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Physiological Impact of Seed Priming with $CaCl_2$ or Carrot Root Extract on *Lupinus termis* Plants Fully Grown under Salinity Stress

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SEEDS of *Lupinus termis* (cv. Gemmeza R_2) were primed by presoaking for six hr in 100% aqueous extract of carrot roots or 10mM $CaCl_2$ solution, sown and left to grow for 30 days on clay-sandy soil (2:1 w/w). Seven-day-old seedlings grown from primed and unprimed seeds were exposed to salinity stress at 150mM NaCl. Salinity stress caused decreases in lengths, fresh and dry weights of roots and shoots, leaf water content and photosynthetic pigments. Total soluble sugars, proteins, alkaloids, malondialdehyde (MDA), catalase and peroxidase activities and ascorbate content were elevated by salinity stress. Priming of lupine seeds with $CaCl_2$ or carrot root extract reversed all of the previous mentioned decreases and increases. Transmission electron microscope results revealed that salinity caused detachment of the plasma membrane from cell walls, degeneration of chloroplast membranes, disorganization of grana, disappearance of some nucleoli and the appearance of some abnormal nuclei. Seed priming preserved the intact cell wall structure, integrity of chloroplast membranes, normal grana organization and nuclear structure with well-defined nucleoli, comparable to those of the control seedlings.

Keywords: Antioxidants, Calcium, Carrot, *Lupinus termis*, Priming, Salinity, Ultrastructure.

Introduction

Salinity is a major abiotic stress which limits plant growth and productivity as a result of water deficit, ionic toxicity, nourishment disorders, oxidative stress, alteration of metabolic processes, membrane disorganization and reduction of cell division and enlargement (Zhang & Shi, 2013). The foremost harmful impact of salinity is the accumulation of Na^+ ions in plant tissues thus inhibiting the uptake of K^+ which is an essential macronutrient for plant growth and development, leading to low productivity and may even cause death (Gupta & Huang, 2014). Among the more deleterious effects of salinity stress on plants are the significant reductions in various growth parameters (Rahneshan et al., 2018), photosynthetic pigments and activity (Negrão et al., 2017), antioxidants (Akladious & Abbas, 2013), the drastic changes in carbohydrate, lipid and protein profiles (Kasim & Hamada, 2003; Sadak et al., 2017), as well as several ultrastructural alterations (Acosta-Motos et al., 2017; Navarro et al., 2007). Bejaoui et al. (2016) have shown that salinity resulted in lipid peroxidation in leaves as indicated by significant increases in malondialdehyde (MDA) content, swelling of thylakoids in the mesophyll tissue of *Sulla carnosa* and in the spongy tissue of *Sulla caronaria*.

According to Morgan et al. (2014), calcium plays a vital role in the regulative mechanisms that plants activate to rectify the adverse effects of salinity, to assist in maintaining structural and functional integrity of membranes and to regulate ion homeostasis.

The harmful effects of different abiotic stresses are often counteracted by utilization of natural plant extracts like carrot root extract (Abbas & Akladious, 2013). Carrot (*Daucus carota* L.) contains an abundance of growth stimulating compounds like vitamins (A, B1, B2, B6, C, D and E), amino acids, sugars, carotenoids, flavones, proteins and fibers as revealed by the HPLC analysis (Kasim et al., 2017).

Lupine (*Lupinus termis*) is among the most important legume crops in Egypt with high nutritional and medicinal values owing to the

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relatively high protein and oil contents of its seeds (35-45% and 10-15%, respectively), as reported by Akladious & Hanafy (2018). Hence, the aim of the present study was to evaluate the impact of salinity stress on the growth, physiological processes and foliar ultrastructure of *Lupinus termis* and to investigate the role of seed priming with either aqueous extract of carrot roots or $CaCl_2$ solution in the alleviation of possible harmful effects of salinity stress.

Materials and Methods

Seeds of *Lupinus termis* (cv. Gemmeza R_2) were provided by the Agricultural Research Center, Giza, Egypt and were selected for apparent uniformity of size and form. Fresh carrot roots were obtained from the local market.

Carrot root extract was prepared according to Sofowora (1982) with some modifications (Kasim et al., 2017) as 200g/L and considered as 100% extract. A 10mM aqueous calcium chloride ($CaCl_2$) solution was prepared.

Based on the results of a preliminary experiment, lupine seeds were divided into four groups, the first group was primed by soaking for six hrs in tap water (as control), the second group was left unprimed to represent the stress treatment, the third and fourth groups were primed for six hrs in 100% carrot root extract and 10mM $CaCl_2$ solution, respectively.

