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Short Communication

Effects of Salinity on Warm-season Turfgrass Species Collected in a Mediterranean Environment

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Abstract

Background and Objective: The demand for salt-tolerant turfgrass is becoming more pressing. In Italy, many turfgrass species have been introduced from foreign countries, but they have shown a low adaptability to the prevailing Mediterranean climatic conditions. The main objective of this study was to evaluate the effects of salinity on warm-season turfgrass species collected in a Mediterranean environment and exposed to salinity in hydroponic culture. **Materials and Methods:** The experiment was conducted in Italy in a temperature-controlled glasshouse, where samples of 25 different macrotherm specimens of *Cynodon dactylon* and four commercial cultivars (Transcontinental, Yukon, Panama and Seaspray) in rectangular plastic pots were exposed to saline conditions. Plants were subjected to one level of salt stress corresponding to 150 mM NaCl from the addition of NaCl. **Results:** Conditions of salinity were shown to have a depressing effect on all of the measured parameters: The leaf area, dry weight, dry weight of roots and root/shoot ratio. Salinity conditions resulted in a great increase in the leaf concentration of sodium and chlorine, but the ability of the plants to limit the accumulation of sodium ions in the leaf tissue varied enormously between the different compared accessions. **Conclusion:** The data produced in this study demonstrate a fair amount of variability in response to salinity in terms of growth among the studied ecotypes. We have identified three accessions from the turfgrass material collected in the Mediterranean area that appear to be relatively less salt-sensitive.

Key words: Salinity stress, saline ion toxicity, shoot growth, root dry weight, Mediterranean environment, *Cynodon dactylon* L.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity causes significant stress to plants. Approximately 50% of all irrigated land (230 Mha) is affected by salinity[1]. The presence of salts in the soil may arise for various reasons such as from intrinsic soil components, the use of low-quality water for irrigation or the excessive use of fertilizers[2]. In Italy, many turfgrass species have been introduced from foreign countries but they have shown a low adaptability to the prevailing Mediterranean climatic conditions[3]. The lack of certified propagation material is the main critical element of the turfgrass sector. The plant material comes almost exclusively from environments different from those of the Mediterranean and the unsatisfactory performance is due mainly to the high susceptibility to summer stress and reduced growth in winter. Therefore, the availability of salinity-tolerant genetic material has become a strong need for the sector of turfgrasses in the Mediterranean area, especially with the increasing spread of turfgrass landscapes in arid and seashore areas[2]. Indigenous species of turf are an important source of genetic variability that can provide performance advantages in terms of tolerance to salinity. In fact, some recent studies have shown the high phenotypic variability of indigenous genetic resources[4-7]. Moreover, there is still little information available on the response of warm-season turfgrasses to salinity[8-14].

In general, the mechanisms of adaptation and tolerance to salinity that a plant can exhibit can be varied. These mechanisms include root ion exclusion[15], ion compartmentalization at the plant level[16,17], salt excretion through salt glands[18] and ion partitioning in the vacuole and cell wall[19,20]. Some warm-season turfgrasses are known to be salt tolerant[9-12,21] and some species are classified as halophytes[22]. Halophytes have developed various mechanisms of adaptation to salinity, including osmotic adjustment through ion partitioning in the cell vacuoles, the accumulation of compatible organic solutes, succulence and salt secreting glands and bladders[18,23,24]. Some turfgrass species also have salt glands from which they eliminate excess saline ions by excretion[25,26]. These structures are epidermal and bicellular, comprising a basal cell inserted into the epidermis leaf and a cap cell covered with a continuous cuticle[27]. From leaf tissue, salt is transported through the small cell to the swollen stalk of the vesicular cell. Over time, the salt concentration increases, eventually causing the cell to burst and release its contents. In the *Poaceae* family, roughly thirty species are equipped with the ability for excretion and among these is *Cynodon dactylon*. Usually, the excretion rate of Na^+ and Cl^- ions is correlated to the tolerance and is negatively correlated to the tissue concentration of these ions[22]. Physio-morphological and structural changes in bermudagrass showed the ability of this turfgrass to prevent water loss and improve tolerance under salinity conditions by bulliform cell formation[28]. Absolute salinity tolerance of turfgrasses does not exist due to the high number of factors involved (soil, environment, plant age, etc.) therefore, to reduce the impact of these factors on turfgrass response to salinity, many experiments have been undertaken with hydroponic solutions under controlled conditions[21,22]. An understanding of the growth response of turfgrass to salinity could be useful for screening and breeding the grasses for higher salt tolerance[29]. The main objective of this study was to evaluate the effects of salinity on warm-season turfgrass species collected in a Mediterranean environment, using plants exposed to saline conditions through hydroponic culture.

