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EFFECTS OF DIFFERENT SALINE WATER QUALITIES ON THE ESTABLISHMENT OF ZOYSIAGRASS (*Zoysia tenuifolia* WILLD.)

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Abstract

Salinity of irrigation water is one of the main environmental constraints limiting the geographical distribution of several species. In order to understand and to cope with critical environmental constraint, a pot experiment was undertaken to investigate the potential effect of irrigation with various natural saline waters gathered from arid and semiarid regions; on rooting of stem cuttings, growth and on the physiological responses of Mascarene grass (Z*oysia tenuifolia* Willd.). The obtained results indicate that rooting of stem cuttings was successfully established even under a relatively high salinity. Moreover, growth under saline conditions was not inhibited as indicated by the no significant effect of the various treatments applied neither on aerial nor on root dry weights. Although the continued irrigation of Z*oysia* plantlets with saline waters, the photochemical efficiency of PSII and the Na^+ and K^+ uptake were not altered. Platelets kept almost a K^+/Na^+ ratio > 1. Results suggest that *Zoysia* tolerate the various applied treatments which will ease their agro management and optimize their production in arid and semi arid regions.

Key words: nutrient balance, saline irrigation waters, stem cuttings, *Zoysia tenuifolia*

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1. Introduction

Water scarcity and salinity are major global environmental problems, especially in arid regions which commonly encounter these problems (Glenn et al., 1997; Perulli et al., 2019). Worldwide, more than 20% of the cultivable land is affected by salinity. Due to climate change and anthropogenic activities, the salt affected area is tended to increase continuously (Munns and Tester, 2008).

It has been shown that the Mediterranean region face an ever increasing aridity basically due to the over years exacerbated climate changes (Chaves et al., 2009). In addition, the swift increase of urbanization in arid and semi arid regions was not favorable and has created a rude competition for fresh potable water. This contributed to its turn to a drastic increase in the use of non conventional water and saline water for turfgrasses irrigation which constitutes an environmental limiting factor for these species.

Salinity affects different physiological and metabolic processes of plants. The responses to high salt concentrations are often accompanied by a variety of symptoms, such as the decrease in leaf area, increase of leaf thickness and succulence, abscission of leaves, necrosis of root and shoot, and decrease of internode lengths (Kozlowski, 1997; Masina et al., 2019; Parida and Das, 2005). Turfgrasses are recognized as a prominent element of the urban landscape and are widely used to cover parks, lawns and sports fields. The use of turfgrasses offers numerous functions besides aesthetic effects, which are suitable to maintain mental health, turf grasses are also utilized in slopes stabilization (Raven et al., 1999). Drought is a result of decrease of water potential due to higher solute concentration, and therefore higher energy is required to the plant to

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absorb water itself. Root zone compaction is a result of soil structure decay due to excessive amount of Na usually which affect negatively turf quality (Carrow and Duncan, 2011).

Thus, the demand of salt-tolerant turfgrass species and cultivars is needed and valuable for maintaining grass cover in ideal environments in where salinity is a problem or where limited or no fresh water is available for irrigation the saline areas (Dudeck and Peacock, 1984; Harivandi, 1992). One strategy to enhance turfgrasses survival and recovery from salt stress is to use cultivars with superior salinity tolerance (Ashraf, 1994; Flowers and Yeo, 1995; Glenn et al., 1999). The physiological responses of turfgrasses to salt stress are often complex and usually multi-faceted, which makes experiments difficult to design first and then to interpret. In order to optimize salinity tolerance of turfgrasses several criteria could be considered and utilized tolerance namely shoot and root, shoot weights reduction relative to a non-saline control, visual scores of salinity injures such as leaf firing, plant survival, and seed germination (Marcum, 1999). Zoysiagrass is one among the prominent turfgrasses in USA originated from the orient and has been considered as a salt tolerant turfgrass (Harivandi et al., 1992). However, certain variability for salt tolerance was detected among *Zoysia* grass species and genotypes. This variability is mostly attributed to its notable geographical distribution and also attributed to the diversification in genotypes and species (Marcum et al., 1998).

However, little is known about Mascarene grass (*Zoysia tenuifolia* Willd.) behavior under saline environment. The objectives of this research was to study the establishment, the growth and the physiological responses of a newly introduced Mascarene grass in Mediterranean region namely Tunisia under four water salinity levels and to determine thereby the extent of its salinity tolerance.

