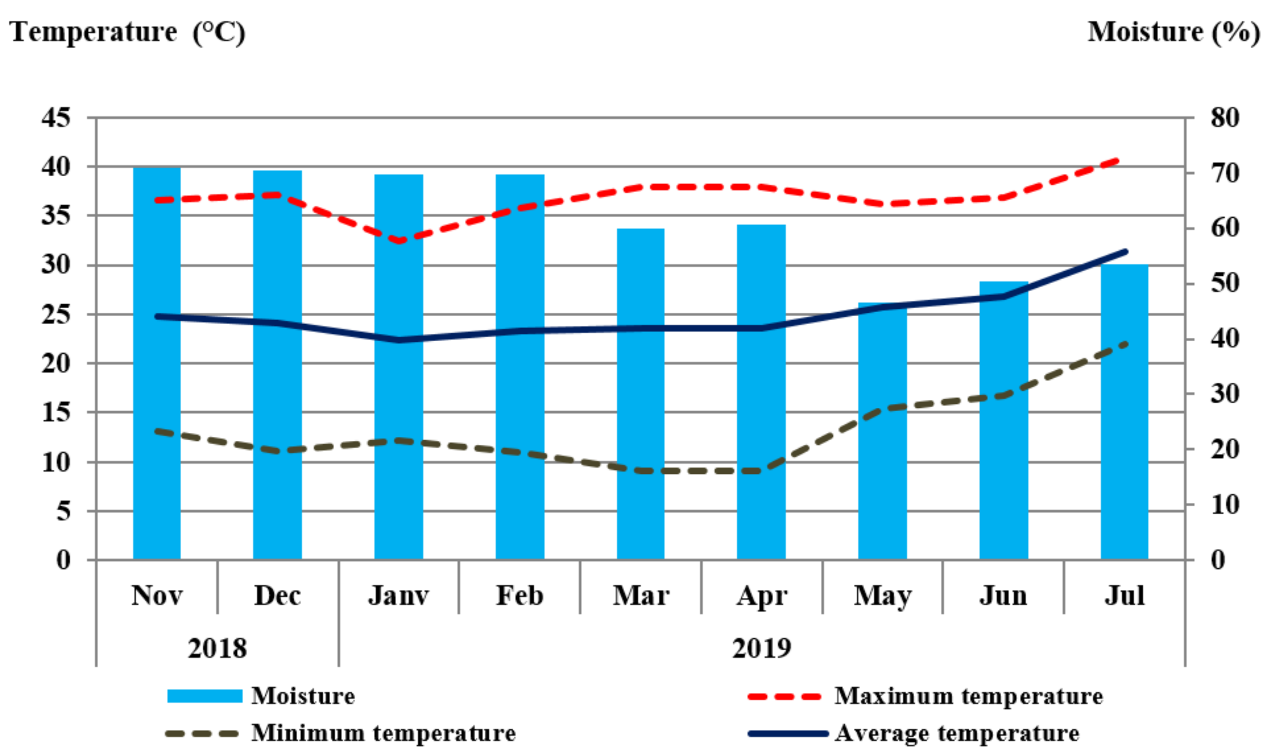

Figure 1. Monthly meteorological data in the greenhouse at the experimental station of the Faculty of Sciences in Oujda for the experimental period from November 2018 to July 2019.

2.4. Studied Parameters

The monitoring of the morpho-physiological behavior of these grasses regarding salt stress was conducted by assessing morphological, physiological, and biochemical parameters used in several studies focusing on plant response to various abiotic stresses. It is worth noting that the initial recordings were conducted after two months from the initiation of the different treatments (from early January to late February).

2.5. Morphological Parameters

2.5.1. Length Growth (cm)

The length growth of the grass and internodes were evaluated each month using a graduated ruler.

2.5.2. Above-Ground Dry Matter of Mowing

At the end of each month, the fresh weight (in grams) was determined by weighing the mowing product directly after harvesting. The dry weight (in grams) was determined after drying the mowing product in an oven set at a temperature of 80 °C for 72 hours.

The dry matter content (%) is derived from the ratio:

$$DM\ (\%) = (DW/FW^{*}\ 100) \qquad (2)$$

Where: DM is the dry matter; DW is the dry weight; and FW is the fresh weight.

2.5.3. Above Ground and Below-Ground Biomass

At the end of the 6-month experiment, the grass from each pot was unearthed, and the above-ground and below-ground parts were separated, cleaned of substrate, dried in an oven at 80°C to constant weight, then weighed. The dry and fresh weights thus determined are expressed in grams.

2.5.4. Determination of Electrical Conductivity

The soil electrical conductivity of each sodium chloride concentration was measured at the end of the trial. Soil electrical conductivity was measured using a 1:5 dilution of soil: water solution, since this technique enables us to determine the amount of salt present in a soil regardless of its degree of saturation [29].

2.6. Evaluation of the Area Covered by Green Grass

2.6.1. Coverage Rate

At the end of each month, photos were taken using a camera to analyze and assess the turf coverage rate. The images were captured in JPEG (Joint Photographic Experts Group) format for processing using ImageJ [26].

2.7. Physiological Parameters

2.7.1. Chlorophyll Pigment

Total chlorophyll content was measured using the method developed by Tran, et al. [30] to asses the impact of saline irrigation on the 6 turf species. 1 g of fresh leaves at the end of the experimental irrigation period were ground and macerated with 10 ml of acetone diluted to 80% in a mortar. To avoid the oxidation, the chlorophyl extracted suspensions were protected from light with aluminum foils once recovered in glass tubes, prior to centrifugation at 2500g for 5 min. The absorbance of recovered surnatant was measured at 645 and 663 nm with a UV-vis spectrophotometer (Humterlaba Inc. Reston, Va, USA). The following formulas were used to calculate the total chlorophyll concentration (a+b):

$$[Chl\ (a)] = (12.7 \times DO663 - 2.63 \times DO645) \times V/M \qquad (3)$$

$$[Chl\ (b)] = (22.9 \times DO645 - 4.68 \times DO663) \times V/M \qquad (4)$$

$$[Chl\ (a+b)] = (7.15 \times DO663 + 18.71 \times DO646) \times V/M \qquad (5)$$

Where:
Chl (a): Chlorophyll (a) (mg/g FW);
Chl (b): Chlorophyll (b) (mg/g FW);
Chl (a+b): Total chlorophyll (mg/g FW);
V: Volume of total extract (l);
M: Mass of fresh material (g).

2.7.2. Relative Water Content (RWC)

The determination of leaf relative water content was performed once a month using the method outlined by Barrs [31]. This technique involves cutting the leaves at the base of the lamina and immediately weighing them to obtain their fresh weight (FW). Subsequently, these leaves are placed in test tubes filled with distilled water and placed in a cool, shaded area. The leaves are retrieved after 24 hours and blotted dry with absorbent paper to remove surface water. They are then weighed again to obtain the weight at full turgor (FT). Finally, the samples are placed in an oven at a temperature of 80°C for 48 hours to determine their dry weight (DW). The following formula by Clark and Mac-Caig (1982), as cited by Mouellef [32] is used to calculate the relative water content:

$$RWC\ (\%) = [(FW - DW) / (FT - DW)]^{*}100 \qquad (6)$$

Where:
RWC: Relative water content (%).
FW: Fresh weight (g).
FT: Weight at full turgor (g).
DW: Dry weight (g).

2.8. Biochemical Parameters

2.8.1. Proline

Proline (pyrrolidine-2-carboxylic acid) is one of the twenty primary amino acids that constitute proteins. It acts as an osmoprotector and an important signaling molecule that accumulates in the plant cytosol, functioning in membrane stabilization and protection. Moreover, proline enhances water absorption and reduces the accumulation of toxic ions [33-35]. Proline is highly soluble in water, methanol, and benzene and easily oxidized by ninhydrin or tricetohydrindene [36]. The quantification of the proline-ninhydrin reaction is performed using a spectrophotometer. Proline reacts with ninhydrin to form a colored complex, and the intensity of the coloration is proportional to the amount of proline in the sample. The quantification method for this amino acid follows the procedure adapted by Leport [37]. For the analysis, 100 mg samples are placed in a test tube, and 2 ml of 40% methanol is added. The samples are then heated in a water bath for one hour. After cooling, 1 ml of the extract is mixed with 1 ml of acetic acid and 1 ml of a solution composed of: distilled water (120 ml), acetic acid (300 ml), orthophosphoric acid (80 ml). And added ninhydrin (25 mg). The optical density is measured using a UV-vis spectrophotometer (ISO-TS 3632-1, 2003) at a wavelength of 528 nm. The obtained value is converted into proline concentration using a standard curve established from a series of solutions with known proline concentrations. The contents are expressed in mg of proline per g of FW (fresh weight).

2.8.2. Soluble Sugars

Maintaining normal water potential could be attributed to the accumulation of osmotica such as soluble sugars, which enable seedlings to maintain leaf turgor by reducing water potential and thus promoting water absorption despite the presence of salt in the soil. Simple sugars (glucose, fructose, and sucrose) are extracted by a solvent capable of solubilizing them and inhibiting enzymatic activities that may degrade them. The assay was conducted referring to the method as reported by Sidari, et al. [38].

