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Research Article

Assessing the Effects of Salt Stress on Growing Sulla Plants (*Sulla coronaria*)

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Abstract

This study assessed how the plant *Sulla coronaria* reacted to different salt levels. The experiment was conducted at Tizi Ouzou University in Algeria, using a pot experiment with a substrate of sand, clay soil, and horticultural potting compost. The experiment had 32 plants in four groups, which were then subjected to different doses of saline solution (NaCl) for 15 days. After two months, the plants were harvested, and the following data were collected: root and shoot length, fresh and dry weight, leaf number and area, relative water content, electrolyte leakage, total chlorophyll content, total soluble sugars content, proline content, and the extraction and quantification of hydro-soluble proteins. The results showed that rising salinity significantly reduced the number and leaf area of the shoots, and shoot biomass and length. These results are likely due to the effects of salinity stress on cell structure and metabolism, as evidenced by a decrease in cell hydration (RWC) and membrane integrity. These events possibly contributed to decrease in chlorophyll pigment concentration and soluble protein levels. Stressful conditions can lower plant growth, but Sulla plants can adjust to high salinity by increasing sugars and proline. We found the highest levels of sugars and proline at 9 g/L and 56.27 mg/g DM(Dray Matter), respectively. To generalise about how this species responds to salt stress, we should do more controlled and field studies with other populations to select those responding better to salt stress.

Keywords: Sulla, *Sulla coronaria*, Salinity, RWC, Electrolytes leakage, Chlorophyll, proteins, Soluble sugars, Growth, Osmotic adjustment

Abbreviations: DM: dry mass, FM: fresh mass, MADR: Ministry of Agriculture and Rural Development, RWC: relative water content, TAA: Total Agricultural Area, UAA: Useful Agricultural Area

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1. Introduction

Abiotic stresses cause significant losses in agricultural production worldwide (Jakab et al., 2005). Salinity and drought, in particular have a significant impact on crop yields in arid and semi-arid regions. Saline soils cover 95 million hectares worldwide (Trifi-Farah et al., 2002), 20% of which is irrigated (Flowers & Flowers, 2005), and 15% of which is arable (Munns, 2002). In Algeria, 3.2 million hectares are affected by this issue (Belkhodja and Midai, 2004). The cultivated areas vary from year to year based on diverse factors, such as plot fragmentation and people's shift towards industry and trade. Currently, 1.233,422 hectares are devoted to forage, consisting of both spontaneous and cultivated species, representing 2.80% of the Total Agricultural Area (TAA) and 14.40% of the Useful Agricultural Area (UAA) (Agricultural Statistics, Series B, 2021). The area sown with artificial fodder accounts for 77.82%. These values were much lower during the second decade of this century, indicating 1.83% of the TAA and 2.6% of the UAA (Senoussi & Behir, 2010; Zirmi-Zembri & Kadi, 2016). This is probably owing to the subsidies provided for this type of crop under the national development plan. It should be noted that alfalfa (*Medicago sativa* L.) is the most commonly grown forage species in almost all production systems. According to Azib (2020), the area devoted to perennial Lucerne represents 0.37 to 0.71% of the total area allotted forage crops. Taken as a whole, these fodder crops reach a production level (6157.9 Kg/ha) that is considered insufficient (i.e., a yield of 4380 Kg/ha) (MADR, series B, 2019), which barely covers 5% of the needs of our livestock, which includes more than 36.5 million livestock (cattle, sheep, goats, camels), with an increase of 37% between 2010 and 2020: 1.786,351 cattle, 29.378,561 sheep, 4.929,069 goats, 417.167 camels and 49.911 horses (MADR, series B, 2019). In Algeria, most fodder resources come from fallow land and cereal crop by-products. The so-called natural grasslands are rare. The poor quality of fodder, which is often very cellulosic, and the unavailability of green fodder for long periods of the year add to the negative impact of climatic constraints on livestock yields. *Hedysarum coronarium* L., a species commonly known as Northern Sulla. It is native to the Mediterranean area, where it is widely used as hay, silage and green fodder (Abdelguerfi-Berrekia et al., 1991; Issolah et al., 2012). Sulla belongs to the genus Hedysarum, which is part of the tribe Hedysareae of the subfamily Papilionaceae and the family Fabaceae (Quezel and Santa, 1962; Issolah et al., 2012). It was recently reclassified by Choi and Ohashi (2003) as *Sulla coronaria* (L.) Medik (Issolah et al., 2012; Annicchiarico et al., 2014). It is known by various common names: Sulla, French honeysuckle, Spanish sainfoin. The northern Sulla is an annual to biennial fodder legume native to Mediterranean regions; endemic to the clay-limestone soils of the semi-arid basin Mediterranean region (Fitouri et al., 2011). The importance given to Sulla by farmers and breeders is essentially due to interesting characteristics like high supply of nitrogen as a result of specific symbiotic fixation (about 100 kg/ha/year), satisfactory seed production, which contributes to the enrichment of soil seed banks (Lombardi et al., 2000).