2. Material and methods

2.1. Plant material and growth conditions

A pot experiment was conducted in a greenhouse located at the National Institute of Agronomic Science of Tunisia. Stem cuttings of Mascarene grass (Zoysia tenuifolia Willd.) about 10 cm long were grown in 1 litre pots at a rate of 5 cuttings per pot. The substrate was composed of 1/3 peat, 1/3 sand and 1/3 topsoil. The average day temperature, humidity and light intensity of the greenhouse were 28.5-39.5°C, 70 % and 1500-2000 lux respectively.

Stem cuttings were treated with four waters collected from different regions of Tunisia. Saline waters used for irrigation in four regions of Tunisia were pumped from aquifers, gathered and stored in specific plastic. After analytical analysis (Table 1 and 2), each pot was irrigated with an irrigation volume of saline water (75 ml) twice a week throughout the experiment (35 days).

The experimental design was a randomized complete block with five replicates for each treatment for each block. The pots in the greenhouse were divided into three blocks where treatments were randomized. A total of three replicates were chosen for each physiological measurement (at an average of five stems per replica).

Table 1. Analytical methods for waters analysis

Parameters	*Analytical methods*	*References*
pH	Electrochemistry	ISO10523 (2008)
Conductivity	Electrochemistry	NF EN 27-88 (1994)
Calcium, magnesium, sodium, potassium, chloride, copper, iron manganese, nickel, zinc, chromium, arsenic, selenium, boron	Atomic emission-ICP	ISO 11885 (2007)
Mercury	Atomic emission-ICP	NF EN 1483 (1997)
Fluoride, Chloride	Hydride generation atomic absorption spectrometry	ISO 10304-1 (2007)

2.2. Estimation of plant growth, rooting rate and Fv/Fm ratio

At the end of the treatment period Mascarene grass growth was estimated. Root systems were carefully uprooted and the rooting rate percentage was estimated. Immediately after rooting rate estimation, roots and aerial parts were separated then putted in sacks and left to dry in a drying oven at 70 °C then weighed for dry mass. Efficiency of Mascarene grass PSII under saline conditions was determined by mean of a non-destructive parameters; chlorophyll fluorescence and measured on a fully expanded leaf using a portable fluorometer OS5p+ - Chlorophyll Fluorometer - Opti-Sciences.

Measurements were performed on 3 leaves considered as a replicate and 3 replicates were adopted per treatment. Each leaf was dark-adapted using cuvette clips. F_v/F_m was determined following established procedures (Maxwell and Johnson, 2000). By means of the fluoremeter the initial fluorescence (F0) was measured before excitation of the reaction centres of photosystem II (PSII).

After excitation of (PSII) by a saturation light pulse, the maximal fluorescence (Fm) was measured. The efficiency of the photosystems (Fv/Fm) can then be calculated where Fv=Fm-F0. The greenhouse experiment design was randomized block design with 4 treatments and 3 repetitions.

2.3. Soluble protein measurement

Following Bradford (1976) method, total soluble protein content was determined after 15 days of irrigation with saline waters; bovine serum albumin was used as a standard.

Table 2. Irrigation waters composition

Parametres	*Treatments*			
	T0 (Fresh water)	*T1 (INAT)*	*T2 (Chott-Meriem)*	*T3 (Medenine)*
pH	8.05 - 24.0°C	7.70 - 23.6°C	8.40 - 24.8°C	8.15 - 25.0°C
Conductivity(dS/m)	1.22	3.40	4.64	7.72
Fluoride (ppm)	0.10	1.14	0.79	1.74
Chloride (ppm)	216	776	1.15	2.09
Calcium (ppm)	94.1	219	263	670
Magnesium (ppm)	27.8	91.9	203	310
Sodium (ppm)	104	475	594	$1.03\ 10^3$
Potassium (ppm)	4.38	7.97	2.02	28.6
Cadmium (ppm)	0.002	0.006	0.003	0.003
Copper (ppm)	<0.01	<0.01	<0.01	<0.01
Iron (ppm)	0.027	<0.01	<0.01	<0.01
Manganese (ppm)	<0.01	<0.01	<0.01	<0.01
Nickel (ppm)	<0.01	<0.01	<0.01	<0.01
Zinc (ppm)	0.110	<0.01	0.011	<0.01
Chromium (ppm)	<0.01	<0.01	0.011	<0.01
Arsenic (ppm)	<0.005	<0.005	<0.005	<0.005
Selenium (ppm)	<0.005	<0.005	<0.005	<0.005
Boron (ppm)	0.110	0.793	1.05	1.14
Mercury (ppm)	<0.0002	<0.0002	<0.0002	<0.0002