The principle of the reaction is based on the condensation of neutral oses degradation products by sulfuric acid. The highly concentrated sulfuric acid transforms the oses under heat into furfural derivatives, which give a blue-green coloration with anthrone.

We begin by soaking 100 mg of fresh foliar material in 5 ml of 80% ethanol (v/v) for 24 hours. Next, the obtained extract is diluted by mixing it with 80% ethanol at a ratio of 1:10. Then, 2 ml of this diluted solution are taken and added to 4 ml of 0.2% anthrone reagent (0.2 g of pure anthrone + 100 ml of sulfuric acid (H_2SO_4)). The mixture, containing both the extract and the reagent, must be kept in ice. Subsequently, the tubes are placed in a water bath at 92°C for 8 minutes. After this step, the tubes are allowed to cool for 30 minutes in the dark, and then the optical density is measured at λ= 530 nm using a spectrophotometer UV-vis (Humterlaba Inc. Reston, Va, USA).

The soluble sugar content of the leaves was calculated with reference to a standard glucose range.

2.9. Experimental Design

The experimental design adopted for this trial is a Randomized Complete Block, with three replicates (Figure 2). The design is made up of three blocks corresponding to three replicates, i.e. a total of 12 pots sown for each species studied (number of treatments (4) x number of replicates (3)).

Figure 2. Illustrative photo of the experimental set-up adopted for salt stress (February 2019).

2.10. Statistical Analysis

Results were subjected to descriptive statistical analysis and analysis of variance using SPSS software, version 20. Means were compared using Tukey's method.

3. Results

3.1. Effect of Salt Stress on Morphological Parameters

3.1.1. Internode Length

The results indicate that salt has a depressive effect on final internode length. The length of the internodes is slightly modified under conditions of moderate stress (3 g/l NaCl), particularly for the species *A. stolonifera*, *F. arundinacea and C. dactylon.* The sensitivity to salt stress of *L. perenne*, *P. prantensis and P. clandestinum* species varies with the amount of NaCl applied.

On the other hand, intense salt stress (6 g/l and 9 g/l) is extremely destructive from June onwards (5 months after the start of stress application) for *L. perenne*, *P. pratensis and P. clandestinum* species, and from July onwards for *F. arundinace and C. dactylon* species, which have completely suppressed their activity. During the summer, only *A. stolonifera* maintained a green level of 6 g/l (Figure 3).

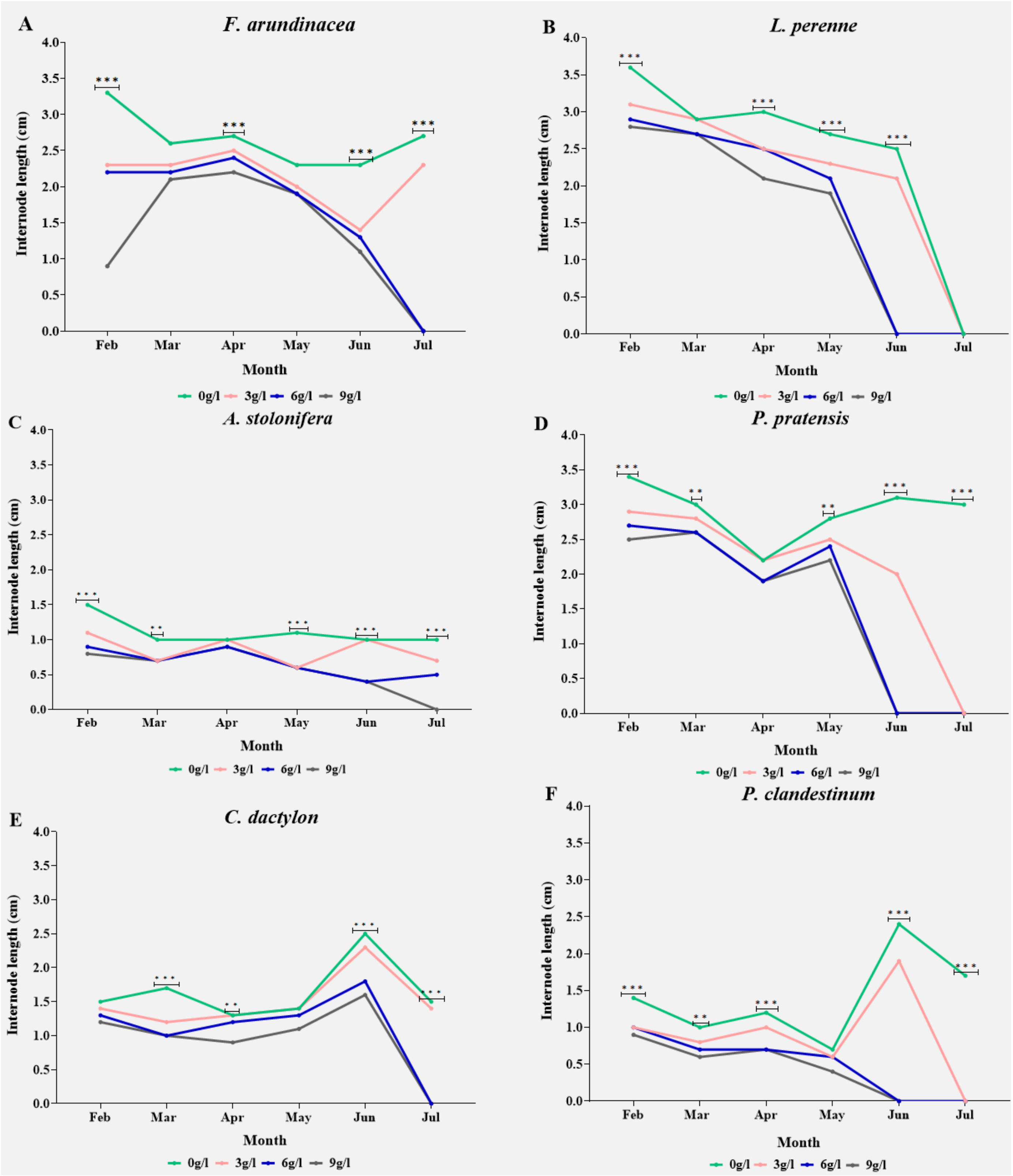

Figure 3. Effect of different levels of salinity (NaCl) on the monthly average internode length among the six studied species.
Note: Significant differences are indicated as follows: * significant ($p<0.05$); ** highly significant ($p<0.01$); *** very highly significant ($p<0.001$).

3.1.2. Grass Length

The results obtained for the length of the produced grass follow the same trend as those recorded for the internode parameter. There is a noticeable reduction for the severe treatments of 6 and 9 g/l for all species and a relative tolerance of the species *A. stolonifera*, *F. arundinacea*, *and C. dactylon* at 3 g/l. Here again, only the species *C. dactylon* was able to maintain length growth at 6 g/l (Figure 4).