MATERIALS AND METHODS

Growing conditions and treatments: The experiment was carried out at the University of Basilicata, Italy (40°N, 15°E), in a temperature-controlled glasshouse with plants being grown in rectangular plastic pots (0.32×0.32 m 0.15 m height) with a volume of 15 L. On 29 April, 2013, samples of 25 different macrotherm specimens of *C. dactylon* (bermudagrass) ecotypes and 4 commercial cultivars (Transcontinental, Yukon, Panama and Seaspray, Table 1) were exposed to conditions of salinity. The ecotypes of bermudagrass had been collected and studied from a morphological point of view in a previous study[5]. The specimens were collected in different coastal and sub-littoral sites characterized by Mediterranean climate with mild winters and hot summers. Stolon fragments were transplanted in honey comb arrangements of Styrofoam containers that were filled with a small quantity of peat. These containers were held at the top of the plastic containers containing 15 L of aerated Hoagland nutrient solution (EC = 2.3 dS m^{-1}, pH = 6.0) formulated with tap water[30]. Solution contained the following nutrients (indicated in mmol L^{-1}): NO_3^- 13.5, NH_4 1.5, PO_4^{3-} 1.0, K^+ 6.0, Ca^{2+} 5, Mg^{2+} 2.0 and SO_4^{2-} 2.0. Styrofoam containers were submerged in the solution to the level of the peat surface. Loss of nutrient solution was compensated by a weekly substitution. The pH of the nutrient solution was adjusted daily to 6.5-7, the solution was constantly aerated and maintained at a constant volume. An automated heating system started each time the air temperature dropped below 18°C, whereas the greenhouse roof opened as soon as the temperature

Table 1: Bermudagrass accessions collected from different regions of Italy

Id	Species	Accession	Region
1	*Cynodon dactylon*	P_P2	Puglia
2	*Cynodon dactylon*	Va	Abruzzo
3	*Cynodon dactylon*	Vb	"
4	*Cynodon dactylon*	Vc	"
5	*Cynodon dactylon*	Ve	"
6	*Cynodon dactylon*	Vf	"
7	*Cynodon dactylon*	Vg	"
8	*Cynodon dactylon*	1R	Basilicata
9	*Cynodon dactylon*	2R	"
10	*Cynodon dactylon*	8R	"
11	*Cynodon dactylon*	G1	Puglia
12	*Cynodon dactylon*	G2	"
13	*Cynodon dactylon*	G3	"
14	*Cynodon dactylon*	3	Basilicata
15	*Cynodon dactylon*	4	Lazio
16	*Cynodon dactylon*	5	Calabria
17	*Cynodon dactylon*	C_O1	Puglia
18	*Cynodon dactylon*	C_O1bis	"
19	*Cynodon dactylon*	AZ_P5	Basilicata
21	*Cynodon dactylon*	Pa4	"
22	*Cynodon dactylon*	A1	Campania
23	*Cynodon dactylon*	A2	"
24	*Cynodon dactylon*	A3	"
25	*Cynodon dactylon*	A6	"
26	*Cynodon dactylon*	A7	"
27(1)	Commercial cv Panama	-	-
28(2)	Commercial cv Transcontinental	-	-
29(3)	Commercial cv Yukon	-	-
36(4)	Commercial cv Seaspray	-	-

exceeded 28°C. An automatic weather station was placed in the greenhouse to measure meteorological data. After 10 days of establishment and turf adjustment to the greenhouse environment, the salt treatment began. Plants were subjected to one level of salt stress, 15 dS m^{-1}, corresponding to 150 mM NaCl through the addition of NaCl (commercial salt). A control treatment was maintained at a level of 2.3 dS m^{-1} as electrical conductivity salt, but with 0 mM NaCl. Each experimental treatment was replicated three times, with the pots being arranged according to a randomized block design with a factorial scheme. In each pot, there were 3 plants for a total of 9 plants per experimental treatment. To avoid osmotic shock to the plants, the NaCl was gradually added to the nutrient solution.

Data collection: Growth measurements were carried out during the experiment and the following morphological parameters were measured biweekly with a digital calliper: Leaf length (l), leaf width (L) and distance between the internodes of the latest fully expanded leaf (i) with five measurements per characteristic plant. Measurements were taken at regular intervals of 14 days. After 60 days, the plants were harvested and the dry matter and leaf number were obtained and counted, respectively. Total dry matter (hypogeous and epigeous) was obtained by drying the samples in a ventilated oven at 75°C until achieving constant weight. The leaf area was measured at the end of the experimental trial with a surface electronic detector (Model 3100, LI-Cor, Inc., Lincoln, NE, USA). At the end of the experiment, the Na^{+} and Cl^{-} ion concentrations in the leaf tissue were measured. Samples were oven dried at 70°C and finely ground. A sub-sample of leaves from each the two treatments was dried, ground and extracted in HNO$_3$ (65% v/v) to measure Na^{+} concentrations in the leaf extracts using a flame spectrophotometer (Flame spectrophotometer, Varian 220 FS). Another sub-sample was ashed at 600°C for one night and sub-samples of dry matter were used for the extraction of Cl^{-}, using a carbonate and sodium bicarbonate solution. The Cl^{-} was measured by titration with a silver nitrate solution.