Every treatment was represented by three replica of plastic pots (25cm diameter and 20cm depth), each was filled with 5kg of clay-sandy soil (2:1 w/w). In each pot, ten primed seeds were sown and irrigated with tap water once daily for 3 days, then twice weekly. At the 7^{th}, 14^{th} and 21^{st} days of sowing, seedlings were irrigated with 150mM NaCl solution to the field capacity. Seedlings were left to grow until 30 days of growth under the environmental conditions (16/8hr day/night at 25°C/15°C± 2 day/night and relative humidity of 65%).

At the end of experiment (30-day- old), growth criteria (root length, shoot height, fresh and dry mass of roots and shoots, water content of leaves and leaf area) were determined. Photosynthetic pigments (Chl. *a*, Chl. *b* and carotenoids) were determined according to the methods described by Metzner et al. (1965). Total soluble sugars and proteins were determined by the methods of Irigoyen et al. (1992) and Bradford (1976), respectively. Total alkaloids were determined following the method of Harborne (1973). The activities of catalase [EC1.11.1.6] and peroxidase [EC1.11.1.7] were assayed according to Kato & Shimizu (1987). The non-enzymatic antioxidant, ascorbic acid (AA) was estimated as described by Oser (1979). Malondialdehyde (MDA) concentration was calculated following the method of Heath & Packer (1968). All the previous measurements were determined in lupine leaves. Specimens of leaves were prepared for TEM using the procedures of Reynolds (1963).

Statistical analysis of results were presented as the mean of three replicates and standard deviation (SD). Data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) to determine the significance of difference using CoState (6.311) statistical software program for Windows. Comparison of the main effects was performed using the Least Significance Difference (LSD) from the control (Bishop, 1983).

Results

Growth criteria

Figure 1 indicates that salinity stress caused a highly significant decrease in root length and shoot height, with percentages of 37.75% and 32.66%, respectively, relative to the control. Similarly, fresh mass of roots and shoots were decreased by 70.66% and 58.8%, respectively, while their dry masses were reduced by 42.8% and 51.7%, respectively, compared to the control (Figs. 2, 3). A similar trend was recorded in leaf area and water content, where they were reduced by 37.75% and 24.43%, respectively, relative to the control (Figs. 4, 5).

Priming of lupine seeds with either $CaCl_2$ or carrot extract led to a significant recovery and increased all the determined growth parameters, compared with the corresponding stressed plants, The ameliorative effect of carrot extract was more obvious than that of $CaCl_2$.

Photosynthetic pigments

Figures 6, 7 show that salinity caused highly significant reductions in Chl. *a*, Chl. *b*, total chlorophyll and carotenoids, where the percentages of decreases were 33.6%, 40.2%, 35.7% and 50%, respectively relative to the control. Priming lupine seeds with either $CaCl_2$ or carrot extract showed a significant amelioration of the harmful effects of salinity, where Chl. *a*, Chl. *b*, carotenoids and total chlorophyll were considerably elevated by 77.2%, 101.6%, 84.3% and 104.6%, respectively compared to the stressed plants. The increase was more pronounced in case of carrot extract compared to $CaCl_2$.

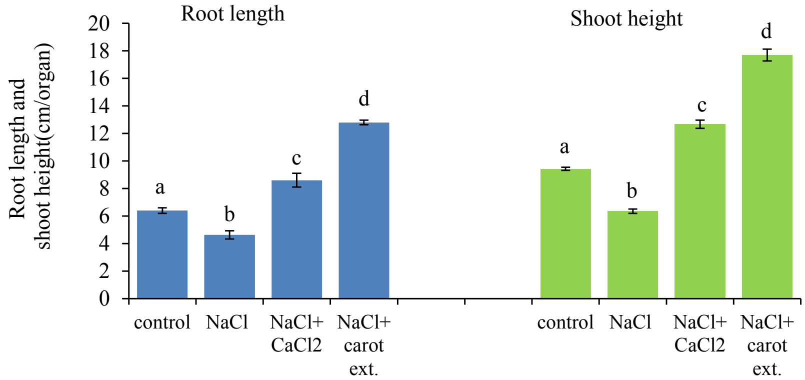

Fig.1. Effect of seed priming with $CaCl_2$ or carrot root extract on root length and shoot height of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

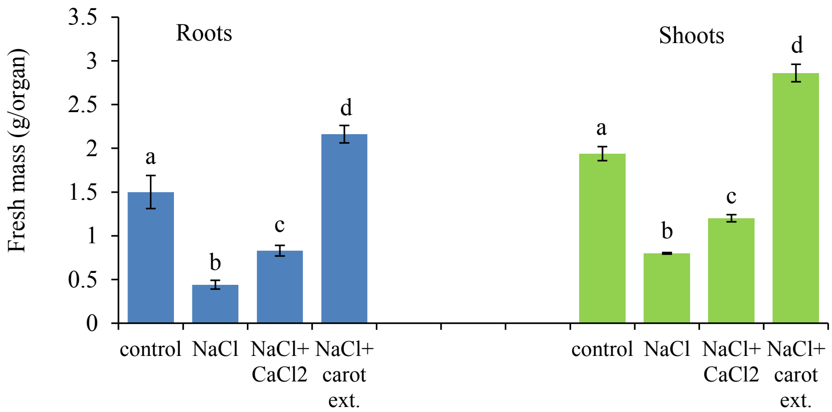

Fig. 2. Effect of seed priming with $CaCl_2$ or carrot root extract on fresh mass of roots and shoots of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