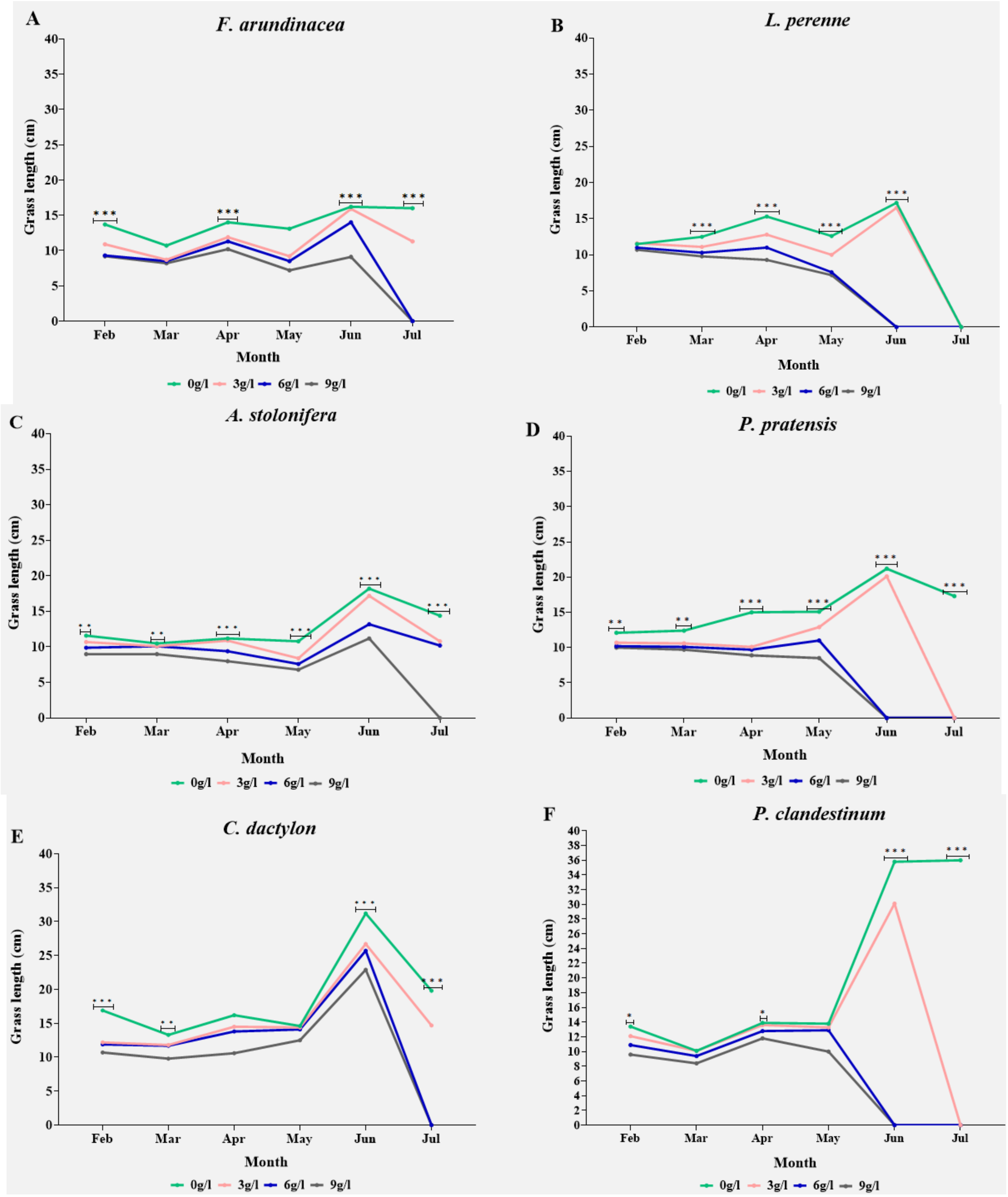

Figure 4. Effect of different levels of salinity (NaCl) on the monthly average grass length among the six studied species.

Note: Significant differences are indicated as follows: * significant (p<0.05); ** highly significant (p<0.01); *** very highly significant (p<0.001).

3.1.3. Effect of Salt Stress on Mowing Dry Matter

For all studied species, both the control and lawns irrigated with water containing 3 g/l responded with a higher production of dry matter compared to other treatments. Conversely, lawns treated with higher concentrations of NaCl (6 g/l and 9 g/l) exhibited low production of dry matter, leading to grass death during the last two months of the trial, except for *A. stolonifera* at the 6 g/l NaCl dose. Significant differences were observed between treatments throughout the trial period (Figure 5). Species *A. stolonifera* appears resistant to doses of 3 g/l and 6 g/l of NaCl, while species *F. arundinacea and C. dactylon* seem resistant to a dose of 3 g/l. However, species *L. perenne*, *P. prantensis*, *and P. clandestinum* are sensitive to saline stress regardless of the NaCl dose applied.

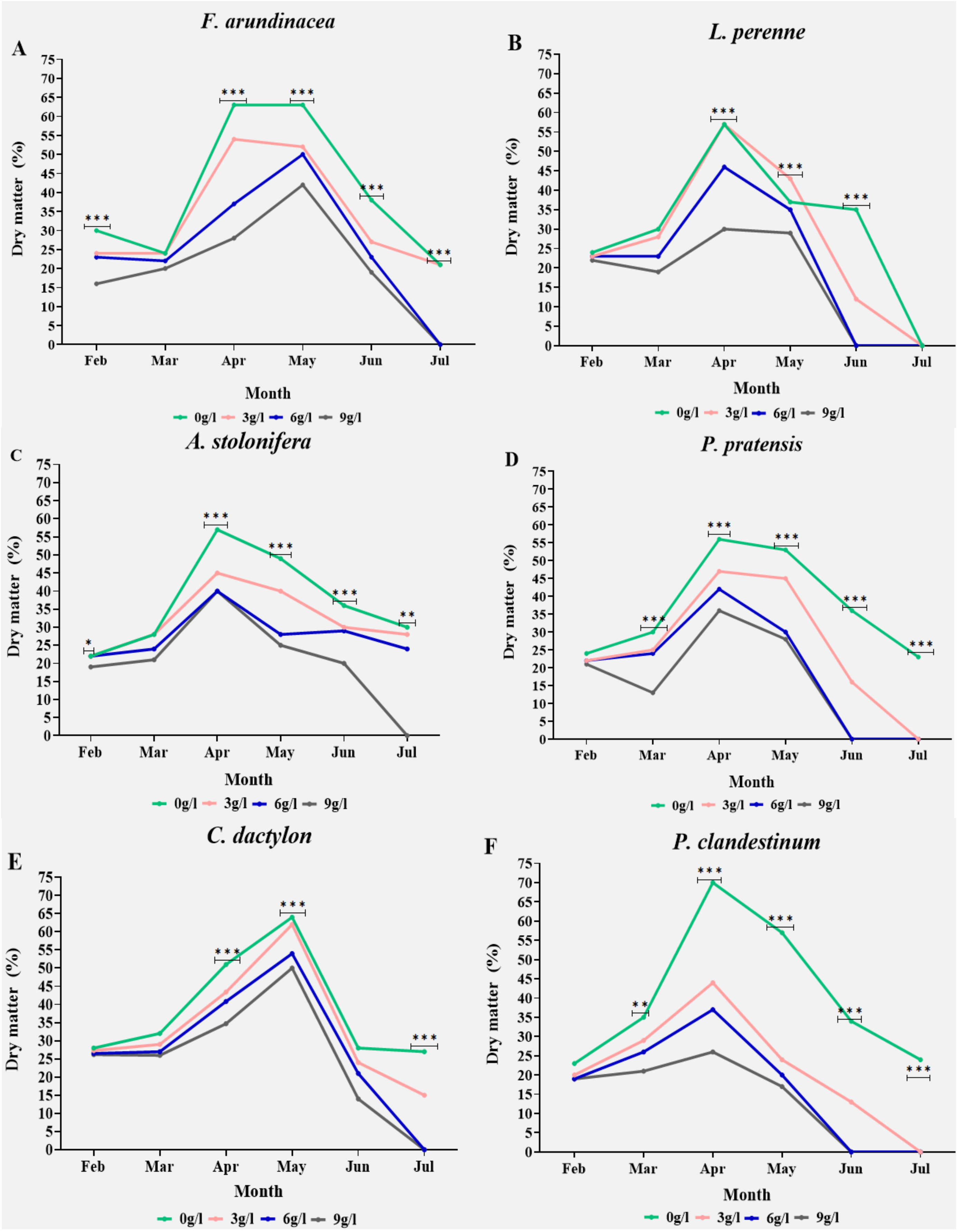

Figure 5. Effect of different levels of salinity (NaCl) on the monthly average dry matter among the six studied species.

Note: Significant differences are indicated as follows: * significant ($p<0.05$); ** highly significant ($p<0.01$); *** very highly significant ($p<0.001$).

3.1.4. Effect of Salt Stress on Above Ground and Below-Ground Biomass

The results shown in Figure 6 (A-B) demonstrate that the application of severe salt stress leads to a reduction in dry biomass both above and below ground. It is noted that for all species, severe treatments with 6 g/l and 9 g/l of NaCl caused increasingly significant reductions in biomass towards the end of the trial. Significant differences between treatments were observed for all species (Figure 6).

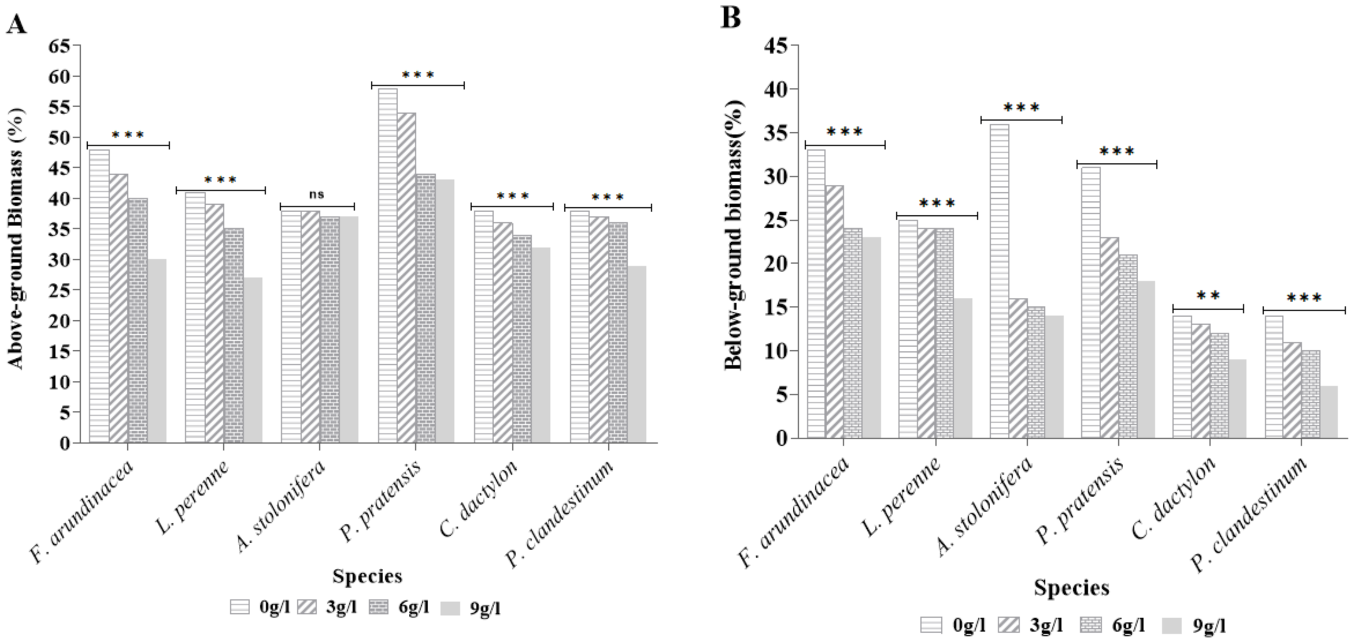

Figure 6. Effect of different salinity levels (NaCl) on the monthly mean above-ground (A) and below-ground (B) biomass for the six species studied.