Statistical analysis: The experimental design was a factorial randomized block with three replications. Bartlett's test was applied to establish the homogeneity of the variance and the data were then subjected to an analysis of variance (ANOVA). The experimental results underwent statistical analysis using Sigma Plot 11.0 for windows (Systat software Inc., San Jose, CA, USA). Significant differences were identified by Tukey's test, with levels of significance of 5 and 1%.

RESULTS AND DISCUSSION

Growth responses: The conditions of salinity had a depressing effect on all measured parameters (leaf area, dry weight, root/shoot ratio, etc.) as widely shown in Fig. 1 and Table 2. The leaf area on average was reduced to 296 cm^{2} from 824 cm^{2}, whereas the dry weight on average was reduced to 4.5 from 8.1 g plant^{-1}. A significant effect of salinity was observed on the dry matter of all studied accessions and a significant difference was observed among the accessions regarding the ability to support the salts provided in the nutrient solutions (Table 2). The interaction effect (salinity × accession) on the leaf area parameter (Table 2) was also significant. The root to shoot ratio on average, increased as an effect of the conditions of salinity, as expected. This ratio increased on average to 0.55 g g^{-1} from 0.33 g g^{-1}. For this parameter, only salinity resulted in a significant effect (Table 2). In some cases, the salt treatment increased the root/shoot ratio as an effect of the different dry matter allocation among the different parts of the plants (Table 2). The dry weight percentage reduction was also calculated with respect to the control for all accessions that were exposed to

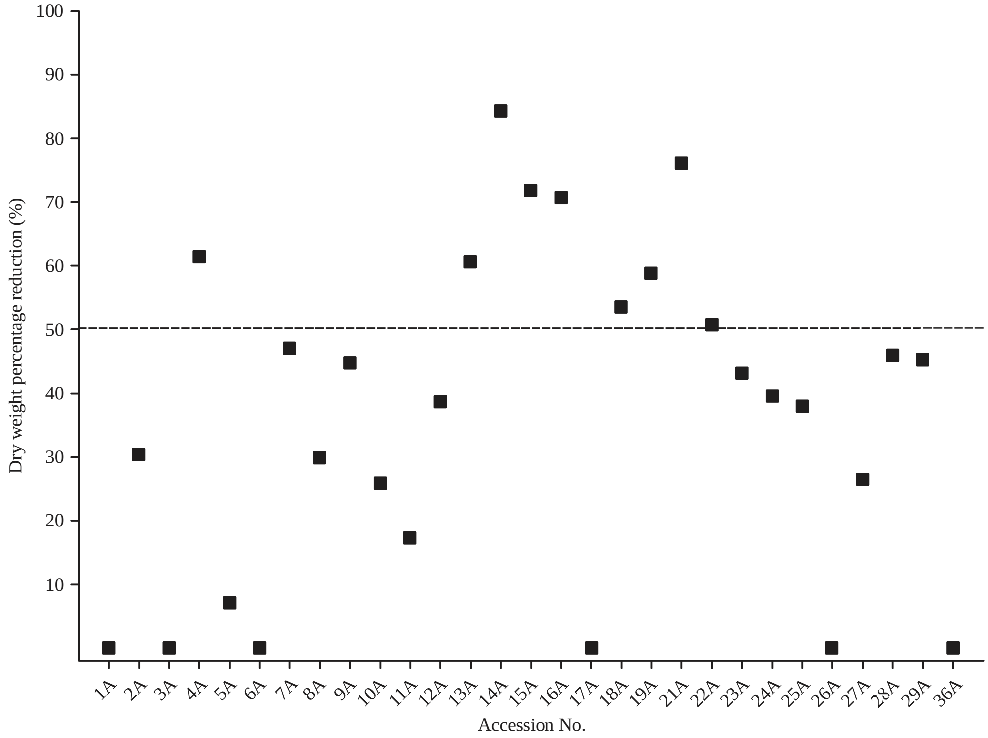

Fig. 1: Dry weight percentage reduction respect to the control calculated in all 29 accessions exposed to salinity

Table 2: Summary of the statistics for dry weight, leaf area and root to shoot ratio of bermudagrass accessions exposed to salinity