Frozen fresh leaves samples were homogenized at 4°C in mortal with 100 mM potassium phosphate buffer (pH 7.8) containing 0.1% (w/v) PVPP. The obtained homogenate was centrifuged then (14,000×rpm for 30 min at 4°C) and the supernatant was collected and used to determine protein content.

2.4. Na^+ and K^+ contents measurement

As a destructive parameter, Na^+ and K^+ contents were determined at the end of the experiment, aerial parts were harvested, oven-dried (70°C, 48h) and finally powdered. The powdered Mascarene grass aerial parts were then incubated for 12 h in a mixture of a concentrated nitric acid (HNO_3) and chlorate acid ($HClO_3$) and then completely digested (300°C, 6h). Ions contents were measured by means of a flame photometer (ICPES, Flame Photometer 410, and Sherwood, UK).

2.5. Statistical analysis

Analysis of variance was performed for all measurements with the Statistical Analysis System 9.0 (SAS) software (Chicago, IL, USA). Differences between means were tested using Least Significance Difference (LSD) test at 0.05.

3. Results and discussion

Results related to rooting percentage indicates that all Mascarene grass cuttings irrigated with the four saline waters remained viable and exhibited a rooting rate relatively superior to 60% with no significant differences between control and treated cuttings (Table 3). The recorded average across treatments is ordered respectively by 76 % (T0), 68 % (T2) and 63 % (T1 and T3). The rate of turfgrasses establishment is very important, especially in areas subject to abiotic stress like water salinity. That present results showed that Mascarene grass may be used to provide rapid cover when irrigated with relatively saline water. Most turfgrasses adapted in the different regions in Tunisia are established vegetative from sod or stolons. Root development is a very important factor, not only for the establishment of the turfgrass but also on its tolerance to traffic and wear, especially when used in playgrounds. The general effect of salinity in plants is reduced growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves (Shannon and Grieve. 1999). This indicates that under saline conditions, the vegetative establishment of Mascarene grass is estimated to be possible.

After the stress period (35 d), aerial and root dry weight showed no significant variation between treatments. The recorded values varied from 0.15 to 0.25 g DW. It has been described by many researchers that growth as result of several physiological mechanisms was inhibited and this after exposure to salt stress (Munns, 2002). In our study, no evident symptoms of salinity were observed in Mascarene grass growth. The obtained results could be considered as a primary indicator of salinity tolerance. Chlorophyll fluorescence was measured to reflect electron transport in the photosynthetic system of Mascarene grass plants.

Table 3. Results of ANOVA testing the effects of saline waters on the different tested parameters

	Rooting rate	*Aerial dry weight*	*Root dry weight*	*F0*	*Fv/Fm*	*Protein content*	*K^+ content*	*Na^+ content*	*K^+ / Na^+ Ratio*
Treatments (T)	ns	ns	ns	ns	ns	***	*	ns	*

** Significant at the 0.05 probability level. *** Significant at the 0.001 probability level. ns, not significant at the 0.05 probability level*

Abiotic stress such as salinity is known to have an influence on different plant fluorescence parameters (Osorio et al., 2012). In the present study, fluorescence parameters showed no significant differences among treatments. After 35 days of exposure to stressful conditions, all Mascarene grass previously exhibited an *Fv/Fm* ratio close to 0.8 indicating that the applied salt treatments did not alter the performances of Mascarene grass PSII. Considering the fact that turf color is of paramount importance to turfgrass it is worth note that Mascarene grass kept almost a bright color throughout the experiment. Our supporting results, include those of Schiavon et al (2011) and Schiavon et al (2012) with their study on warm- and cool-season turfgrasses namely bermudagrass and seashore paspalum. They found that the irrigation with saline from a subsurface drip irrigation system turfgrasses are capable to maintain adequate turf quality.