Note: Significant differences are indicated as follows: * significant ($p<0.05$); ** highly significant ($p<0.01$); *** very highly significant ($p<0.001$).

3.1.5. Effect of Salt Stress on Substrate Electrical Conductivity

Measurements of electrical conductivity (EC) at the end of the trial showed a considerable increase in this parameter for all treatments. The results of the evolution of substrate EC show that the lowest substrate salinization was recorded in the control and the highest in the 9 g/l treatment. The high final electrical conductivities can be explained by the cumulative effect of the different watering solutions, leading to grass death at the end of the trial. Results were significant between treatments for all species (Figure 7).

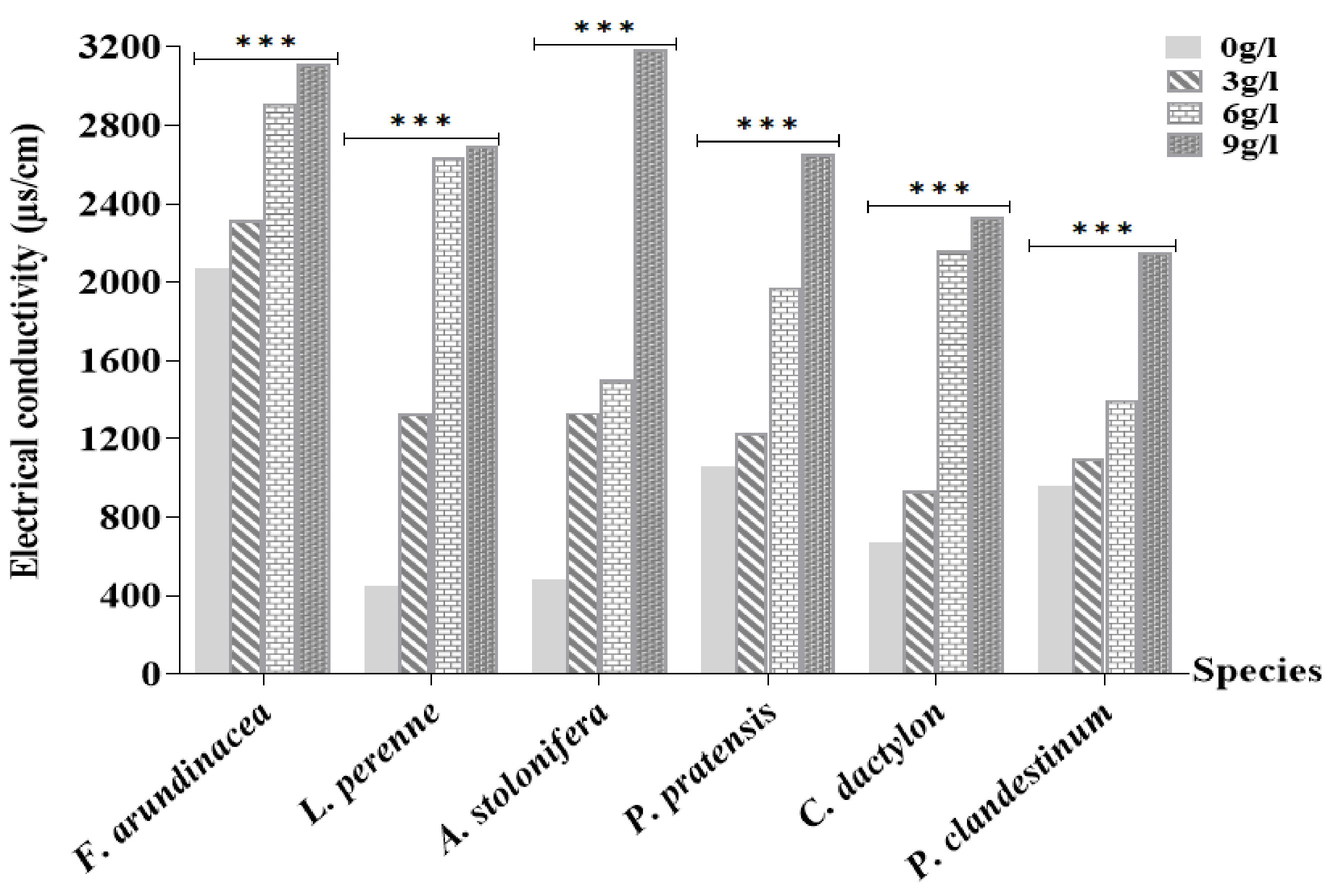

Figure 7. Effect of different salinity levels (NaCl) on monthly mean electrical conductivity in the six species studied.

Note: Significant differences are indicated as follows: *** very highly significant ($p<0.001$).

3.1.6. Effect of Salt Stress on Ground Coverage Ratio

The ground cover ratio by the turfgrass (Figure 8) decreased as the levels of salt stress increased, for all species. This reduction in coverage was observed as early as March for species *L. perenne*, *P. prantensis, and P. clandestinum*, particularly at high stress levels (6 and 9 g/l). For the same stress levels, the impact of salinity on this ratio only became apparent from May onwards for species *A. stolonifera* and *C. dactylon*, suggesting a relative tolerance for the latter. At 3 g/l, the reduction in the cover ratio was most pronounced for species *L. perenne and P. prantensis* as early as March. Species *A. stolonifera* and *L. perenne* continued to provide good ground cover until July, when a decline in this coverage began (Figure 9).

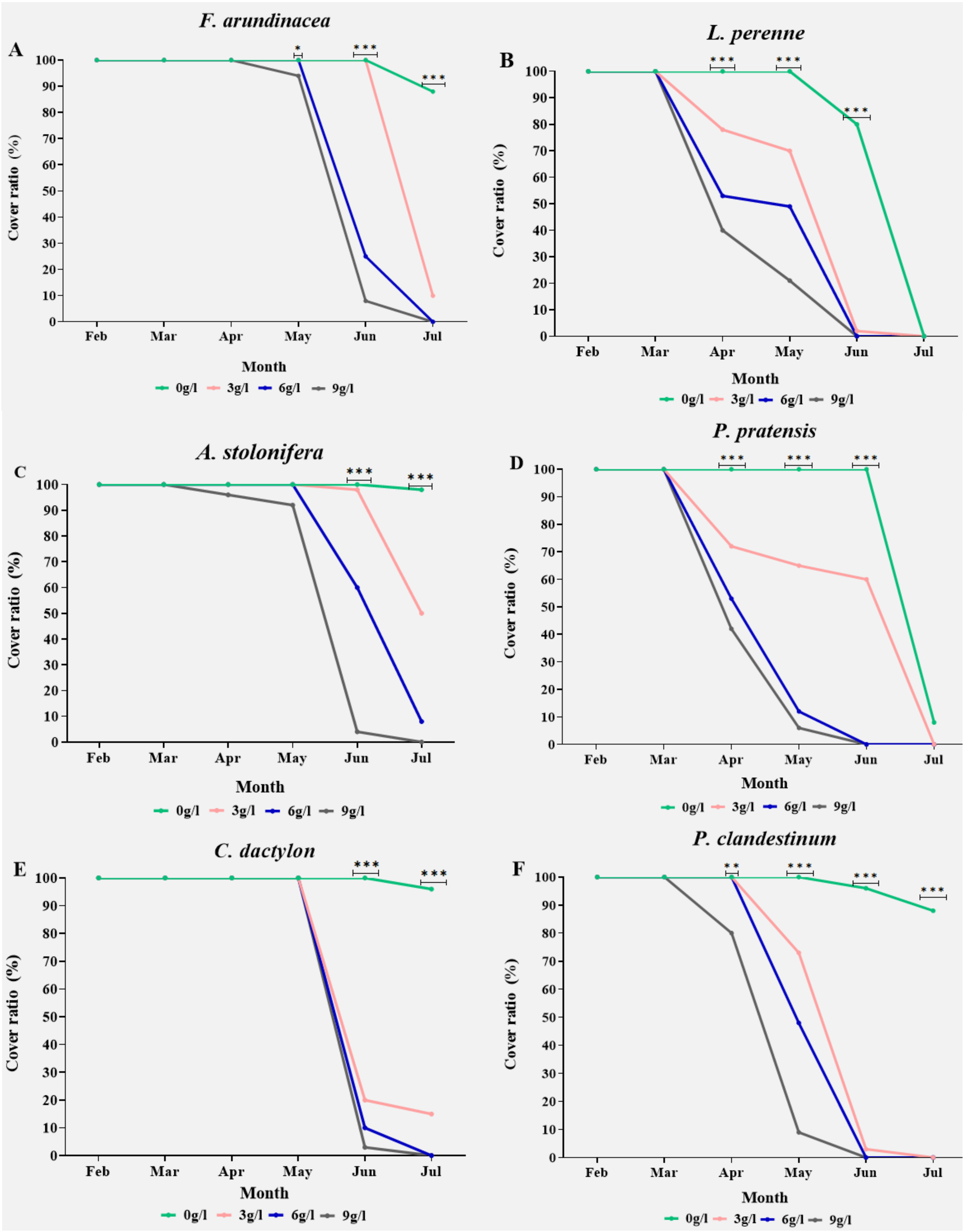

Figure 8. Effect of different salinity levels (NaCl) on the monthly average cover rate for the six species studied.

