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Effect of Induced Salt Stress on Growth of *Lygeum spartum* L.

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***Abstract*:** The esparto (*Lygeum spartum* L.) is a perennial poaceae which is of significant ecological interest in the fight against the advance of the desert and desertification because of developed root system and a salinity-tolerant species. The present study aimed at to study some parameters of biological adaptation to salinity in *Lygeum spartum* Land to observe the effect of salinity by adding increasing concentrations of NaCl (0, 50, 100 mM). The results obtained show the negative effect of NaCl on the growth and development of the plant, salt stress led to a decrease in the growth of the plant, decrease in the concentration of flavonoids and antioxidant activity which indicate increase in the rate of inhibition of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and an increase in the rate of malondialdehyde (MDA). These results confirm that esparto is a model plant that has an adaptive and salinity-tolerant capacity.

***Keywords*:** Salt stress, *Lygeum spartum*, Growth, Oxidative stress, Flavonoids

Soil salinity is one of the environmental factor affecting vegetation cover and land. Salinity leads to soil degradation and erosion (Ferreira et al 2021). Numerous studies confirmed the deleterious effects of salinity on soil properties, microflora, seed germination, plant growth, and soil-dwelling organisms (Sahab et al 2021). In addition salinity stress causes osmotic stress, nutrient imbalance, ion toxicity, increased reactive oxygen species (ROS) production, decreased photosynthesis and reduced plant productivity (Kordrostami and Rabiei 2019). Salinity-induced oxidative stress in the form of ROS adversely affects plant growth and productivity (Kumar et al 2018). Plants trigger an antioxidant defense system through non-enzymatic compounds, such as ascorbic acid, glutathione, α-tocopherol, carotenoids and flavonoids (Caparos et al 2019) and is identified salt-tolerant species. The level of tolerance is important to fight abiotic stress. Tolerant and hardy grass species have been (Kumar et al 2016, Liu et al 2022).Among these is *Lygeum spartum* L. species is of interest because of its tolerance to environmental in combating the advance of the desert and desertification due to its developed root system. *L. spartum* L. is a perennial poaceae serves as a natural barrier against advancing sand and desertification in the Algerian high plateaus. Nedjimi (2009) showed that salt tolerance strategies of *L. spartum* L. is achieved by appropriate osmotic adjustment involving accumulation of ions and glycine betaine. Physiological behavior of *L. spartum* L. in a salty environment shows an osmotic adjustment in this species which is associated with a significant accumulation of sodium (Na^+) and chlorine (Cl^-), while the accumulation of soluble sugars contributes partially to osmo-regulation (Nedjimi 2013). But until there is no study which has been carried of biological adaptation defense system to fight the effect of salinity on growth of *Lygeum spartum* L.

The objective of this study is to demonstrate the influence of a salt stress on certain physiological and morphological parameters of the plant by the induction of salt stress by different concentrations of NaCl and to measure of some plant growth parameters, flavonoids, antioxidant activity and lipid peroxidation.

MATERIAL AND METHODS

Plant material: The seeds of *Lygeum spartum* L. harvested in the region of El Bayedh (El_Kheiter) in June 2021 and were disinfected with 2% sodium hypochlorite for 3 minutes, then rinsed with sterile distilled water and then placed in plastic petri dishes 10 cm in diameter and 1.3 cm thick. The seeds were germinated in an oven at 30°C. Afterwards were placed in pots; containing the soil taken from the El_Kheiter station. The pots are placed in the greenhouse then watered daily with a nutrient solution consisting of K_2HPO_4 (0.5 mM), $MgSO_4$ (0.5 mM), H_3BO_3 (25 mM), $MnSO_4$ (2 mM), $ZnSO_4$ (2 mM), $CuSO_4$ (0.5 mM) for 7 days at a rate of 5ml per pot.

Induction of salt stress: After 7 days, the pots of *L. spartum* L. seedlings were divided into 3 batches, each batch containing 60 seedlings. The first batch was watered with the

nutrient solution for 7 days at the rate of 5 ml per pot, 3 times per week for 60 days and kept as control. The second batch was sprayed with a 50 mM NaCl solution for 60 days, 3 times a week. The third batch was sprayed with a 100 mM NaCl solution for 60 days, 3 times a week.

Measurement of the aerial part and the underground part: After 60 days of growth, the lengths were measured. For the measurement of the fresh mass, the seedlings were dug up. The fresh weight of the seedlings was weighed. For dry weight, the seedlings were dried in the open air, and placed in an oven at 30°C, from the 5th day the dry weight was estimated after stabilization of the dry weight.

Estimation of flavonoids: The extraction of flavonoids was made after crushing the seedlings of *Lygeum spartum* L. 10 g of plant powder were extracted using 100 ml of 80% methanol for 2 h at room temperature. Then centrifugation was carried out for 5 minutes at 3500 rpm. The quantification of flavonoids was carried out by a colorimetric method adapted by Dirar et al (2019) with slight modification. The method is based on the complexing of flavonoids with aluminum trichloride. A standard range is produced with catechin (Sigma-aldrich) (5-10-15 and 25 µg/ml). The results are expressed in mg of catechin equivalents per gram of extract (mg EC/g of extract).