Significant changes in soluble protein content in leaves of Mascarene grass were observed after a short term (15 d) of irrigation between the different levels of salinity treatments (Fig. 1).

In response to T1 (3.40 dS/m) and T2 (4.64 dS/m) soluble proteins were accumulated. While protein content in Mascarene grass plantlet treated with T3 (7.72 dS/m) was not affected compared to control and exhibited relatively similar protein content. In our investigation, the accumulation of protein observed could be attributed to the tolerance level exhibited by Mascarene grass plantlets to the applied treatments. Omar et al. (1993) reported that exposure to sodium chloride (NaCl) stress negatively affect protein synthesis and lead to a decrease of soluble protein content. Thus, in order to overcome stress effects and thereby protect their cells plants accumulate proteins (Wang et al., 2003).

A certain number of physiological disturbances are generally observed under salt stress which include a decrease of osmotic and turgor potential, ion toxicity, and a nutritional imbalance (Alshammary et al., 2004). It has been pointed out that the ion toxicity noted under salinity stress is associated with the excessive uptake mainly of Na^+ and could lead to the inhibition of plant growth by disturbing the normal function of photosynthesis (Muhling et al., 2002). Selective transmembrane ion redistribution and regulation of Na^+ and K^+ across plant cells is considered as a prominent factor directly involved in salt tolerance of plants (Du-Pont, 1992; Ashraf and Harris, 2004). Going through the data presented in Fig. 2, it appears that at the end of the stress period Na^+ was not accumulated ($p > 0.05$).

These observations may be linked to the capacity of the aerial part of Mascarene grass to compartmentalize easily the Na^+ ions under salt stress and thereby contributing to an osmotic adjustment under stressful conditions. Qian et al (2000) studies support our findings. They noticed that Na^+ content in 29 zoysiagrass (*Zoysia* spp.) measured under salt stress was varietal dependent. They found that some cultivars of *Zoysia* accumulated Na^+ in their shoot while other cultivars exhibited a lowest decrease in Na^+ content and have attributed this variability to a relatively higher capacity of these cultivars to eliminate Na^+. Marschner (1995) reported that K^+ has a prominent role not only in plant growth and development but also in osmotic adjustment and cell turgor maintenance. In plants, this cation counterbalances the negative charge of anions, and intervenes crucially in enzymes activation implicated in the metabolism and proteins and carbohydrates synthesis and also plays an important role in the regulation of stomata movement (Marschner, 1995).