Note: Significant differences are indicated as follows: * significant (p<0.05); ** highly significant (p<0.01); *** very highly significant (p<0.001).

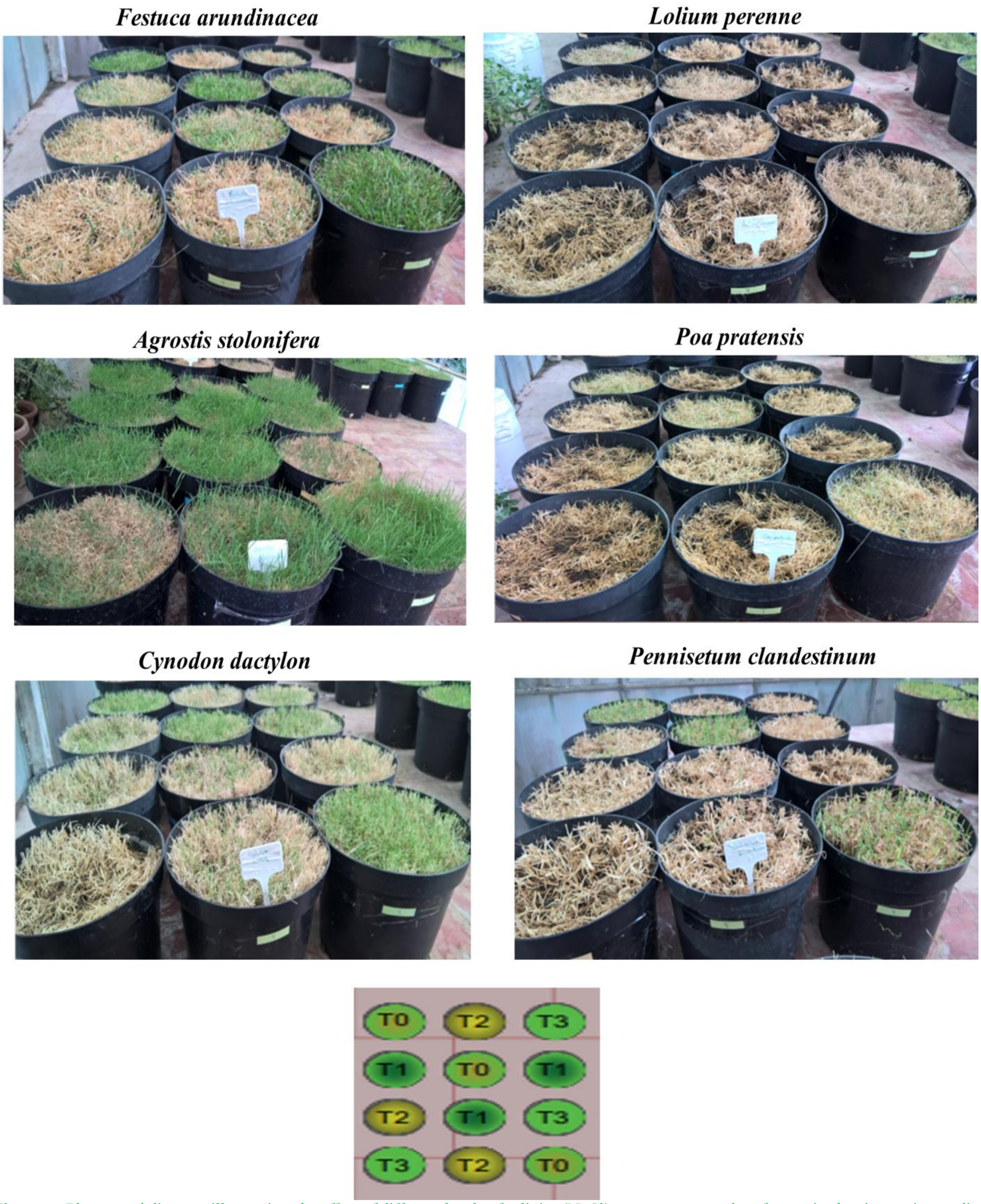

Figure 9. Photos and diagram illustrating the effect of different levels of salinity (NaCl) on greenness and turf cover in the six species studied (July 31, 2019) on greenness and turf cover in the six species studied (July 31, 2019); (T0: 0 g/l ; T1: 3 g/l ; T2: 6 g/l ; T3: 9 g/l).

3.2. Effect of Salt Stress on Physiological Parameters

3.2.1. Total Chlorophyll

Overall, increasing salinity tends to decrease the total chlorophyll content for all species. Indeed, the moderate level of stress (3 g/l) recorded concentrations exceeding those of the control (0 g/l) for all species. However, the application of high salinity levels (6 g/l and 9 g/l) leads to a decrease in total chlorophyll for all species. Significant differences between treatments are observed throughout the trial period (Figure 10).

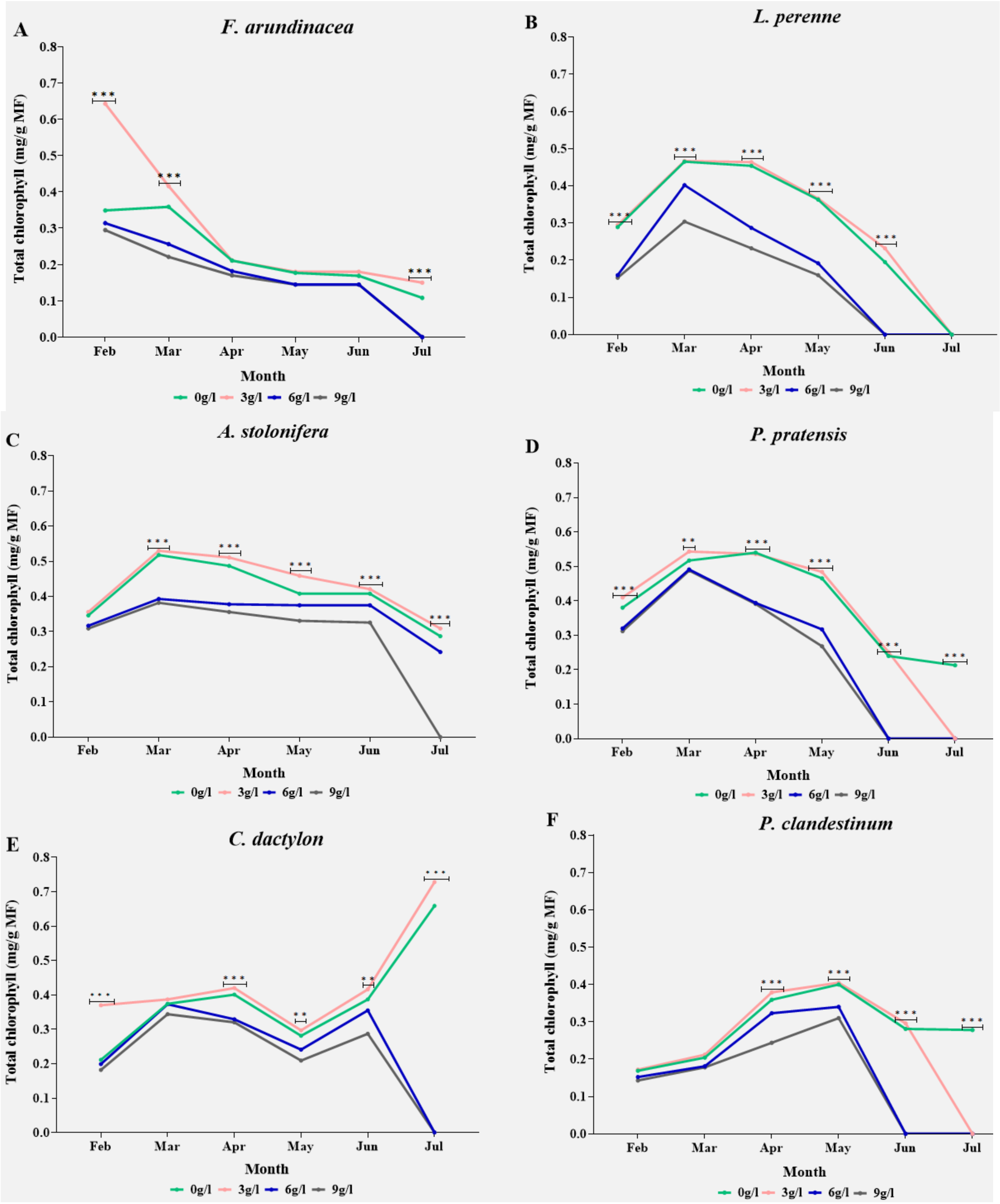

Figure 10. Effect of different salinity levels (NaCl) on monthly mean total chlorophyll (a+b) in the six species studied.