Sulla is a precious plant for hot countries; it is likely to provide great services in the Mediterranean basin and in particular in Algeria (Denaiffe & Denaiffe, 1996). According to Abdelguerfi-Berrekia et al. (1991) and Issolah et al. (2012), this species is composed of about 86% dry matter, a considerable protein content varying between 15 and 23%, and 12.5% mineral matter. Another interest attributed to Sulla is the large contribution of mineral and organic matter to the soil. In particular, Sulla is an excellent crop precedent for cereals (Ben Jeddi, 2005; Slim et al., 2018). These authors mention the richness of Sulla in proteins (22.5 g/kg DM), lipids, and minerals. They also note a digestibility very close to that of other cultivated fodder legumes such as alfalfa. The rotation system based on Sulla/wheat has improved the protein content of seeds and stabilized the quantity and the quality of cereal production, particularly in semi-arid areas (Ben Jeddi, 2005). At the end of their biological cycle, plants of the Sulla genus have the potential to be used as a soil improver, particularly for sandy soils that are naturally poor in organic matter (Salhi, 2008). The high mineral content of this plant (one ton of the plant exports 25 kg of nitrogen, 18.7 kg of phosphorus pentoxide and 25 kg of potassium oxide to the soil) means it can improve soil fertility, water and nutrient retention, and moisture conservation.

It can also prevent erosion by incorporating it into the soil as green manure (Fitouri-Dhane, 2011). According to a recent study, integrating *S. coronaria* residues as organic amendments in production systems is considered an effective solution to the fertility problems of degraded soils and a kind of improvement in organic carbon mineralization (Bouajila et al., 2014). The extant literature has noted that the cultivation of *S. coronaria* has a beneficial effect on the agronomic and physicochemical characteristics of the soil, such as structure, permeability, and cation exchange capacity (Trifi-Farah et al., 2002). It is, therefore, recommended as a means of improving the value of fallow land, particularly in the case of marl soils. The level of green production is a key factor in determining the species' level of interest. These developmental capabilities allow the Sulla to cover a large part of the fodder calendar when the other species are dormant (Abdelguerfi, 2002). Biological fixation is an essential phenomenon, comparable to photosynthesis, because it allows the production of an organic compound from atmospheric nitrogen. It makes it possible to reduce the application of synthetic nitrogen fertilizers while enriching the soil in nitrogen (Julier & Huyghe, 2010). In normal conditions, the farmer should not apply mineral nutrients to the cultivated Sulla, because this element is obtained through a symbiotic way, with a rate of 78.2 to 82.7% of the nitrogen needs of this plant coming from atmospheric fixation (Abdelguerfi, 2002). This proportion can exceed 90% in the presence of efficient specific rhizobial strains (Casella et al., 1984). This process is characterized by the presence of bacterial strains able to convert molecular nitrogen into ammonium, which can be assimilated by the plant partner. Some species of the Sulla genus are known for their richness of metabolite compounds specially condensed tannins Bonanno et al., 2011). This feature explains its effect on the reduction of methane emission of livestock grazing, the risk of bloat in cattle and the need for anthelmintic treatments in sheep. Except, few studies investigated the response of Tunisian origins of *H. coronarium* to salinity conditions (Dallali et al., 2012); the majority of studies were interested in the ecological and morphological (Abdelguerfi-Berrekia et al., 1991), and genetic aspects of this plant (Tilaki-Ghasem et al., 2016; Tibaoui et al., 2017). The Italian researchers showed that the ecotypes collected from the central regions of the country present morphological and physiological potentialities, which allow them to widely adapt to environments prone to cold and drought (Annicchiarico et al., 2014). The much-publicized morphological diversity within the genus Sulla highlights the importance of expanding our knowledge of the ecophysiological behaviour of the genus Sulla under different environmental conditions.