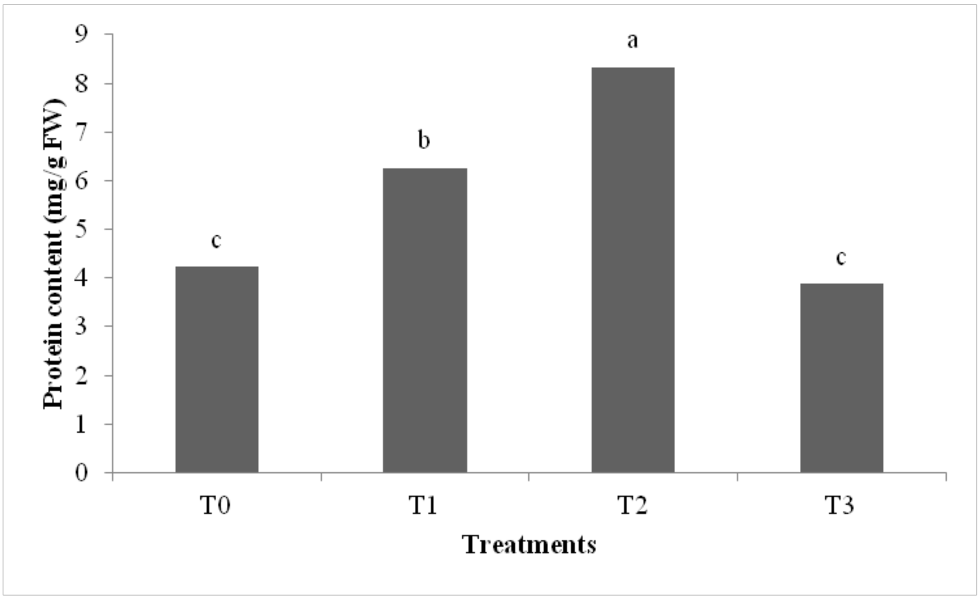
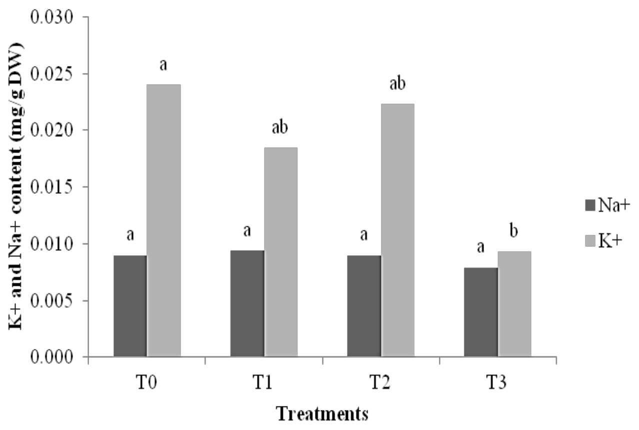
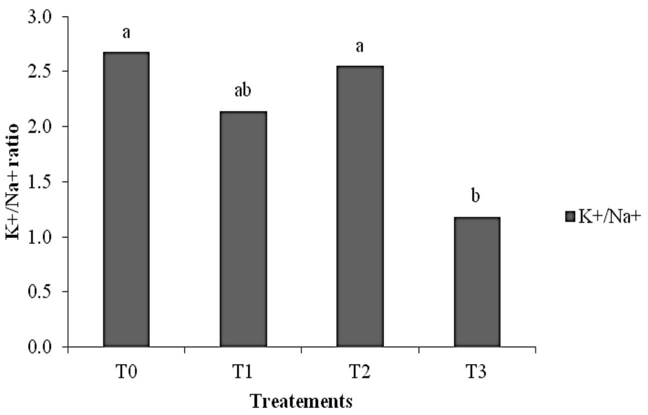


Fig. 1. Soluble protein content of Mascarene grass during 15 days of irrigation with saline waters; T0 (1.22 dS/m), T1 (3.40 dS/m), T2 (4.64 dS/m) and T3 (7.72 dS/m). Different letters in the same column indicate significant differences ($p < 0.05$)



(a)



(b)

Fig. 2. Na^+, K^+ content (a) and K^+/Na^+ ratio (b) of Mascarene grass during 35 days of irrigation with saline waters; T0 (1.22 dS/m), T1 (3.40 dS/m), T2 (4.64 dS/m) and T3 (7.72 dS/m).
Different letters in the same column indicate significant differences ($p < 0.05$)

According to the statistical analysis a significant difference was noticed in aerial K^+ contents of *Zoysia*. Under high salt treatment T3 (7.72 dS/m) a drastic decrease ($p < 0.05$) with 61.1% comparing to control was noticed, which may be considered as a potential disturbance of the membrane systems of *Zoysia*. Moreover, K^+ uptake content in aerial part was accumulated and did not show significant differences between treatments T1 (3.40 dS/m), T2 (4.64 dS/m) and control.

This accumulation under stressful conditions could be considered as one character of the plant adaptation. Especially that K^+/Na^+ ratios in all treated aerial parts were > 1 which suggesting also a better adaptation ability of Mascarene grass to the applied salt stress. To strength our suggestions, Wyn Jones, (1981) affirmed that under salt stress a K^+/ Na^+ ratio of >1 in mesophytes plant organs was the required ratio needed to ensure normal functioning. Maathuis and Amtmann, (1999) distinguished a relatively high K+ /Na+ ratio in the leaves as a marker of salt tolerant.

4. Conclusions

The identification of salt tolerant turfgrass cultivars suitable for Mediterranean region climate is challenging due to scarcity of fresh water for irrigation. One of the most urgent strategies that should be applied is the use of salinity tolerant turfgrasses and this due to the increasing use of effluent or poor quality waters for turfgrass irrigation in Tunisia.

Irrigation of Mascarene grass cuttings during 35 days of culture with saline water that reaches 5300 ppm had no effect on these parameters. Mascarene grass could be considered as a tolerant species, however, further experiment are necessary to assess the plant salt tolerance in different soil conditions for stress extended duration.

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