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The *Lepidium sativum* L. (Brassicaceae) recovery ability post salinity stress exposure

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1 ABSTRACT

Salinity causes a major threat on the agriculture nowadays and can alter the global vegetal production's map. The present work focuses on the physiological behaviour of *Lepidium sativum* L. (Garden cress) under salt stress condition and its post-stress recovery. In order to better understand the effects of salinity on the growth of plants, the recovery experiments were conducted in *L. sativum* by measuring physiological parameters on seedlings prior to salt (NaCl) treatment and after culturing them on a salt-free nutrient solution. This study indicates that removal of NaCl from the media results in resumption of growth activity. The increase in leaf biomass exceeds the roots. The results indicated a significant increase in protein, sugar and proline contents that is equivalent to the ones in untreated controls. In addition, the high endogenous accumulation of Na^+ ion, decreased significantly after NaCl suppression, especially in roots. Salt ions provoked an increase in the NH_4^+ concentrations in the leaves and roots parallel to high increase of proteolytic enzymes activities involved in the protein degradation. This was in accordance with the pronounced decrease of dry weight by salt in leaves and roots. Also, protease activity decreased during the recovering time. On the basis of these results, *L. sativum* specie can be able to dilute the effects of NaCl toxicity in cellular compartment during the recovering period. An important conclusion of this work is that a transient contamination of the culture medium by salinity or pollutants is not necessarily followed by a significant depreciation in the product yield and tolerance.

2 INTRODUCTION

Salinity is one of the major environmental stress, limiting crop production. Plant species are diverse in their ability to tolerate saline soils. Each year, 10 to 15% of soils are affected by salinity (Zhang *et al.*, 2017). In arid and semi-arid regions, the salinity result in a high water evaporation from the soil (Munns and Tester, 2008) and irregular and insufficient rainfall (Mezni *et al.*, 2002). Moreover, polluted irrigation water and excessive use of fertilizers are also among the soil salinization causes (Sdouga *et al.*, 2019). Plants respond to salinity with many changes, revealing the multifactorial mechanisms of tolerance and adaptation to this constraint like morphological and anatomical modifications (Poljakoff-Mayber, 1988), physiological (Parida and Das, 2005) and biochemical (Brugnoli and Lauteri, 1991) mechanisms involving enzymatic activity (Chaffei *et al.*, 2004). The response of plant to salinity depends on the species, the variety, the salt concentration and the development stage of the plant (Ben Naceur *et al.*, 2001). In addition, plants develop several mechanisms to ensure

their growth and development cycle. Some glycophyte species use the exclusive strategy linked to exclusion and compartmentation of salt (Zid and Grignon, 1991; Alem and Amri, 2005). While, halophyte species adopt inclusive strategy linked to osmotic adjustment, ion homeostasis and accumulation of soluble osmolytes (Zhang *et al.*, 2019). Salinity affect many morphological and physiological function and mechanisms in plant like growth (Zhang *et al.*, 2016), plant water status (Munns *et al.*, 2005), photosynthetic pigments and activities (Chen *et al.*, 2015; Wang *et al.*, 2017; Goussi *et al.*, 2018), and chloroplast ultrastructure (Islam *et al.*, 2017). A lot of work has focused on the identification and the use of salt-adapted species or cultivars in saline areas (Debez *et al.*, 2013; Ben Hamed *et al.*, 2016) or the use of treatments to alleviate the effects of salinity stress to ensure crop production, in such adverse conditions. However, very little information about the recovery of plant, after salt exposure, is available. In fact, the majority of studies on plant response to salinity don't incorporate the plant ability to recover from stress. However, studying the post-stress behaviour is fully justified from an environmental and fundamental point of view, since it would provide a better understanding of the adaptive strategies of plants in their natural environment. Indeed, the plant is not continuously subjected to this kind of stress, as salt leaching following precipitation can occur. The present work focuses on the physiological behaviour of *L. sativum* plant under and post salt stress.

3 MATERIALS AND METHODS

L. sativum plant growth parameters, dry weight, height, Na^+ content in leaves and roots, and plant symptoms of NaCl toxicity, were measured at 10 days after NaCl exposure. *L. sativum* plants in each treatment were harvested and rinsed thoroughly with distilled water, separated into leaves and roots, then dried at 80°C and weighed. Soluble protein, total soluble sugars, proline and NH_4^+ contents was performed. In addition, protease enzyme activity (EC 3.4.21.62) was determined.

