



Interactive effect of salinity and zinc stress on growth and photosynthetic responses of the perennial grass, *Polypogon monspeliensis*



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ABSTRACT

A glasshouse experiment was designed to investigate the interactive effect of zinc from 0 to 300 ppm and/or 150 mM NaCl on the growth and the photosynthetic apparatus of *Polypogon monspeliensis* from two Tunisian provenances (North and South) seedlings by measuring Growth, photosynthesis, chlorophyll fluorescence and chlorophyll concentration after 30 days of treatments. These parameters were severely reduced by this heavy metal. The zinc excess involves the degradation of photosystem II and the chlorophyll decomposition. However, these effect of Zn concentrations alleviated by 150 mM of NaCl. Adequate Zn also prevents uptake and accumulation of Na and Cl⁻ in shoot, by increasing membrane integrity of root cells. Our results show that at the same time, the variation of these parameters depends on the seeds origin, Zn stress and saline conditions.

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1. Introduction

Heavy metal pollution in the environment is a major ecological concern due to its impact on human health through the food chain (Thounaojam et al., 2012). Among metals, zinc (Zn) is an essential micronutrient required for many enzymes involved in numerous physiological and metabolic processes of plants. Zn is also known to have a stabilizing and protective effect on the biomembrane against oxidative and peroxidative damage, loss of plasma membrane integrity and also alteration of the permeability of the membrane (Bettger and O'Dell, 1981). Moreover, Zn can alleviate other metal-induced oxidative stress (Upadhyay and Panda, 2010). However, the excess of Zn in organisms can lead to toxicity including nutrient imbalances, growth inhibition, leaf chlorosis and photosynthesis impairment (Todeschini et al., 2011; Cambrolleí et al., 2012). Thus, Zn contaminated soils cause injury to soil microorganisms, reduce crop yield and hence are dangerous for food chains (Hassan and Aarts, 2011). Despite this, certain plant species have evolved heavy metal tolerance and can grow in Zn-contaminated soils, remain-

ing unaffected by the elevated Zn levels (Ernst et al., 2000). For example, it is well known that salt-marsh plants can tolerate and accumulate high contents of heavy metals (e.g. Mateos-Naranjo et al., 2008a). Weisany et al. (2014) found that the application of zinc on salinity stress exposed plants has a positive effect on plant growth and increasing of the K, P, Ca, Fe, and Zn concentration and Ca/Na ratio.

Polypogon monspeliensis (L.) Desf. (Annual rabbits foot grass; Poaceae) is an annual grass that expands episodically in high salt marshes of southern California. When rainfall is sufficient to lower soil salinities, the numerous seeds of this species germinate and establish seedlings (Callaway and Zedler, 1998; Kuhn and Zedler, 1997). Since annual grasses are not a natural component of this community, its presence is obvious during the dry summer, when its pale dead stems contrast with evergreen halophytes. In experiments, *P. monspeliensis* produced higher biomass than a native associate, *Salicornia virginica* (Callaway and Zedler, 1998). This species is potentially useful as an ornamental, food and forage (Khan and Qaiser, 2006). Yet, how abiotic stresses impact this species is still poorly documented. Mahmood et al. (1996) reported that 100 mM NaCl salinity led to a drastic reduction in seed germination of *P. monspeliensis* originating from Pakistan. Interestingly, in Tunisia the geographical distribution of this species extends from the humid bio-climatic area to the Sahara. In several

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regions, *P. monspeliensis* grows in sediments featuring extremely high concentrations of metals. These are produced from industrial activities like mining, smelting, refining & manufacturing process. The industries discharge their effluents into coastal water bodies and contribute to a variety of toxic substances on living organisms in food chain (Dembitsky, 2003) by bioaccumulation and bio-magnification (Manohar et al., 2006). To date, the physiological impact of elevated concentrations of metals on this species remains unknown; however, this knowledge is necessary in order to understand its limits of phytotoxicity and, ultimately, its potential for use in the forage or phytoremediation of areas contaminated by metals. Many publications report on the effect of Zn or salinity of poacee plants, but there limited work dealing with the interaction of Zn/salinity on plants. According to Pandey et al. (2015), plants activate a specific and unique stress response when subjected to a combination of multiple stresses. These combined constraints may cause a variety of plant responses which can be additive, synergistic or antagonistic.

The main objectives of the present study were to understand some of the physiological mechanisms involved in the response of plants to a combination of stresses, we investigate the effect of Zinc and/or moderate salinity (150 mM NaCl) on the photosynthetic apparatus (PSII chemistry), gas exchange characteristics, photosynthetic pigments, mineral matter (ash), calcium, magnesium, phosphorus and nitrogen accumulated in *P. monspeliensis*.

2. Materials and methods

2.1. Plant material

2.1.1. Seed collection, sowing and seedling pretreatment

Fully ripened seeds of *P. monspeliensis* from two Tunisian provenances were harvested from July to August 2012 and stored at 4 °C (in darkness) for six months. After that, seeds were sown in plastic pots (11 cm of diameter) filled with perlite and placed in a glasshouse with controlled temperature of 21–25 °C, 40–60% relative humidity and natural daylight (minimum and maximum light flux: 250 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). Pots were carefully irrigated with 20% Hoagland's solution (Hoagland and Arnon, 1938) as necessary during one month. All the pots received the same irrigation.