Estimation of antioxidant activity: The estimation of the antioxidant activity was carried out by the chemical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test. DPPH is a free radical used to study the structure-antioxidant activity relationship of phenolic compounds (Aree and Jongrungruangchok 2018). Fifty µL of different concentrations of the extracts were added to 1950 µL of DPPH solution (0.025 g/L) dissolved in methanol. After 30 min incubation at room temperature, absorbance is read at 515 nm against a blank containing all reagents except test compound. Ascorbic acid was used as a positive control. Each sample was measured in triplicate. The results were expressed as a percentage of trapping activity (I %).

$$I\% = [(\text{White Abs} - \text{Sample Abs}) / \text{White Abs}] \times 100.$$

Estimation of lipid peroxidation: Lipid peroxidation was estimated by measuring MDA (malondialdehyde) with the TBARS (thiobarbituric active species) (Burri et al 2019). MDA is one of the products of the decomposition of polyunsaturated fatty acids under the effect of free radicals released during stress. The assay is based on the formation in an acidic medium (pH 2 to 3) and hot (100°C) between one MDA molecule and two molecules of thiobarbituric acid (TBA) of a pigment colored pink, absorbing at 530 nm. Lipid peroxidation was estimated by measuring the MDA content. Fifty mg of sample was ground and homogenized in 2 mL of 1% w/v trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 rpm for 10 min at 4°C. 0.5 mL of the supernatant was mixed with 1.5 mL of thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90°C for 20 min. Absorbance was read at 532 nm. The MDA content was determined using the extinction coefficient at 155 mm/cm. All experiments were repeated 3 times.

RESULTS AND DISCUSSION

Seed germination and length of the aerial part and the underground part: The germination rate of Lygeum *spartum* L. seeds was 100%. The various saline treatments applied to the seeds after 60 days of growth influenced the growth and development of the seedlings, The elongation of the root axis and of the hypocotyl in the presence of NaCl, indicated that elongation of the aerial part and underground part of the plant was slowed down compared to the elongation in the control. The application of 50 mM of NaCl caused a slight reduction in root elongation, and reduction was more marked for the roots at 100 mM of NaCl. The hypocotyl showed significant reduction at 50 mM of NaCl compared to the control plant which stabilizes despite the increase in the doses of NaCl to 100 mM (Table 1). The result of application of high concentrations of NaCl from 50 mM to 100 mM, resulted in decrease of fresh weight of young seedlings obtained after 60 days of germination compared to the control plants. The results showed that 50 and at 100 mM, increased, dry weight of young seedlings obtained after 60 days of germination as compared to control (Table 1).

Flavonoids : The quantitative analysis of the extracts of the aerial parts of *L. spartum* L. was carried out by spectrophotometric determination of flavonoids (Fig. 1). The results are expressed in mg catechin equivalent/g DW. The flavonoid content obtained from a calibration curve (y=0.040 x +0.006; R2 = 0.998) established that with increasing

Table 1. Length of the aerial part and the underground part, fresh and dry weight of control and stressed plant (50, 100 mM NaCl)

	LAP	LUP	FW	DW
Control	25.85±0.70	26.81±1.05	0.72±0.48	0.15±0.006
50 mM NaCl	22.35±0.80	26.42±1.12	0.29±0.01	0.15±0.006
100 mM NaCl	21.34 ±0.87	24.98±0.98	0.3±0,01	0.19±0.009

concentrations of catechin .The flavonoid content in the control extract is higher (0.34 mg CA Eq/g DW) as compared to 50 mM extract (0.29 mg CA Eq/g DW (and 100 mM extract (0.012 mg CA Eq/g DW).

Antioxidant activity: The DPPH assay was used to estimate the antioxidant activity of the different extracts obtained from the different treatment (control, stressed at 50 Mm and 100 mM). The test is based on the capacity of the anti-radical substances present in the extracts to reduce the DPPH free radicals, ascorbic acid was used as a positive control to check the reactivity of the solution. The different treatment have variable anti-free radical activities towards the DPPH free radicals. The control extract shows a low anti free radical activity between 21.78 and 76.16 % compared to the 50 mM extract (45.97 and 85.14) and the 100 mM extract (95.12 %) at the concentration 6 mg/ml. all these extracts showed their possession of an antioxidant power (Fig. 2).

Lipid peroxidation: There was a variability in the accumulation of MDA in *Lygeum spartum*; in control batch there, accumulation of 1.09 µmol/g of MDA was observed. The plants stressed at 50 mM of NaCl have an accumulation of 1.1 µmol/g of MDA plants stressed with 100 mM of NaCl present 1.3 µmol /g of MDA (Fig. 3).