3.1 Determination of soluble sugars: The extraction is realized starting from 25 mg of dry vegetable matter in the presence of 5 ml of 80% ethanol. The samples are placed in a water bath at 70°C for 30 minutes. After cooling, the samples are centrifuged at 6000 g for 15 minutes. Subsequently, 25 µl of supernatant is removed, and added to 5 ml of anthrone solution (anthrone + sulfuric acid + distilled water) in test tubes under a fume hood. After, these tubes are put in a water bath at 100 °C for 10 minutes, then placed in the ice. The reading of optical density is at 640 nm (Yemm and Willis, 1954).

3.2 Determination of proline: It is a colorimetric assay based on the proline-ninhydrin complex from a mixture of about 100 mg powdered vegetable material and 1.5 ml of sulfosalicylic acid. The mixture is centrifuged at 12 000 g for 20 minutes at 4°C. The assay is carried out by mixing 500 µl of the extract with 500 µl of sulfosalicylic acid, 1 ml of reagent composed of ninhydrin, glacial acetic acid and phosphoric acid, and 1 ml of concentrated acetic acid. Tubes are incubated in a water bath at 100°C for 1 h and then cooled to 4 °C. The acid mixture catalyses the proline-ninhydrin reaction during which the solution turns red. To separate the phases, 2 ml of toluene are added. The optical density is determined at 520 nm and the proline concentration is determined by reference to a calibration curve, which is expressed in µg/mg FW (Bates *et al.*, 1973).

3.3 Total soluble protein: Soluble proteins are assayed according to the method of Bradford (Bradford, 1976). The principle of this method is the formation of complexes between Coomassie blue and the basic protein residues. A volume of 10 µl of protein extract is added to 200 µl of Bradford reagent. After 5 minutes of incubation at room temperature and in the dark, the absorbance is measured at 595 nm and compared to that of a standard range of bovine serum albumin (BSA).

3.4 Ammonium (NH_4^+) assay: A mass of 0.5 to 1 g of the plant material, previously frozen

in liquid nitrogen, is ground at 4 °C in a mortar in the presence of 2 ml of H_2SO_4 and 0.5% of polyclart AT. The ground material is centrifuged for 15 min at 30 000 g. The obtained supernatant is placed for 30 min at 37 °C and the absorbance is measured at 620 nm (Weatherburn, 1967).

3.5 Determination of protease activity: Protease activity was adapted from Brouquisse *et al.* (2001). In a mortar, 100 mg of fresh plant material already stored in liquid nitrogen is mixed with 1 ml of extraction buffer containing (50 mM Tris-HCl, pH=7.5, 5% mercaptoethanol, PVP). The centrifugation is carried out at 140 000 g for 30 minutes at 5 °C. The supernatant obtained will be used for the determination of proteases by the addition of reaction medium (270 µl of 0.4% azocaetin, 270 µl of enzymatic extract, 270 µl of incubation buffer). The whole is subjected to an incubation of 3 h at 37 °C whose reaction is stopped by the addition of 320 µl TCA 5%. The optical density is read at 330 nm following centrifugation at 15 000 g for 10 minutes at 5 °C and for enzymatic activity test.

3.6 Statistical analysis: Data shown are means of three replicates for each treatment. Analysis of variance between treatments means was carried out with the SPSS 10.0 program. Means were compared using the Duncan's test at $P < 0.05$.

4 RESULTS

4.1 Growth parameters: Plant growth was reduced in NaCl treatments. Leaves showed chlorosis symptoms when grown at 100 mM NaCl, and root browning was also observed in salinity treatment (Fig. 1).