2.1.2. Stress treatments

After one months of seedling culture, plants were treatments with Zn or Zn/salt (ten pots per treatment): 0, 100 and 300 ppm Zn. The treatment with 0 ppm Zn was considered the control treatment. Zinc treatments were established by combining 20% Hoagland's solution and ZnSO₄ of the appropriate concentration. The control, 0 mmol l⁻¹ Zn treatment, had exactly 0.002 mmol l⁻¹ Zn, as Hoagland' solution contains a small amount of Zn as an essential trace nutrient. Salt treatments were uniform (150 mM NaCl) were added to the pots with or without Zn treatment.

2.2. Chemical analysis of plant samples

At the of experiment, four and six entire plants (roots and shoot, respectively) from each treatment were dried at 80 °C for 48 h and then weighed, ground with a porcelain grinder, and then used for nutrient and heavy metal analyses. For the anion analysis, *P. monspeliensis* roots and leafs were dried, diluted, and injected into a Dionex-D-100 ion chromatograph. An ionpac AS 124–4 mm (10–32) column and AG 14 (4350 mm) guard column were used. The flow rate was 1 ml m⁻¹, with 0.5 mM Na₂CO₃ and 0.5 mM NaHCO₃ as eluent. The anion concentration was measured with a conductivity detector and quantified with Chromleon/Peaknet 6.40 software by comparing peak areas with those of known standards. For cation

analysis, an ICP plasma analyser (IRIS Intrepid II XDL, Thermo Electron Corporation) was used. Plant heavy metals (Zn²⁺) were determined by flame atomic absorption spectrometry (AAS) in a UNICAM 969 atomic absorption spectrometer (Thermo Elemental, UK) after nitric-perchloric acid (2:1) digestion to a maximum of 210 °C for at least 2 h (Abrisqueta and Romero, 1969).

2.3. Chlorophyll fluorescence

Chlorophyll fluorescence was measured in random, fully developed penultimate leaves (n=12, two measurements per plant) using a portable modulated fluorimeter (FMS-2; Hansatech Instruments Ltd., UK) after 30 days of treatment. Light- and dark-adapted fluorescence parameters were measured at dawn (stable, 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ambient light) and at mid-day (1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to investigate whether Zn concentration affected the sensitivity of plants to photoinhibition.

Plants were dark-adapted for 30 min, using leaf clips exclusively designed for this purpose. The minimal fluorescence level in the dark-adapted state (F_0) was measured using a modulated pulse (<0.05 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 1.81 s), which is too small to induce significant physiological changes in the plant. The stored data were an average taken over a 1.6 s period. Maximal fluorescence in this state (F_m) was measured after applying a saturating actinic light pulse of 15,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.7 s. The value of F_m was recorded as the highest average of two consecutive points. Values of the variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0 and F_m .

This ratio of variable to maximal fluorescence correlates with the number of functional PSII reaction centres, and dark-adapted values of F_v/F_m can be used to quantify photoinhibition (Krivoshcheva et al., 1996). The same leaf section of each plant was used to measure light-adapted parameters. Steady state fluorescence yield (F_s) was recorded after adapting plants to ambient light conditions for 30 min. A saturating actinic light pulse of 15,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.7 s was then used to produce the maximum fluorescence yield (F_m) by temporarily inhibiting PSII photochemistry.

Using fluorescence parameters determined in both light- and dark-adapted states, the following was calculated: quantum efficiency of PSII [$F_{PSII} = (F_m' - F_s)/F_m'$] and non-photochemical quenching [$NPQ = (F_m - F_m')/F_m'$; Redondo-Gómez et al., 2006].

2.4. Gas exchange

Gas exchange measurements were taken on random, fully expanded leaves (n = 10, one measurement per plant and four extra measurements taken randomly) using an infrared gas analyser in an open system (LI-6400; LI-COR Inc., NE, USA) after 7 and 30 days of Zn/salt treatment. Net photosynthetic rate (A), intercellular CO₂ concentration (C_i) and stomatal conductance to CO₂ (G_s) were determined at an ambient CO₂ concentration of 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 20/25 °C, 50 ± 5% relative humidity and a photon flux density of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A, C_i and G_s were calculated using the standard formulae of Von Caemmerer and Farquhar (1981). Photosynthetic area was approximated as the area of a trapezium. The water use efficiency (WUE) was calculated as the ratio between (A) and the transpiration rate [mmol (CO₂ assimilated) mol⁻¹ (H₂O transpired)].

2.5. Photosynthetic pigments

At the end of the experimental period, photosynthetic pigments in fully expanded penultimate leaves (n = 10, one measurement per plant and four extra measurements taken randomly) from each treatment were extracted using 0.05 g of fresh material in 10 ml 80%

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