Accordingly, the present work was carried out in this context aims to contribute to increasing knowledge about the ecophysiological characterization of this species in the objective of searching its advancement, protection and conservation, as it is threatened by human trophic pressures such as urbanization, frequent cutting before fruiting, and overgrazing. The objective of this study was to evaluate the effects of salinity on the growth of *S. coronaria* by assessing the morphological, physiological and biochemical responses of plants to this salinity stress.

2. Materials and methods

2.1. Research method

The experiment consisted of a pot culture trial of plants grown under a greenhouse. The pods of *S. coronaria* used in this study were collected at the stage of full seed maturity (browning of the pods), corresponding to the second half of June, from uncultivated plots of approximately 1000 m^2, this area is part of INRAA (Algerian National Institute Agronomic Research), geographical territory (INRAA station of Oued-Ghir department, Bejaia, North-East of Algeria in the altitude of 250 m), bounded by latitude 36°42'38"N and longitude 04°58'38"E.This area is part of the sub-humid bioclimatic stage.

In the laboratory, seeds were stored in the freezer at 5 ± 2°C in paper bags be safe the humidity for 3 months. Seeds were extracted by manual shelling. Seeds with holes or visible signs of fungal attack, bruising, or smaller than average size were removed. Selected healthy seeds were stored in paper bags at around 5 ± 2°C.

Seed viability was tested using the densimetry test, which consists of placing each batch of seeds to germinate in a container of distilled water; seeds that fall to the bottom are considered viable, and those that rise to the surface are non-viable and are removed from the batch. Mechanical scarification (light rubbing between two sheets of sandpaper) was performed to avoid any tegumental dormancy. The seeds were then treated with a 5% solution of sodium hypochlorite for 5 minutes, and then thoroughly rinsed with distilled water before germination.

The present experiment was carried out in the greenhouse located at the Faculty of Biological and Agronomical Sciences, Mouloud Mammeri University of Tizi Ouzou, Algeria (Altitude: 196 m; Latitude: 36.7°N and 4.06°E). Five seeds have been sown in each plastic pot (continence of 1000 mL) in a substrate consisting of a mixture of one-third horticultural soil, one-third topsoil and one-third sand (washed and sterilised). A total of 128 plants were tested with four doses (0, 3, 6 and 9 g/L) of NaCl with 32 replications for each treatment. At the beginning of the experimentation, all the pots were watered with 250 ml of nutrient solutions according to Wacquant (1974), which corresponds to the field capacity of the substrate. Two weeks after sowing (corresponding to the vegetative stage of the plants). The plants were divided into four samples. The 1st set was still watered with nutrient solution, which served as a control, while the 2nd, 3rd, and 4th sets were watered with nutrient solution to which 3 g/L, 6 g/L and 9 g/L NaCl were added gradually. After two months of cultivation, corresponding to a typical growth stage, the plants were harvested and taken back to the laboratory, where they were dusted and evaluated for morphological, physiological, and biochemical traits.

2.2. Morphological parameters

2.2.1. *Leaf count*

The number of leaves per plant was noted from a sample of 12 plants.

2.2.2. *The leaf area*

(cm^2) was measured by Mesurim Pro® (version 3.4) Software using digitised images taken with a standard scanner.

2.2.3. *Organs length*

Twelve plants per treatment were sampled for homogeneity, harvested at the end of the experimental period, and divided into two parts: roots and shoots. The roots were carefully rinsed with water and then quickly dried with absorbent paper. The length of the aerial parts and roots was measured with a ruler. For the measurement of root volume, the procedure consisted of the submersion of the root to be measured in a container of water, after which the resulting change in the scale reading is taken as an estimate of the volume of the root.

2.2.4. *Organs weight*

The fresh weight of the shoots and roots was measured as soon as the plants were harvested, then these organs were dried in the incubator at 80°C for two days and the dry weight of the shoots and roots was measured.