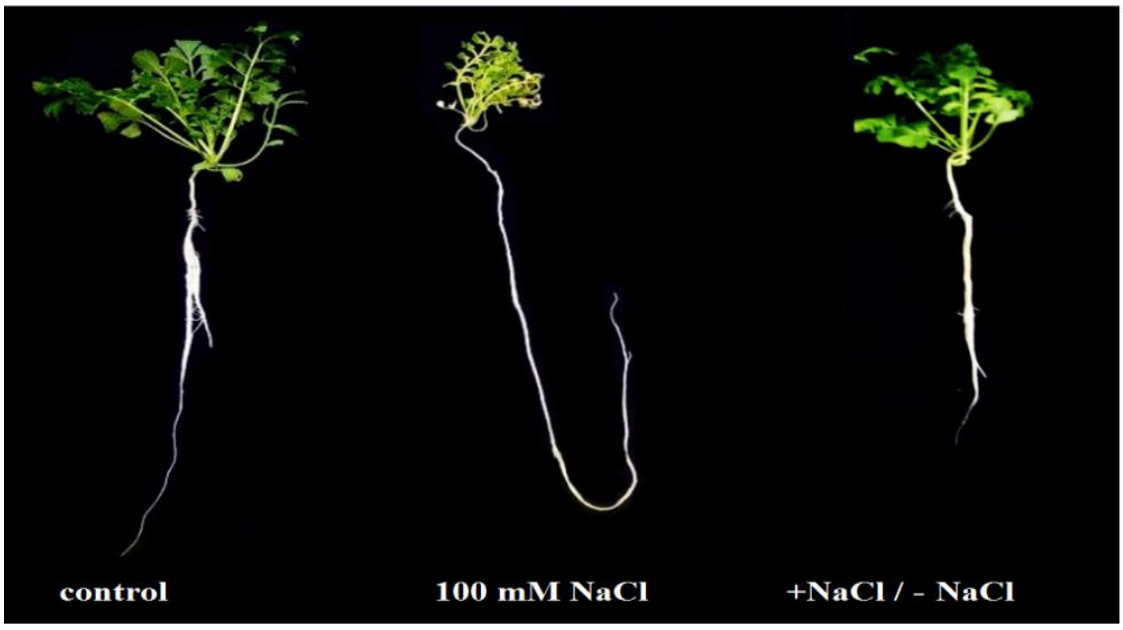

Fig. 1. NaCl and recovery effects on morphological state of *Lepidium sativum* 10 days after treatment

Moreover, a significant increase in the growth state was obtained after NaCl removal. At 100 mM of NaCl treatment, dry weight decreased significantly compared to the control values, and decreases were higher for leaves (-23.71%) than roots (19.89%). After NaCl removal, a significant increase in dry matter was obtained compared to salt treatment. The results showed an insignificant decreases of dry matter in leaves (-10.16%) and roots (-8.28%) compared to control condition (Table 1).

Table 1. NaCl and recovery effects on dry weight (DW), Na^+ content in leaves and roots of *Lepidium sativum* 10 days after treatment

	Leaves			Roots		
	Control	**+100 mM NaCl**	**+/-100 mM NaCl**	**Control**	**+100 mM NaCl**	**+/-100 mM NaCl**
Dry weight (mg)	34.25b	26.13a	30.77ab	153.88d	123.27c	141.14d
Na^+ (µeq/g DW)	2×10^3a	36.6×10^3d	31.1×10^3cd	6.6×10^3b	36.6×10^3d	26.22×10^3c

Means values followed by the same letter (s) are not significantly different at $p \leq 0.05$

4.2 Sodium content: Na^+ content increased significantly in all organs at 100 mM of NaCl in the nutrient solution (Table 1). The Na^+ content was more pronounced in leaves than in roots. In fact, the values were 18.3 and 5.6 times more important respectively in leaves and roots compared to control. However, when salt is removed from the nutrient solution, the increase of Na^+ becomes less important than in presence of 100 mM of NaCl, but remains higher than the control with values 15.6 and 4 times more important respectively in leaves and roots (Table 1).

4.3 Ammonium content: Figure 2 shows the NH_4^+ accumulation in plant. In fact, this ion is increased in all organ of *L. sativum* plant in presence of 100 mM of NaCl. The increase of ammonium content was 196.76% and 145.6% respectively, in leaves and in roots. The return of the plants to the nutrient solution devoid of NaCl leads to a decrease in the NH_4^+ content compared to 100 mM NaCl treatment. But the level of NH_4^+ remains more important than in control condition.