Note: Significant differences are indicated as follows: * significant ($p<0.05$); ** highly significant ($p<0.01$); *** very highly significant ($p<0.001$).

3.2.2. Impact of Salt Stress on Relative Water Content

The data from Figure 11 indicate that the presence of NaCl led to a decrease in leaf water content. Indeed, for all species, the lowest rates of relative water content were observed in lawns subjected to severe NaCl treatments (6 g/l and 9 g/l). Significant differences were noted between treatments throughout the trial period (Figure 11).

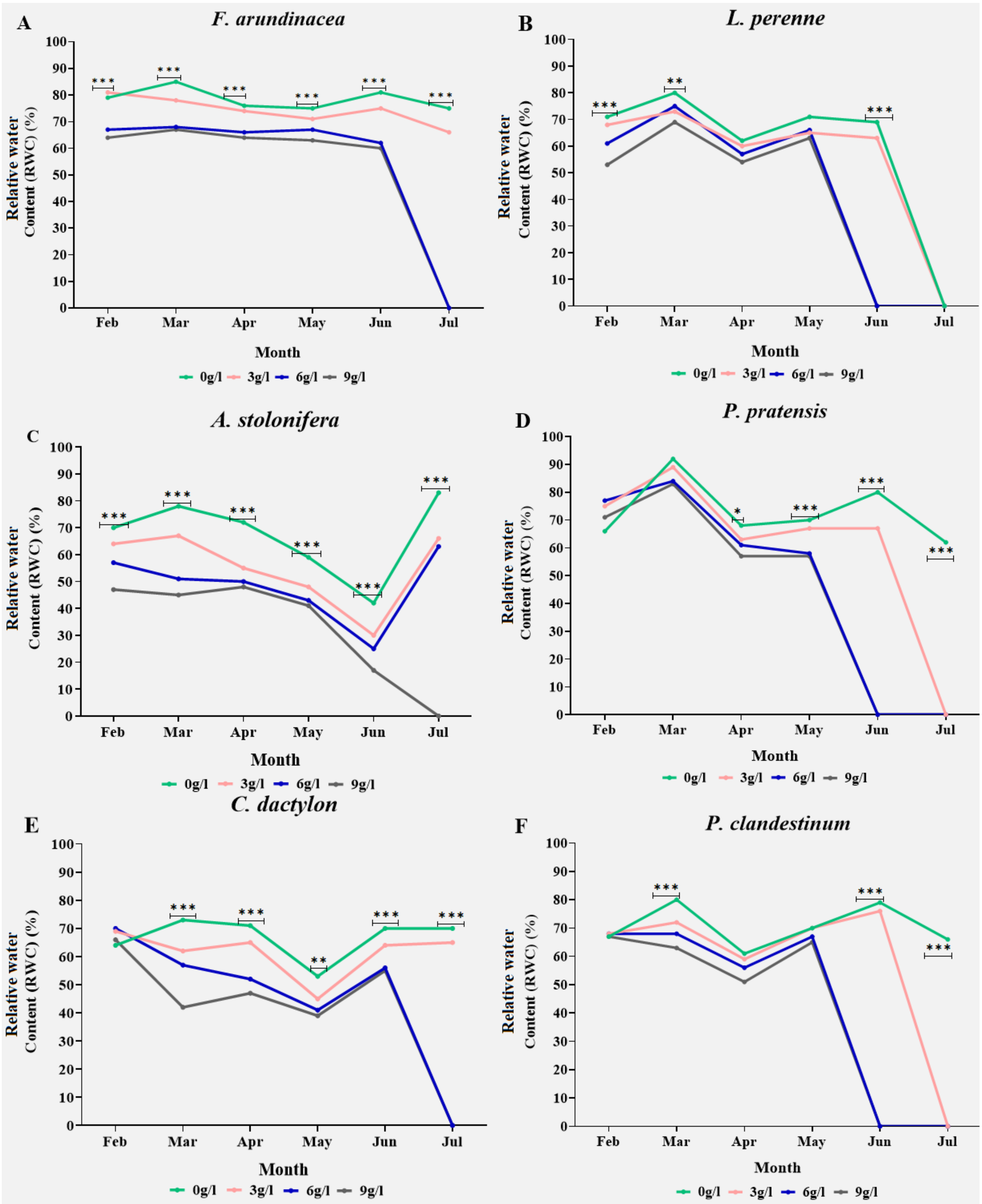

Figure 11. Effect of different salinity levels (NaCl) on monthly mean relative water content in the six species studied.

Note: Significant differences are indicated as follows: * significant (p<0.05); ** highly significant (p<0.01); *** very highly significant (p<0.001).

3.3. Effect of Salt Stress on Plant Biochemical Parameters

3.3.1. Proline Content

The effect of NaCl on proline content reveals that irrigating grass with high concentrations of NaCl induces a significant accumulation of this solute. This accumulation is generally proportional to the NaCl concentration in the irrigation water. The highest proline content was recorded for the 9 g/l NaCl treatment in all species. There were significant differences between treatments throughout the test period (Figure 12).

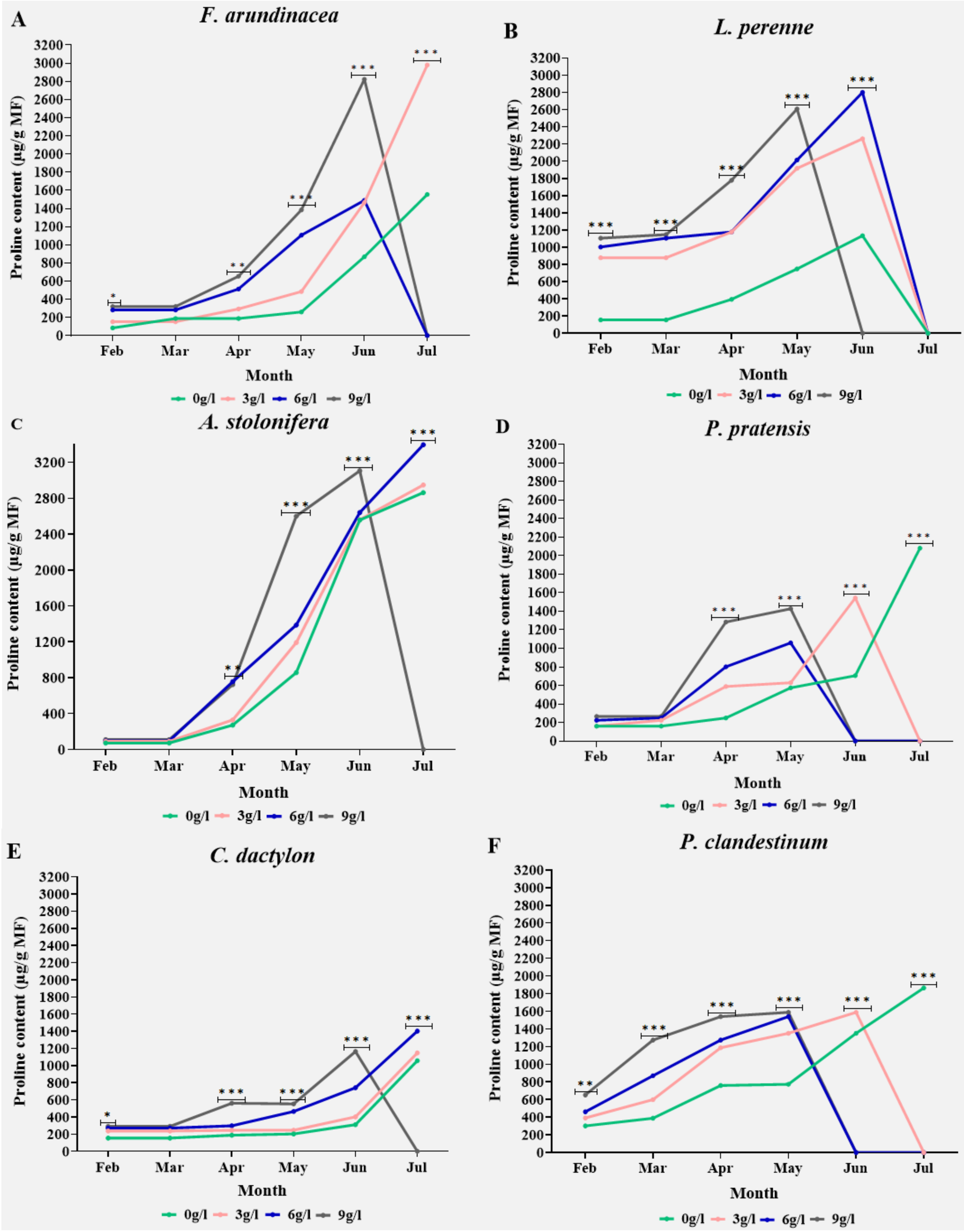

Figure 12. Effect of different salinity levels (NaCl) on monthly mean leaf proline content in the six species studied.