2.3. Physiological parameters

2.3.1. *Relative water content*

First, leaves from a sample of five plants were picked randomly and weighed immediately to record the fresh weight (FW). Then they were placed in test tubes containing distilled water, sealed well, wrapped in aluminium foil to maintain saturated relative humidity, and stored in a cool, dark place. After 24 h, the leaves were removed, dabbed on absorbent paper to remove any water on the surface and weighed again to obtain the full turgid (saturated) weight (TW). The leaves were then wrapped in aluminium paper and placed in an incubator at 80ºC for 48 h, and then weighed to obtain the dry matter weight (DW). The relative water content (RWC) was calculated according to Barrs & Weatherley (1962):

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) \times 100/(\text{TW} - \text{DW})$$

Where:
RWC: Relative Water Content.
FW: Fresh Weight;
DW: Dry Weight.
TW: Turgid Weight

2.3.2. *Total Chlorophyll*

For each NaCl treatment, using the method of Bruinsma (1961), samples were taken at harvest from fresh leaves of the same rank at stem level. Optical density of the extracts was read at 652 nm. Total chlorophyll content was estimated according to the following formula:

$$\text{Total Chlorophyll content (µg/g of FW)} = \frac{\text{Vol.Ac.(mL)} \times \text{value of absorbance} \times 10}{\text{DW (mg)}}$$

Where:
Vol. Ac.: Volume of acetone extract;
FW: Fresh Weight;
DW: Dry Weight

2.3.3. *Electrolyte leakage*

The method of Thiaw (2004) was used to assess electrolyte leakage (EL). For this purpose, 0.2 g of fresh, small-sized leaf material was collected, to which 5 mL of distilled water was added for each sample. The leaves were then placed at room temperature for 45 min, after which a first measurement of conductivity (C1) was carried out. A second conductivity reading (C2) was made after the samples had been placed at 100°C for 15 min. The electrolyte leakage was then calculated by the following formula:

$$\text{EL (\%)} = \frac{C1}{C2} \times 100$$

Where:
C1 and C2, the first and second measurements of conductivity, respectively.

2.4. Biochemical parameters

2.4.1. *Total soluble sugar content*

Sugars were extracted using the Cerning method (1975), in which a sample of 1 g of dry leaf matter was crushed in 10 ml of 80% ethanol. The extract was centrifuged at 4000 rpm for 10 min after vortex agitation. The first supernatant was recovered, and a second extraction was performed with 5 mL of 80% ethanol, followed by centrifugation at 3000 rpm for 10 min. Volumes of the two extracts were remixed and adjusted with distilled water to a volume of 50 mL. The determination was done on 1 mL of extract to which was added 2 mL of Anthrone reagent (0.2 g of Anthrone powder dissolved in 100 mL of concentrated sulphuric acid). After agitation, the tubes were placed in a double boiler at 100°C for 7 min to allow the staining to develop. After cooling the tubes, the reading was taken with a spectrophotometer (Shimadzu Spectrophotometer UV-101.02) at 630 nm.

2.4.2. *Proline content*

Proline of dry matter was extracted and measured according to Troll and Lindsley (1955) as modified by Bates et al. (1973). Free proline was determined by homogenisation of 0.5 g of dry leaf mass in 10 mL of 3% aqueous sulphosalicylic acid. The obtained homogenate was centrifuged at 200 g for 5 min. An aliquot of 2 mL of the extract was treated with 2 mL of ninhidrine reagent and 2 mL of glacial acetic acid. The mixture was then incubated for 1 h at 100°C in a Bath-Marie. After cooling the tubes by immersing them in ice for 5 min, 5 mL of toluene was added and the whole was agitated by a vortex.

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After 30 minutes of rest, the absorbance is then measured at 528 nm.

2.4.3. *Total soluble protein content*

Hydrosoluble proteins were determined by the Bradford method (Bradford, 1976), which involves the formation of a blue staining complex between the proteins and Coomassie blue (G250). The protein-Coomassie blue complex absorbs at a wavelength of 595 nm. The extract was prepared from 0.5 g of fresh leaf material. Leaves were crushed and centrifuged at 5000 rpm for 15 min, and the supernatant containing the proteins was removed for dosing. To an aliquot of 100 µL of the supernatant, 3 mL of the Bradford reagent was then added, and the mixture was homogenized and then incubated for a few minutes. The reading was taken at a wavelength of $\gamma = 595$ nm after calibrating the spectrophotometer with a blank prepared from 100 µL of distilled water and 3 mL of Biorad reagent.