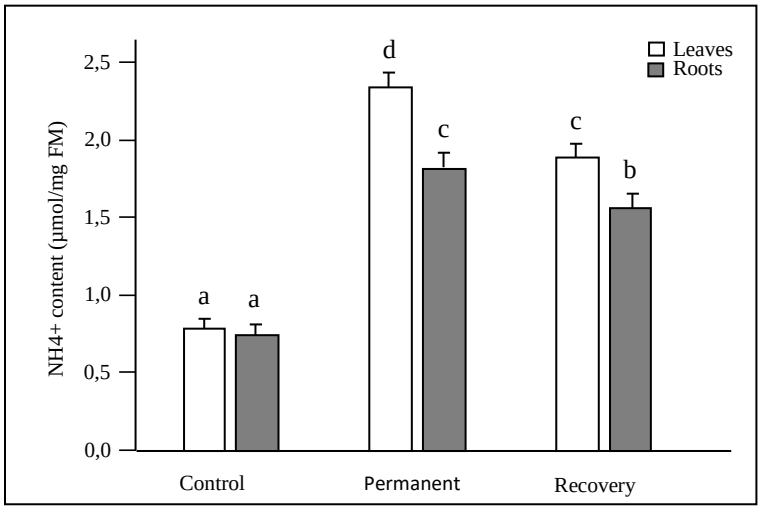

Fig. 2. NaCl and recovery effects on ammonium content in leaves and roots of *Lepidium sativum* 10 days after treatment. Means values followed by the same letter are not significantly different at $p \leq 0.05$

4.4 Proline content: Proline accumulation is one of the adaptive strategies of plant in response to environmental constraints. Plant metabolism is disrupted by saline stress, of which proline is a marker of plant tolerance to abiotic stress. The application of 100 mM of NaCl resulted in a significant increase in proline content at the plant level. The percentage

increase was respectively 93.62% at the foliar level and 181.71% at the root level compared to the control (Fig. 3). The analysis of the obtained results, shows that the removal of NaCl from the culture medium induces a decrease in proline content compared to plants grown on culture medium containing 100 mM NaCl. The reduction was 42.24% at the leaf level and 46.55% at the root level. In addition, the proline content in leaves after NaCl removal increased insignificantly compared to leaves of control plant (+11.84%). However, in roots, the proline content remains more important after NaCl removal than control (+50.58%) (Fig. 3).

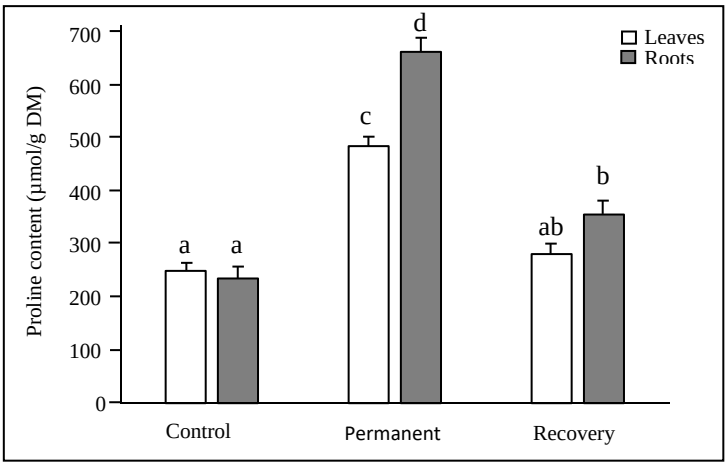

Fig. 3. NaCl and recovery effects on proline content in leaves and roots of *Lepidium sativum* 10 days after treatment. Means values followed by the same letter are not significantly different at $p \leq 0.05$

4.5 Soluble sugar and protein contents: The accumulation of soluble sugars increases in plant at 100 mM NaCl compared to control condition (Fig. 4). This increase was more pronounced in roots (+22%) compared to leaves (11.29%). After removal of salinity, the soluble sugar content shows values close to those obtained on the controls plant, especially for the leaves.

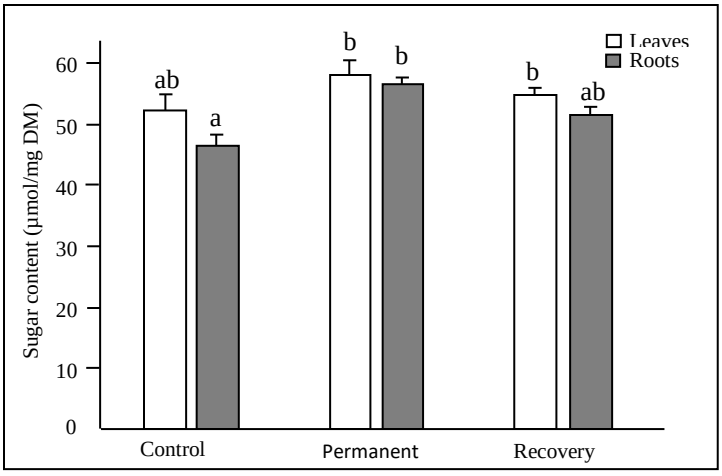

Fig. 4. NaCl and recovery effects on sugar content in leaves and roots of *Lepidium sativum* 10 days after treatment. Means values followed by the same letter are not significantly different at $p \leq 0.05$.