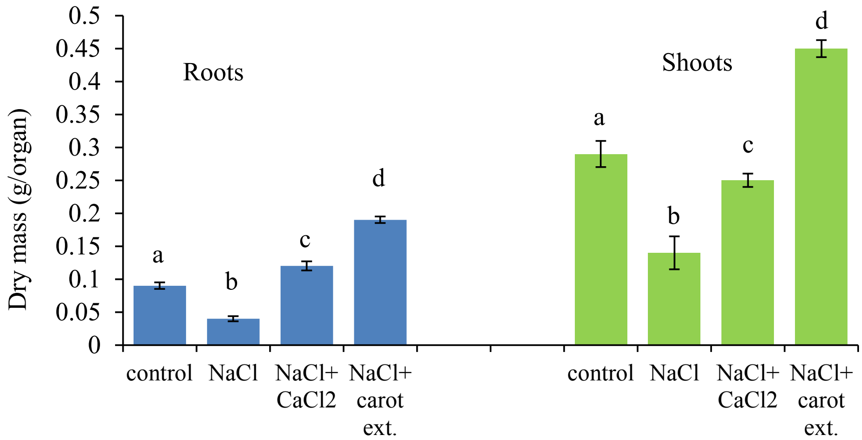

Fig. 3. Effect of seed priming with $CaCl_2$ or carrot root extract on dry mass of roots and shoots of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

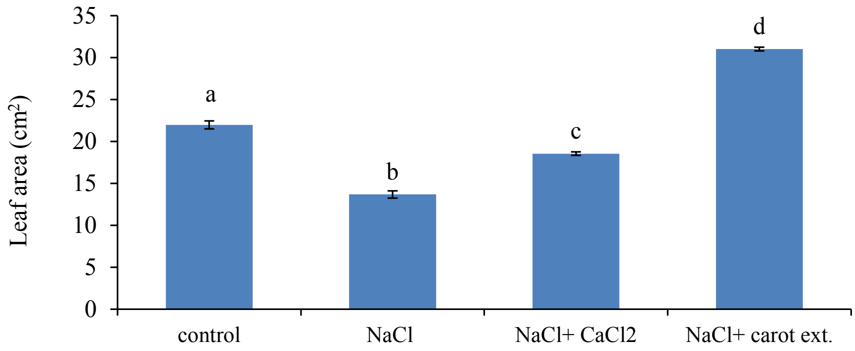

Fig. 4. Effect of seed priming with $CaCl_2$ or carrot root extract on leaf area of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences ($P\leq 0.01$)].

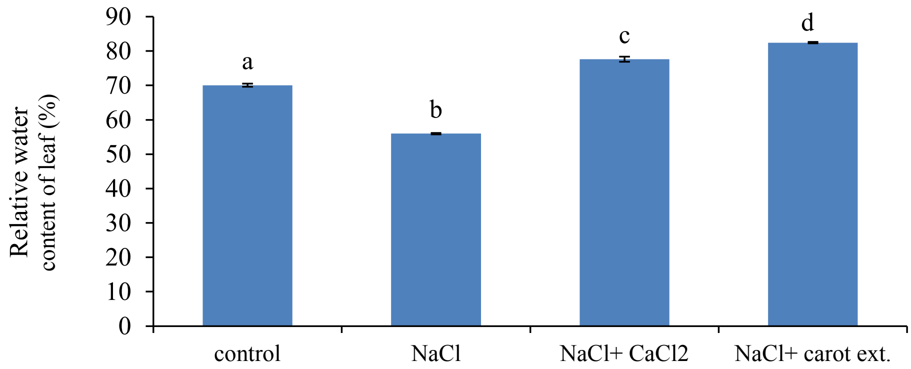

Fig. 5. Effect of seed priming with $CaCl_2$ or carrot root extract on leaf relative water content of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences ($P\leq 0.01$)].