2.5. Statistical analysis

Data for each parameter (morphological and physiological) were subjected to a one-way ANOVA (salinity) with four levels: 0 g/L: control; 3 g/L, 6 g/L, and 9 g/L NaCl; using R- R-Software 3.6.1. Comparisons of the means between the different treatments were made to highlight any significant difference using the Tukey–Kramer HSD test at a 5% significance level.

3. Result

3.1. Influence of salinity on morphological parameters

The results of the measured parameters at the end of the experiment are presented in Table 1. Results showed that all growth parameters decrease with salinity. The salinity stress induced effects on all the growth parameters that were monitored in this essay (Table 1). The mean number of leaves per plant and leaf area was significantly reduced according to the salinity intensity. For instance, the control plants recorded a maximum mean number of leaves per plant (12.6) and leaf area of 6.78 cm^2. The measures demonstrated a significant decrease at 6 g/l, exhibiting respectively a mean value of 8.9 and 3.54 cm2 (Table 1) corresponding to a reduction of 29.36% and 47.78% respectively (Table 2). This effect was accentuated according to the stress in to reaching lowest values at the strong concentration of NaCl, with 6.7 and 2.54 cm2 (Table 1), corresponding to a reduction of 46.82% and 62.53% respectively for number and leaf area (Table 2). The biomass production was also significantly affected by the constraint of salinity. We have recorded in the shoots for example, a decline of length and fresh and dry matter weight. Indeed, we have noted a results of 20.28 cm, 10.20 g and 1.1 g length, fresh and dry weight in the control case which decreased significantly at 6 g/l, respectively to 14.52 cm, 5.37 g and 0.44 g (Table 1) corresponding to a proportion of reduction of 28,40%, 47,35% and 60%. The drop continued until registering respectively the highest reductions of 50.39%, 62.15% and 78.18% (Table 2) at a higher NaCl concentration (9 g/L) compared to the control. According to our results, the response of *S. coronaria* plants to salinity constraint, showed morphological readjustment. Indeed, the root was also affected by salinity stress but it appears to be less affected comparatively to the shoot. For instance, the reduction of root system was just 19% from the level of 6 g/L comparatively to shoot which was more affected with 34% and 50% at 6 g/L and 9 g/L (Table 2). Therefore, after the limit of the medium dose of 6 g/L, the plants reduced the growth of roots, as well as their fresh weight (45.48 %), volume (23.93 %) and dry weight (34%). The root/shoot dry weight ratio showed slightly the same variation as the root growth; in fact, a marginal increase in the ratio was observed at 3 g/L NaCl, with a value of 0.79 g. Subsequently, a slight decrease was observed after the salinity was augmented to 9 g/L (Table 1).

3.2. Influence of salinity on physiological parameters

The results of the measured physiological and biochemical parameters at the end of the experiment are reported in Table 3. As observed in the study of growth parameters, the current investigation also demonstrates a detrimental effect of salt stresses on all physiological and biochemical parameters (Table 3). In the present experiment, under the constraint conditions, the hydric status of leaf cells of *S. coronaria* expressed by relative water content (RWC), showed a highly signifiant ($p \leq 0.01$) reduction, which correlated with the salinity severity. In fact, RWC showed a high percentage in control conditions with 90.05%. The hydration statue was decreased with the elevation of the stress intensity until registering a lowest level of 52.9% (Table 3), corresponding to a drop of 41.54% at 9 g/L, which is still below the vital reference value of the hydric cell status of the plants.

The statistical analysis of the result obtained shows a significant decrease ($p \leq 0.05$) for chlorophylls according to the salinity level.

We have recorded 5.21 µg/g FW in control plants. The significant diminution was noted from the first level of 3 g/L to 4.05 µg/g FW, corresponding to a proportion of 22.26% until reaching the lowest level of 3.27 µg/g FW, corresponding to a decrease of 37.23% at 9 g/L.

At the cellular level, our results indicate a significant alteration in membrane integrity, as evidenced by changes in electrolyte leakage (EL) percentages. Indeed, a gradual

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increase in electrolyte leakage (EL) was observed with an elevation salinity gradient in the substrate. Statistical analysis of EL percentage showed a highly significant variation ($P \leq 0.01$). The results showed the lowest rate of the plants, as reflected by an increase in this value with the degree of salinity in the substrate, reaching a maximum value (43.89%) at the high level of salinity that was tested (9 g/L) (Table 3).