The results show after 60 days of growth a decrease in the length of the aerial and underground parts of the plant, however it is indicate that the aerial parts are more affected than the underground parts. Indeed, salt stress inhibits the growth of and development of plants (Lepengue et al 2010, Silva et al 2014).Similar results were observed in *Hordeum vulgare*, where salt stress reduces the growth of young leaves and roots (El Goumi et al 2014), Moreover, salinity negatively affects the growth of the vegetative apparatus, in *Pistacia vera,* (Benmahioul 2009). Khodarahmpour et al (2012) concluded that aerial parts were more affected than the roots in the presence of salt stress. Wang (2022) observed the reduction in the root, stem, and leaf dry weights exposed to salt stress. This decrease in biomass was also reported by earlier researchers (Benmahioul 2009, El Goumi et al 2014). The decrease in dry biomass can be caused by the increase in Cl- concentration in the tissue (Tavakkoli et al 2011). Shabala et al (2016) and El-Badri (2021) shows that soil salinity is a major abiotic stress factor who negatively affects crop yield by impairing germination, plant vigor and metabolic pathways. Saline condition in halophyte grasses leads to an increase in the content of antioxidants. Indeed saline condition in halophyte grasses leads to an increase in the content of antioxidants (Singh et al. 2015).In addition salinity enhanced the production of ROS, which are highly toxic to the cell, and they disturb cell redox homeostasis. Surplus ROS in the cells facilitates protein and enzyme degradation and lipid peroxidation (Li et al 2017). The flavonoids decrease when the NaCl concentration increases and slight decrease in flavonoids from the treatments at 50 mM NaCl, whereas when the concentration of NaCl doubles, significant decrease of content of flavonoids is observed. Lipid peroxidation were estimated by MDAs by the TBARS test, an indicator of damage caused by stress. There was

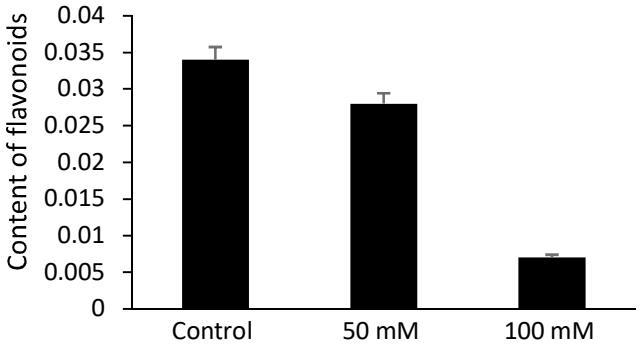

Fig. 1. Flavonoid content by spectrophotometric determination (results are expressed in mg catechin equivalent/g DW)

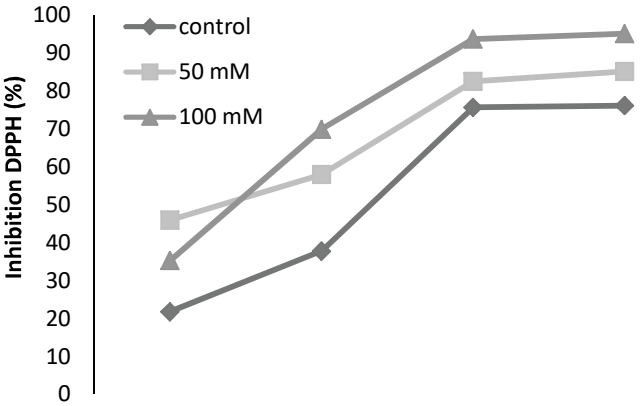

Fig. 2. Percentage of inhibition of DPPH according to the different concentrations of *Lygeum spartum* L

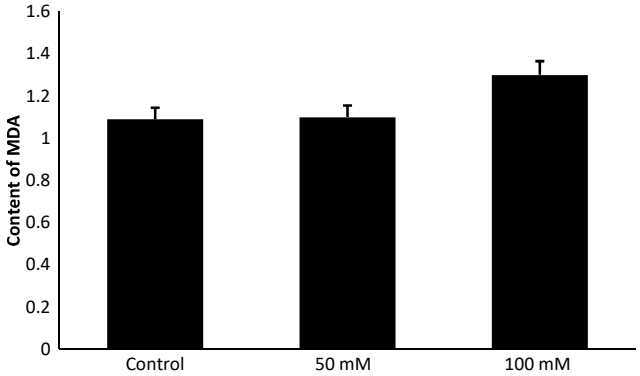

Fig. 3. Determination of content of MDA (results are expressed in µmol/g)

increase in MDA levels compared to controls. The results are similar with induced salt stress in maize, prolong salinity reduced leaf relative water content and leaf water potential and increased malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content (Abdelgawad et al 2016).

CONCLUSION

The induction of salt stress by sodium chloride shows that the development of the aerial and underground system of the plant is affected by the concentrations of sodium chloride, as well as the development of fresh matter, which is strongly affected by sodium chloride. Salinity is a limiting factor for the growth of *L. spartum* L. The effect of salt stress on the growth of *L. spartum* L at the root and leaf emergence stage is visible and the increase in salt concentration leads to a slowdown in growth. The salt stress generates oxidative stress, this results in an accumulation of hydrogen peroxide and lipid peroxidation indicating the instability. The production of reactive oxygen species disturbed the redox status of cells h triggered oxidative stress in *Lygeum spartum* L. The quantitative estimate of flavonoids indicate plant contains significant amount metabolites. Esparto is a model plant for adaptation and tolerance to salt stress.

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