Parameters	Dry weight (g)	Standard error	Leaf area	Standard error	Root/shoot	Standard error	Na^+ (mg g^{-1})	Standard error	Cl^- (mg g^{-1})	Standard error
Salinity	**	0.48	**	36	**	0.02	*	0.22	*	0.31
Accession	**	1.44	**	139	n.s	-	**	1.66	n.s	-
Salin×Acces	n.s	-	**	196	n.s	-	**	2.33	n.s	-

**Highly significant (p<0.01), *Significant (p<0.05), n.s: Not significant

the salinity, as shown in Fig. 1. As seen from this Fig. 1, 9 accessions show a dry weight percentage reduction greater than 50%, which means that they have a low tolerance to conditions of salinity because their growth processes and dry matter accumulation were strongly compromised. The remaining 19 accessions, while having growth process reductions of less than 50%, show great variability in terms of percentage reduction (Fig. 1). A lower percentage reduction in growth was found in the accession numbers 1, 3, 6, 17 and 26, as well as in the commercial cultivars (accession No. 27, 28, 29 and 36).

Uptake of Na^+ and Cl^-: Salinity conditions have resulted in a great increase in leaf concentrations of sodium and chlorine. In general, the Cl^- concentration was higher than the Na^+ concentration in the salinized treatments. The Cl^- leaf tissue concentration on average, increased to 25.2 mg g^{-1} compared to the control, where the Cl^- concentration was found on average to be 9.8 mg g^{-1} . The maximum Cl^- concentration (43.9 mg g^{-1}) was measured in accession No. 9. There were no significant differences between accessions with regard to Cl^- uptake (Table 2). The Na^+ leaf tissue concentration increased on average, 12.0 and 1.2 mg g^{-1}, respectively, for the treatment and control plants. The maximum Na^+ leaf tissue concentration (30.3 mg g^{-1}) was measured in accession No. 9. The minimum Na^+ leaf tissue concentrations (3.8 and 6.1 mg g^{-1}) were measured in accession No. 25 and 7. The ability to limit the accumulation of sodium ions in the leaf tissue varied enormously among the different accessions (Table 2).

Salinity tolerance is known to be a complex trait[31]. Absolute tolerance of turfgrass accessions cannot be determined due to the complexity of the response to salinity and the many factors involved. For turfgrass species in conditions of salinity, osmotic adjustment is a common response, but only tolerant genotypes show shoot saline ion exclusion (Na^+ and Cl^-) together with a minimum but adequate osmotic adjustment[22,32]. Moreover, the use of different criteria to measure the salinity tolerance complicates comparisons between turfgrass species[22]. Examples of such criteria are as follows: Shoot weight[33], shoot weight reduction relative to non-salinized plants[34], root weight or length[35], shoot/leaf length[36], shoot visual injury[37], plant survival[38] and seed germination[39]. In this experiment, the significant reductions in dry matter, leaf area and root/shoot ratio is in agreement with results from other researchers who have highlighted the close relation between sensitivity to salinity and a severe reduction in growth processes[31,40]. The less compromised the growth processes are, the greater the salinity tolerance of the genotype is considered to be[22].

Recently, the critical roles of biomass partitioning and of root growth and morphology in crop adaptation to salts were shown[41]. Obviously, turfgrass species are different from crops, but root growth in turfgrass species was studied to understand the physiological modifications of the grass in response to salinity[35,40]. The first effect of salinity is experienced by the plant in the rhizosphere, for this reason, selection criteria for salt tolerance should include root measurements[40]. In this experiment, despite having measured a significant increase in the root/shoot ratio (Table 2), we did not observe significant differences in the effect of salinity on this parameter among the different studied accessions. According to Marcum[22], 50% total dry weight reduction can be used as the reference point for comparisons of salinity tolerance among turfgrass species. As can be seen clearly from the data shown, only some of the studied accessions showed a reduction of at least 50% and in some cases, no reduction in the growth rate was observed, as in the cases of ecotype numbers 1, 3, 6, 17, 26 and 36 (Fig. 1). These results show that the high genetic variability found within the populations of the genera *Cynodon* that we collected in the Mediterranean area is in agreement with the variability observed by Romani *et al.*[42].

CONCLUSION

The data produced in this study demonstrate that there is a fair amount of variability in the responses of the studied ecotypes to salinity in terms of growth. Obviously, this study represents an initial screening of plant material collected in the Mediterranean environment. We have identified three accessions from the turfgrass material collected in the Mediterranean area that appear to be relatively less salt-sensitive. They could be used in breeding programmes to obtain salt tolerant bermudagrass variety for the Mediterranean area. Further studies are needed to better characterize the above ecotypes of *C. dactylon* before proposing them to the turfgrass market and discouraging the use of alien species in Mediterranean environments.

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