According to the present experimentation, a potentiality of osmotic adjustment was highlighted in *S. coronaria* plants. We have observed an increase in soluble sugars and proline with the elevation of the salt concentration in the irrigation solution. We have observed that the lowest amount of soluble sugars (28.12 mg/g DM) and proline (0.04 µg/g DM) were recorded in control plants. However, EL in control plants with 24.81%. A significant increase in the percentage of Electrolyte Leakage was recorded from a concentration of 3 g/L of NaCl. The impact on membrane integrity is becoming increasingly evident in from the salinity level of salinity of 3 g/L, a significant increase was observed with 37.43 mg/g DM and 0.29 µg/g DM, respectively, for soluble sugars and proline (Table 3).

The influence of salinity constraint on nitrogen metabolism revealed statistically significant damage, expressed by a decrease in soluble protein content from a salinity of 3 g/L (6.2 mg/g DM). A protein content diminution was progressively continued until a level of 4.25 mg/g DM was reached, which was registered at 9 g/L NaCl, corresponding to a 48.73% drop compared to control plants (Table 3).

Table 1. Effect of salinity on morphological traits (Mean ± SD) of *Sulla coronaria*

Salinity	Root Length (cm)	Shoot Length (cm)	Leaf Number	Leaf Area (cm^2)	Roots Volume (cm^3)
0g/l (Control)	37.58± 9.38 b	20.28± 1.78 b	12.6±1.64 a	6.78± 0.86 a	11.70± 0.82 a
3g/l NaCl	48.52± 8.21 a	24.56± 4.75 a	11.1±2.80 a	5.47± 0.43 b	11.30± 1.16 a
6g/l NaCl	30.45±8.40 c	14.52± 2.16 c	8.9±1.83 b	3.54± 1.51c	8.90 ± 1.45 b
9g/l NaCl	30.35±3.79 c	10.06± 2.23 d	6.7±1.69 c	2.54± 0.45 d	6.25± 1.68 c

Table 1. Continue

Salinity	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Root Dry Weight (g)	Shoot Dry Weight (g)	Root/Shoot Dry Weight ratio
0g/l (Control)	5.87± 1.59 a	10.20± 1.53 a	0.98± 0.23 a	1.10± 0.27 a	0.55±0.19 c
3g/l NaCl	6.82± 1.14 a	8.11± 1.09 b	0.87± 0.29 b	0.80± 0.24 b	0.79±0.12 a
6g/l NaCl	3.20± 1.00 b	5.37± 1.52 c	0.35± 0.11 c	0.44± 0.15 c	0.65±0.11 b
9g/l NaCl	2.05± 1.04 c	3.86± 0.86 d	0.22± 0.06 c	0.24 ± 0.08 d	0.46± 0.1 d

Means with the same letter are not significantly different at 0.05

Table 2. Variation rate of the morphological parameters of *Sulla coronaria* plants according to salinity stress

Parameters	Abbreviation	NaCl Concentration		
		3g/L	6g/L	9g/L
Leaf Number	LN	NS	29.36% ↓	46.82% ↓
Leaf Area (cm^2)	LA	19.32% ↓	47,78% ↓	62.53% ↓
Root length (cm)	RL	29.11 % ↑	19.0% ↓	19.0% ↓
Roots Volume (cm^3)	RV	NS	23.93% ↓	46.58% ↓
Shoot Length(cm)	SL	31.1% ↑	28.4% ↓	50.39% ↓
Root Fresh Weight (g)	RFW	NS	45.48% ↓	65.07 % ↓
Root Dry Weight (g)	RDW	11.22% ↓	34.0% ↓	77.55% ↓
Shoot Fresh Weight(g)	SFW	20.49 % ↓	47.35 % ↓	62.15% ↓
Shoot Dry Weight (g)	SDW	27.27% ↓	60% ↓	78.18% ↓
Root/Shoot Dry Weight Ratio	RDW/SDW	X 1.43 ↑	X 1.18 ↑	X 1.19 ↓

The arrow direction indicates a reduction or increase of data compared to the control