The soluble protein content was slightly increased in plant at 100 mM of NaCl (Fig. 5). This increase was 13.87% in leaves and 6.94% in roots compared to control organs. However, after removal of salinity, the soluble protein content shows values comparable to those obtained on the controls plant or even less important.

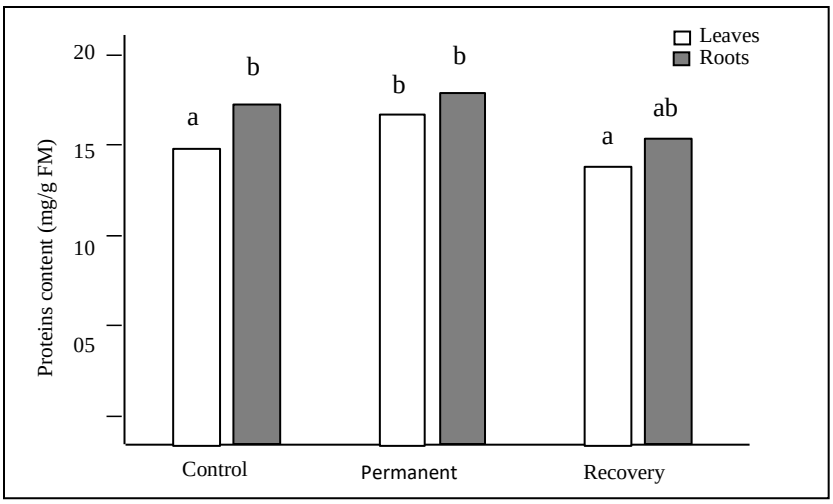

Fig. 5. NaCl and recovery effects on protein content in leaves and roots of *Lepidium sativum* 10 days after treatment. Means values followed by the same letter are not significantly different at $p \leq 0.05$.

4.6 Protease activity: The activity of protease enzyme involved in protein degradation in roots and leaves extracts of *L. sativum* plant was measured (Fig. 6). Activities of protease enzyme increased 1.5 times both in leaves and in roots, with 100 mM of NaCl treatment. Leaf and root extracts, from plants grown after removal 100 mM of NaCl, showed significant decreases in the protease activities almost the same values of control plants (Fig. 6).

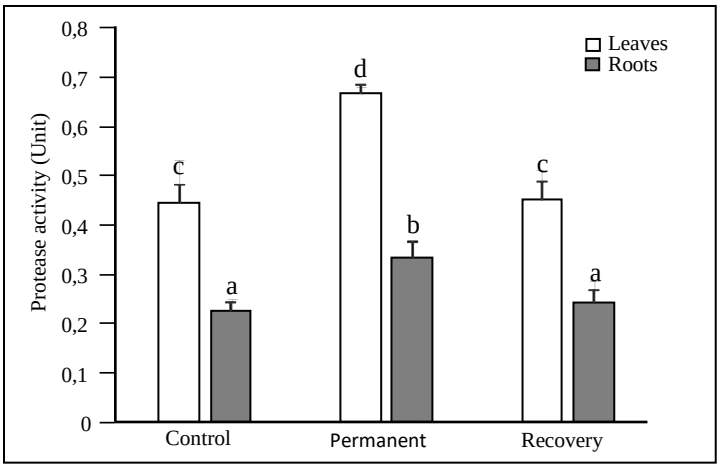

Fig. 6. NaCl and recovery effects on protease activity in leaves and roots of *Lepidium sativum* 10 days after treatment. Means values followed by the same letter are not significantly different at $p \leq 0.05$.