Note: Significant differences are indicated as follows: * significant ($p<0.05$); ** highly significant ($p<0.01$); *** very highly significant ($p<0.001$).

3.3.2. Soluble Sugar Content

The analysis of the results presented in Figure 13 reveals that the effect of salt stress led to a significant accumulation of soluble sugars. This accumulation increased from February to July. Indeed, the highest values for this type of osmoticum were observed under high salinity conditions (6 g/l and 9 g/l NaCl) and towards the end of the experimentation period for all species, until the depletion of the grass towards the end of the trial. Significant differences exist between treatments throughout the trial period (Figure 13).

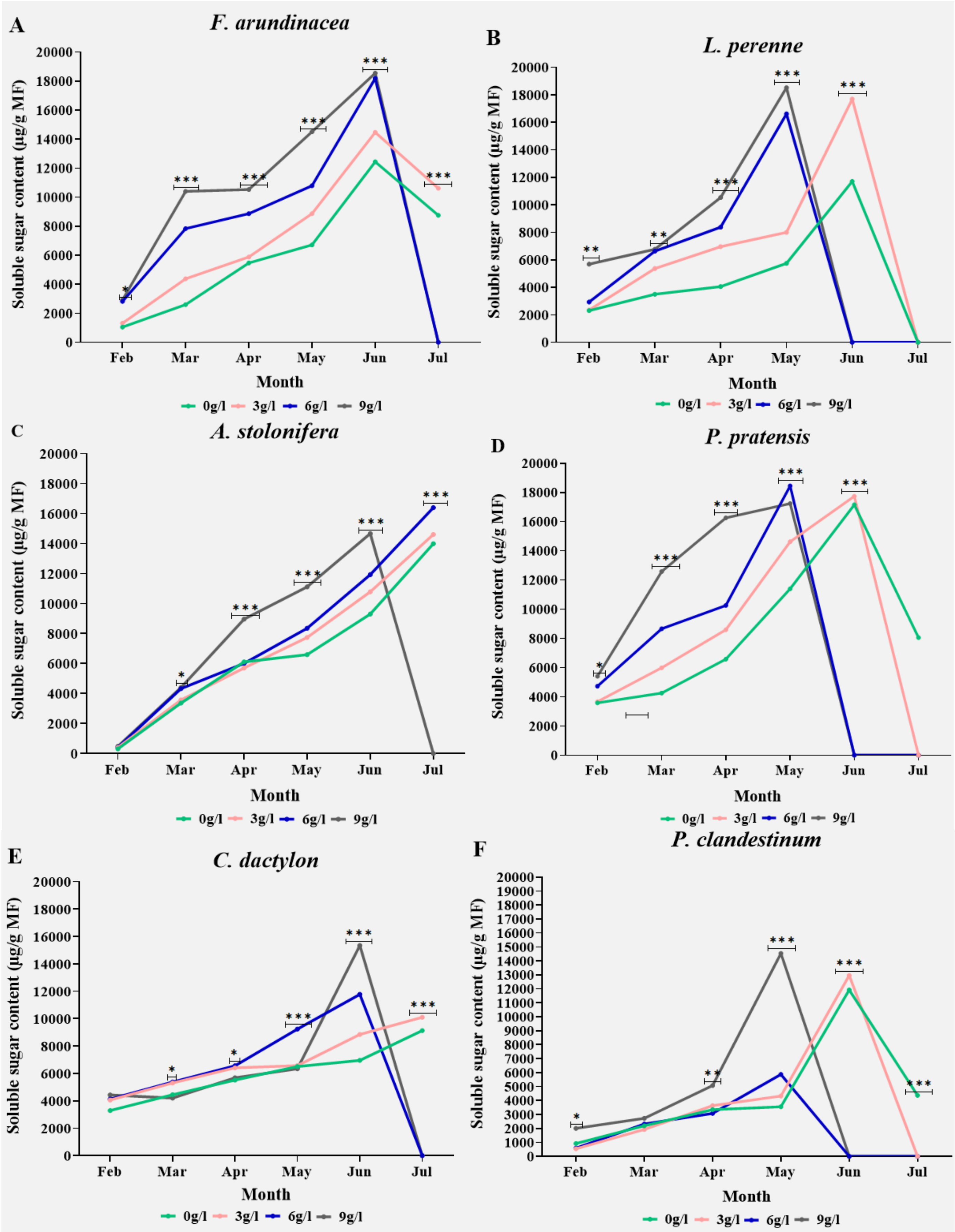

Figure 13. Effect of different levels of salinity (NaCl) on the monthly average soluble sugar content among the six studied species.

Note: Significant differences are indicated as follows: * significant ($p<0.05$); ** highly significant ($p<0.01$); *** very highly significant ($p<0.001$).

4. Discussion

The results show that the six turfgrass species studied show contrasting responses to salt stress for most of the parameters analysed. Observation of the behavior of grasses under salt stress revealed that irrigation with NaCl-enriched water caused a reduction in morphological parameters, such as the length of blades and internodes. Similar reductions were observed for dry matter and above- and below-ground biomass, with a marked drying out of the grasses when irrigated with solutions containing 6 g/l and 9 g/l NaCl, from the fourth month after exposure to the stress, except for Agrostis stolonifera, which resisted the 6 g/l NaCl concentration. In addition, more pronounced reductions in turf cover were noted under severe stress (6 g/l and 9 g/l NaCl), particularly during the last two months.

These results are consistent with those of Bizhani and Salehi [39]; Guo, et al. [40] and Xu, et al. [41] who reported that leaf elongation rate of tall fescue, shoot growth of seashore paspalum (*Paspalum vaginatum*), shoots and roots of Bermuda grass (*Cynodon dactylon*), as well as shoots of Kentucky bluegrass (*Poa pratensis*) are significantly inhibited by salt stress. In addition, the cover rate is a key indicator for assessing the visual quality of turf, and in

Kentucky bluegrass, this rate decreases significantly under the effect of salinity [42]. Previous research has also shown that the main signs of stress in plants subjected to saline irrigation include a stunted appearance, a decrease in root biomass and drying out of the aerial parts, leading to reduced growth [43]. This reduction can be explained by the increase in osmotic pressure in the environment due to NaCl, which limits water uptake by the roots. This abiotic stress disrupts turfgrass growth and development by inhibiting water uptake, creating osmotic and ionic imbalances at the root-soil interface [44-46]. Under moderate salinity conditions, some salt-tolerant species in *Cynodon spp.*, *Paspalum vaginatum*, *Puccinellia distans* and *Zoysia matrella* are able to maintain their shoot growth, on the contrary, sensitive species shoot growth is stopped [13, 24, 47]. *Lolium perenne* 'Belida', *Festuca rubra commutata* 'Casanova', *Poa pratensis* 'Evora', and *Festuca rubra* trichophylla 'Smyrna', cool-season turfgrasses, millipedes (*Eremochloa ophiuroides*) and when irrigated with saline solutions have maintained the turf quality at the low to moderate salt concentration, whereas at higher concentrations leaf width and length significantly decreased [14, 15].

In addition, all species studied (*Distichlis spicata*, *Festuca arundinacea*, *Poa pratensis*) showed an increase in root/stem ratio at all salinity levels. Of these species, only glasswort (*Distichlis spicata*) maintained a relatively stable above-ground biomass despite an increase in soil electrical conductivity (EC) from 2.5 to 25 dS m^{-1}. An initial increase in root biomass was observed in alkaligrade and glasswort up to 15 dS m^{-1}, while tall fescue maintained its root mass at the same EC level [24]. A similar stimulation of root growth was observed in a halophytic grass, *Sporobolus virginicus*, when the NaCl concentration reached up to 1.0 M [17].

The changes in the morphological characteristics of turfgrass under salt stress are directly linked to physiological changes, in particular the stability of cell membranes and the production of reactive oxygen species (ROS). Physiological and molecular studies have been carried out to gain a better understanding of the mechanisms by which turfgrass responds to salt stress. It has been reported that malonyldialdehyde (MDA) concentration and electrolyte leakage (EL) increase under NaCl in perennial ryegrass and Kentucky bluegrass, indicating damage to cell membranes [42, 48, 49]. Under salt stress, the plant first undergoes osmotic stress linked to external salts, then ionic stress when salts accumulate to toxic levels inside the cells, the latter phase being decisive in the genetic differentiation of plants' ability to tolerate salinity [50]. However, some studies suggest that osmotic and ionic stress simultaneously affect plants exposed to salt stress [51-54].