Table 3. Effect of salinity on physiological and biochemical traits (Mean ± SD) of *S. coronaria*

NaCl Concentration	Relative Water Content (%)	Total Chlorophyll (µg/gFM)	Electrolytes Leakage (%)	Soluble Sugars Content (mg/gDM)	Proline Content (µg/gDM)	Protein Content (mg/gFM)
0 (Control)	90.5±1.65 a	5.21±1.78 a	24.81±1.50 d	28.12±3.07 d	0.04±0.01 d	8.29±1.00 a

3 g/l	83.2±7.74 b	4.05±0.28 b	31.41±2.06 c	37.43±5.99 c	0.29±0.02 c	6.20±0.31 b
6 g/l	57.8±3.36 c	3.80±0.31 c	36.46±1.54 b	45.55±6.10 b	0.67±0.04 b	5.29±0.43 c
9 g/l	52.9±3.36 d	3.27±0.31 d	43.89±1.26 a	56.27±4.06 a	1.36±0.36 a	4.25±0.98 d

Means with the same letter are not significantly different at 0.05

4. Discussion

This experiment evaluated the effects of salt on *Sulla coronaria* plants. We observed that an increase in salt content in the growth substrate led to a decrease in the average number of leaves per plant, as well as the leaf area and the growth rate of other plant parts, resulting in a reduction in overall biomass production. This effect was accentuated with increasing salt stress intensity. However, at the lowest level of salinity (3 g/L NaCl), the plants showed a slight increase in growth traits. This plant response could be attributed to morphological adjustments potentiality on aerial part of plants and their root systems. These adjustments enabled the plants to mitigate the adverse effects of salinity on their growth. Consequently, an increase in root length and an improvement in the RDW/SDW ratio were recorded at a moderate rate of stress. From the mediums salinity level (6 g/l), the plants manifested a sensitive and negative effect on growth revealed by a significant reduction in all the morphological traits expressed by a progressive decrease of the leaf number and area, the length and weight of the plant's organs. The present findings were consistent with previous studies on various species, including Tunisian populations of *S. coronaria* and S. carnosa (Dallali et al., 2012), S. flexuosa (Medjebeur, 2018), and clover (Ben Khaled et al., 2003).

As De Rossi et al. (2021) found, the Algerian population of S. *coronaria* showed more efficient morphological adjustment in salinity conditions than the Italian population. Indeed, our findings revealed a limited impact on the root systems, with no more than a 19% increase, whereas the Italian population was more affected, experiencing a severe 47% increase in stressful conditions, as well as an increase in the LR/LS ratio. The effect of salinity on plant morphology is due to osmotic and toxic effects. A high salt concentration leads to a reduction in the hydric potential of the soil substrate, mineral nutrition disequilibrium and ion toxicity damage to plant cells. As a result, a reduction in cell turgor pressure has been observed, which restricts water uptake and cell elongation, resulting in reduced leaf area (Jampeetong & Brix, 2009). Glycophyte species have evolved to minimise the surface area of their aerial parts exposed to water loss through the process of evapotranspiration, and then maintain the hydration level of their tissues by reducing the number and size of their leaves. This results in a decline in overall biomass. Salt exposure significantly affects the integrity of leaf cell membranes, as indicated by elevated electrolyte leakage in *S. coronaria* leaf tissue. These findings are consistent with those reported by Dallali et al. (2012) in Tunisian populations of *S. coronaria* and S. carnosa, as well as in cotton and sorghum. Studies have shown that the loss of membrane integrity contributes to photosynthetic damage induced by salinity by causing electrolyte leakage (Freitas et al., 2011; Munns & Tester, 2008). Numerous studies have noted a strong correlation between lipid peroxidation and electrolyte leakage, with elevated levels of these parameters recorded in two species of beet. Specifically, heightened MDA and conductivity assessments have been obtained in S. *coronaria* and Medicago truncatula seedlings under osmotic stress.

In the present study, it was observed that salinity reduced chlorophyll content by 22-37% in S. *coronaria* leaves. This corroborates the findings of Dallali et al. (2012) and De Rossi et al. (2021). According to Nunkaew et al. (2014) and Ben Khaled et al. (2003), the deterioration in chlorophyll content could be attributed to the impact of salt on the chloroplast ultrastructure and inhibition of the synthesis of a chlorophyll precursor, 5-aminolevulinic acid.