5 DISCUSSION

Salinity is a major stress affecting plant growth and development (Bouaouina *et al.*, 2000). The response of plants to saline stress is studied, in this work, by evaluating morphological, physiological, biochemical and mineral parameters. The study of the growth and mineral nutrition of *L. sativum* grown in the presence of 100 mM of NaCl shows that the response varies according to the type of organ. In fact, at 100 mM of NaCl, a significant reduction was observed especially for the leaves growth. The inhibition of growth activity by NaCl is a general behaviour characterizing glycophytes plants. However, the biomass reduction of *L. sativum* at 100 mM is not very important compared to the control condition, which indicates consequently a relative tolerance of this specie to saline stress. In fact, the plant can avoid damage by reducing growth (Zhu, 2002). It was an adaptive capacity necessary for the survival of a plant exposed to abiotic stress. In addition, many studies explain that the plant growth reduction under saline conditions may be due to disruption of water supply and/or excessive accumulation of toxic ions in tissues (Wang *et al.*, 2015). Also, an increase on the biomass allocation to the roots was observed in response to stress due to water deficit (Farhat *et al.*, 2019). This study shows that the exposure of plants to a 100 mM dose of NaCl leads to a little increase in protein synthesis, which proves that salt stimulates alternative pathways of mineral nitrogen reduction and uptake. In addition, the study results show an increase of protease activity only on the leaves. This result proves the change in cellular metabolisms (anabolic and catabolic) in presence of salinity. The high ammonium accumulation in plant may be related to this metabolism change under salt stress. In addition, the changes induced by saline stress on the accumulation of certain metabolites have been estimated, such as proline, since it is one of the adaptive strategies triggered by the plant in response to environmental stresses. For many Monocotyledons and Eudicotyledons, proline accumulates in both glycophytes and halophytes (Zhang *et al.*, 2019). Proline is also involved in the regulation of gene expression in response to saline stress (Debez *et al.*, 2013; Ben Hamed *et al.*, 2016). This study results are in agreement with these works. A very significant increase in proline content was reported at the leaf and root levels. This increase was more pronounced in roots compare to leaves. The accumulation of soluble sugars is one of the adaptive strategies under stress conditions (Parida and Das, 2005; Debez *et al.*, 2013). The presence of NaCl stimulates the accumulation of soluble sugars mainly at the root level. Many studies have shown that the accumulation of soluble sugars is necessary to stabilize membranes and proteins, neutralize the osmotic imbalance imposed by the saline constraint (Ashraf and Foolad, 2007) and thus improve carbonaceous metabolism by providing carbonaceous skeletons. The analysis of growth and biochemical parameters after removal of NaCl from the nutrient base solution indicates that the plant is able to restore its growth and mechanisms. In addition, the analysis of physiological parameters after return to the base nutrient solution without NaCl indicates that the plant is capable of restoring its stress indicators. In fact, a decrease in proline, sugar contents and protein contents, both in leaves and in roots is noticed. On the other hand, despite the alteration of various physiological and biochemical parameters under saline stress, this species has shown a strong capacity for recovery following stress removal. All parameters studied were significantly restored, and the recovered plants on a salt-free environment were largely similar to the control plants. Similar results were obtained in halophyte plant under salt stress (Ellouzi *et al.*, 2013). This recovery capacity linked to photosynthetic protection, chlorophyll biosynthesis, restoration of nutritional status, reduced proline and sugar content and increased enzyme activities (Ben Fattoum *et al.*, 2016). It should be noted that little studies have assessed the ability of plants to recover after exposure to saline stress. Other studies have shown that the ability to recover after exposure to water deficiency or salinity is related to deep arrangements at the proteome

level. Indeed, proteomic studies have revealed specific proteins that contribute to salt tolerance and survival of plants under abiotic stress (Debez *et al.*, 2012; Wang *et al.*, 2015; Farhat *et al.*, 2019). These proteins are linked to photosynthesis, osmotic and ionic homeostasis. The main results in this study show clearly the physiological and biochemical capacity of leaves and roots of *L. sativum* plants to dilute the Na^+ concentration in cellular compartment after removal of NaCl and revealed the recovery ability of this specie to salt stress.

6 CONCLUSION

In conclusion, the NaCl toxicity effects were significantly alleviated such as physiological and biochemical parameters after salinity removal in *L. sativum*. This recovery ability is linked firstly to reducing endogenous Na^+ levels, then increasing the leaves and roots biomass, and also resulting to the less Na^+ accumulation and more osmotic and ionic homeostasis.

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