In the same context, Smillie and Nott [55] and Hessini, et al. [56] have shown that the presence of soluble salts in the soil solution lowers its osmotic potential, causing a state of physiological 'drought' characterised by a reduction in plant metabolic activity. This drop in osmotic potential limits water uptake by the roots, reducing cell turgor pressure and leading to plasmolysis. However, some plants quickly adjust their osmotic potential in response to the external environment, maintaining a favourable water gradient. Inclusive plants store chloride and sodium ions, whereas in exclusive plants these ions are not retained to any great extent and are transported to the roots via the xylem [57]. Numerous studies confirm that osmotic adjustment is a central mechanism for the survival and growth of plants subjected to salt stress [58-61].

Our observations of physiological and biochemical parameters, including total chlorophyll, relative water content (RWC), proline and soluble sugars, reveal that high concentrations of NaCl in the irrigation water led to a significant decrease in chlorophyll pigment content and RWC in all the species studied, particularly at concentrations of 6 g/l and 9 g/l NaCl. Conversely, an increase in the accumulation of proline and soluble sugars in the leaves was observed as salinity increased.

These results agree with those of Uddin, et al. [62] who reported a decrease in chlorophyll content and RWC, accompanied by an increase in proline with increasing salinity in *Paspalum vaginatum*, *Zoysia japonica*, *Zoysia matrella*, *Digitaria didact*yla and *Cynodon dactylon*. These observations have also been made for other monocotyledons such as rice, wheat and maize [63-66].

Under stress conditions, the decrease in osmotic potential can be due to dehydration processes as well as the accumulation of organic and inorganic solutes [67, 68]. This osmotic adjustment mechanism has been identified as an important tolerance strategy in *Paspalum vaginatum* and *Agrostis stolonifera* [40, 69, 70]. In response to stress, organic solutes such as soluble sugars and proline, as well as inorganic ions such as Na+ and K+, play an essential role in the osmotic adjustment of turfgrasses [25, 71]. These mechanisms enable plants to maintain leaf turgidity, thereby reducing water potential and promoting water uptake despite soil salinity [72]. In addition to its role as an osmolyte, proline contributes to the stabilisation of subcellular structures, captures free radicals and acts as a buffer to regulate redox potential during stress situations [73]. The accumulation of soluble sugars, in particular, is a frequent phenomenon in response to salt stress [74]. This accumulation could be linked to enzymatic changes affecting carbohydrate metabolism, as observed in rice plants subjected to salinity, where a drop in fructose-1,6-bisphosphatase activity led to an accumulation of sucrose, thereby increasing salt tolerance by increasing cellular osmolarity and carbon reserves [75].

Knowledge of the use of osmotic adjustment and its components (organic and inorganic osmolytes) in turf grasses remains limited [76]. In our study, the most marked impact of salt stress on morphophysiological parameters was a significant reduction in species cover during the last three months of the growing season. This reduction in the density of plant cover resulted in a deterioration in the aesthetic quality of the turf.

Species that demonstrated salt tolerance included *Agrostis stolonifera*, *Festuca arundinacea* and *Cynodon dactylon*, particularly at a concentration of 3 g/l NaCl. These species showed an ability to maintain a higher cover rate than the other species. In comparison, *Lolium perenne* proved to be the most sensitive to salt stress conditions, whatever the dose applied.

Plants react in a variety of ways to salt stress through morphological, physiological and metabolic responses, which can considerably reduce crop yield and quality [77, 78]. Plant resistance to salinity results from two complementary processes: avoidance and tolerance. Avoidance mechanisms limit the excessive translocation of salts to sensitive leaf tissues, while tolerance mechanisms increase the survival capacity of plants in the presence of salts accumulated in these tissues [79]. This involves various physiological adaptations such as root growth stimulation, osmotic adjustment, ion exclusion and regulation, and ion sequestration and excretion. These resistance mechanisms can coexist within the same species and are common to most salinity-tolerant plant species [78].

Many turfgrass species considered moderately tolerant or tolerant to salt stress often show an increase in root/stem ratio when subjected to salt stress [24]. This is explained by an increase or maintenance of rooting, while shoot growth decreases in response to salinity, which may constitute a tolerance mechanism.

Salinity resistance within the Poaceae family encompasses a wide range of salinity levels, and turfgrass species employ a multitude of resistance mechanisms. These include the maintenance of stomatal conductance, anatomical and morphological adaptations, drought and heat tolerance, signalling cascades, minimisation of Na^+/Cl^- toxicity, a high K^+/Na^+ ratio, as well as nutrient and osmotic balances, active control of ionic accumulation, and the activation of tolerant genes, accompanied by the production of osmolytes.

In response to a limited exposure to salinity levels, salt-tolerant species like halophytic plants and particularly warm season grasses maintain or increase roots growth [24, 77, 80] driving to a higher root biomass which is expected to limit the osmotic stress and salt accumulation in shoots tissues, while storing them in the roots where there are limited negative effects. The 4 studied species Bermuda grass, Saint Augustine grass, seashore paspalum and Korean lawngrass irrigated for two years with salty water with an electric conductance of 15 dS m^{-1}compared to the control irrigated with water at 2.5 dS m^{-1}. Additionally those species which maintained the roots growth, were also able to maintain the shoot quality [77].

The exclusion of salt ions from shoots, combined with osmotic adjustment, is one of the most widespread mechanisms for avoiding salt stress in C3 and C4 turfgrass species [81]. This process consists of preventing the translocation of Na^+ to the shoots to minimise the toxic effects associated with salinity [82]. Numerous studies have demonstrated a negative correlation between shoot Na^+ and Cl^- concentrations and the relative salinity tolerance of C3 and C4 turfgrass species [22, 79, 80]. This relationship has been successfully exploited to predict salinity tolerance in *Cynodon spp.* and *Zoysia spp.* [10, 83]. Various sodium exclusion mechanisms have been identified, including Na^+ efflux from roots, Na+ compartmentalisation in root cell or mesophyll vacuoles, and control of Na^+ accumulation and release by xylem parenchyma cells [82].

Roots have a developed system to regulate the uptake of water with selected ions from soil solution such as Na^+, K^+ and Cl^- which are essential elements to maintain the osmotic pressure of plant cells, which is directly linked to the capacity of the plant to absorb water. Salt tolerance has been correlated with the capacity to maintain the ratio K^+/Na^+ [79, 80]. This is possible thanks to an increased uptake of K^+ by the roots while reducing its cells leakage and reducing the transport of Na^+ from roots to shoots [80]. *Paspalum* spp, a halophytic grass retrieved in the coasts when exposed to salt present an increased level of Na^+ of 15-fold compared to non-stressed plants in the shoots, and 25-fold in the roots, associated with a decrease of K^+ of 26.02% and 69.68% respectively. Calcium ions have probed to play a key role being a secondary messenger for signaling salt stress indirectly activating membrane and tonoplast antiporters [84]. Thus, when roots are exposed to NaCL there are modifications in cytosolic calcium to increase Ca^{2+} levels. Another mechanism of resistance to salt stress is the compartmentalization of Na^+ ions in organs like vacuole, reducing their concentration in the cytoplasm, thanks to the overactivation of membrane tonoplast, thank to calcium and also some 'compatible solutes' such as proline and glycine betaine facilitate this translocation into the vacuole [79, 85]. This process protects the cell structure and the osmotic balance. Proline, besides this protecting function, contributes to membrane stability and maintains photosynthetic activity [79, 86]. On the other side, when adding glycine betaine in the solution with NaCl, *Lolium perenne* presented a reduced accumulation of Na^+, a maintained level of the ratio K^+/Na^+ and increased level of enzymes with antioxidant activities like catalases, superoxide dismutases and ascorbate peroxidase which prevents cell membrane damage [87].

5. Conclusion

The present study examined the physiological and morphological responses of six turfgrass species (four temperate and two tropical) under conditions of salt stress.

The results indicate that the most salinity-tolerant species are *A. stolonifera*, *F. arundinacea and C. dactylon*, particularly at a dose of 3 g/l NaCl. These species also demonstrated an ability to maintain a higher cover rate than the other species. On the other hand, *L. perenne* proved to be the most sensitive to salt stress conditions, whatever the dose applied. *A. stolonifera* seems to be the best adapted to this stress, particularly at the dose of 3 g/l NaCl, maintaining a high coverage rate throughout the trial. It is important to note that morphological and physiological traits are strongly affected by severe salt stress conditions (6 g/l and 9 g/l NaCl), resulting in zero value records from the fourth month of stress application. Overall, these results provide an insight into the morphological and physiological responses of turfgrass species of temperate and tropical origin to high salinity, offering an indication of their level of tolerance. These preliminary data could encourage physiologists and landscape designers to carry out field trials on a regional and national scale.

Highlights:

- *A.stolonifera*, *F. arundinacea* and *C. dactylon*, are the most salinity-tolerant species.
- The highest salinity-tolerance dose is 3 g/l NaCl.
- *A.stolonifera* is the best adapted to salt stress.
- Severe salt stress conditions resulting in zero value records.

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