The results illustrated proven potential for osmotic adjustment, as indicated by an accumulation of osmotic compounds. We observed an increase in soluble sugars in salt-stressed *S. coronaria* leaves compared to the control group, beginning at 3 g/L of NaCl. This increase was found to be proportional to the salt concentration. These findings are consistent with those of many other studies (Nedjimi and Daoud, 2009; Ramezani et al., 2011).

The accumulation of soluble sugars is often highly correlated with the reduction of starch under moderate stress conditions (Khosravinejad et al., 2009).

Soluble sugars can act as osmolytes, protecting specific macromolecules (such as enzymes) and contributing to membrane stability (Bartels & Sunkar, 2005). In addition to soluble sugars, we have also observed an accumulation of proline. As salt stress intensifies, the proline accumulation becomes more pronounced. Similar observations have been made by several authors in different species, including okra (Habib et al., 2012) and an Italian population of *S. coronaria* (De Rossi et al., 2021). Increased proline levels under stress could be due to stimulated proline biosynthesis and/or inhibited proline oxidation. Other researchers have demonstrated a correlation between the accumulation of proline and the activity of enzymes involved in its biosynthesis, including

pyrroline-5-carboxylate reductase and gamma-glutamyl kinase (Székely et al., 2008; Jallele & Azouze, 2009).
On the one hand, there is the inhibition of the activity of enzymes involved in the catabolism of this amino acid, such as proline dehydrogenase (Jallele et al., 2008). According to the findings of De Rossi et al. (2021), *S. coronaria* exhibits antioxidant activity against ROS. In addition to this, an increase in phenolic content and the expression of genes encoding antioxidant enzymes, such as superoxide dismutase and catalase, has also been reported in *S. coronaria* under stressful conditions. These properties help mitigate the negative effects of oxidative stress induced by drought and salinity (Foyer and Noctor, 2005; De Rossi et al., 2021).

With regard to the effect on nitrogen metabolism, we observed that protein content in *S. coronaria* leaves decreased in plants under salt stress, becoming more pronounced at high NaCl concentrations in the culture medium (Table 1). These results are fully in concordance with the findings reported by Tilaki-Ghassem et al. (2016) in the Iranian population of *S. coronaria.*

The deleterious impact of salinity on nitrogen metabolism and decreases in protein content could result from decreased protein biosynthesis, accelerated protein degradation, decreased amounts of amino acids and denaturation of enzymes involved in protein synthesis (Jallele et al., 2008, Swigonska et al., 2014).

5. Conclusion

The findings of this study demonstrate that salt influences on the growth and photosynthetic activity of the Bejaia population (northern Algeria) of *S. coronaria* plants under experimental conditions. This observation suggests that the species can be considered moderately sensitive to salt. The variation in certain physiological parameters indicates that the Tunisian population exhibits higher tolerance to salt stress in comparison to the population examined in this study, maintaining tissue hydration and biomass production at 8 g/L NaCl. However, the *S. coronaria* of Algerian origin utilised in this study (collected in the Bejaia area: northeastern Algeria) demonstrated superior performance in terms of soil salinity resistance when compared to Italian strains. The cultivation of *S. coronaria* has been demonstrated to exert a favourable impact on the productivity of pastures and fallow land. It has also been demonstrated that the species of the genus Sulla respond in different ways (morphological, physiological, biochemical and at the molecular level) to environmental factors.

As demonstrated by numerous scholars, the biodiversity observed within the Sulla genus is likely attributable to their elevated genomic plasticity and subsequent epigenetic variability.

To generalise the results of the study of the behaviour of this species concerning salinity, and given the high diversity that characterises it, it would be advisable to complement this study with other similar studies on other populations of this species. The aim would be to select those that respond better to salinity stress. Incorporating tolerance as a pivotal factor in the enhancement of this species will contribute to the amelioration of marginal soils, particularly in the highlands, which are designated as a semi-arid bioclimatic zone. This species, along with all those whose growth and physiology depend on the rhizosphere, should be the focus of conservation and protection strategies

Authors' contribution
Authors have contributed equally in preparing and writing the manuscript.

Conflict of data/materials
I certify that there is no actual or potential conflict of interest concerning this article.

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