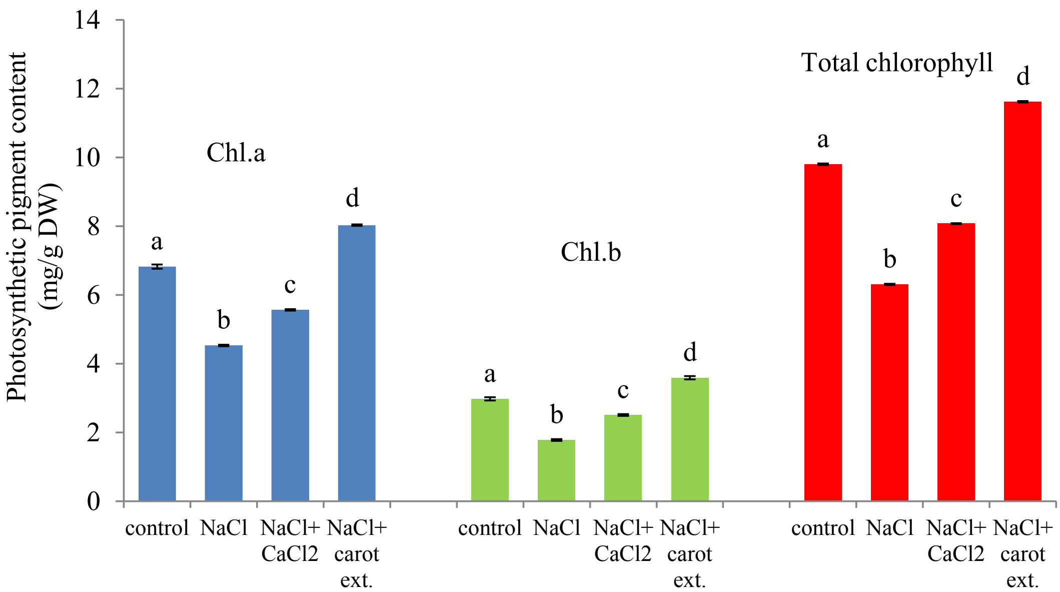

Fig. 6. Effect of seed priming with $CaCl_2$ or carrot root extract on photosynthetic pigment content (Chl. *a*, Chl. *b* and total chlorophyll) of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences ($P\leq 0.01$)].

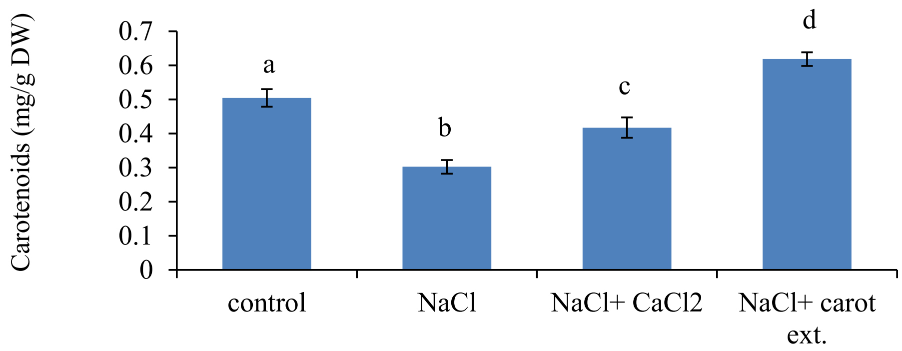

Fig. 7. Effect of seed priming with $CaCl_2$ or carrot root extract on carotenoid content of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

Total soluble sugars, proteins and alkaloids

Figure 8 indicates that salinity caused a 72.1% increase in the total soluble sugar content, while priming of lupine seeds with either $CaCl_2$ or carrot extract resulted in a significant decrease in total soluble sugars, compared to the stressed plants.

Salinity caused a highly significant increase in total soluble protein content of leaves by 14.14%, compared with the control (Fig. 9). Seed priming with $CaCl_2$ resulted in a negligible increase of 0.1% in salinity-stressed lupine leaves, while carrot root extract was more effective (17.89%), compared to the stressed plants.

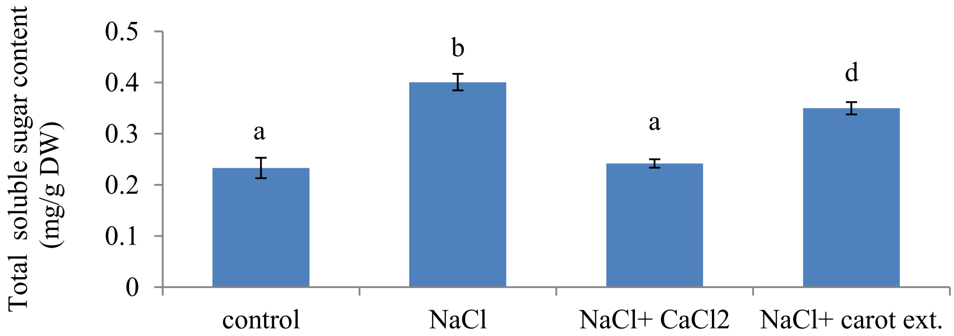

Fig. 8. Effect of seed priming with $CaCl_2$ or carrot root extract on total sugar of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

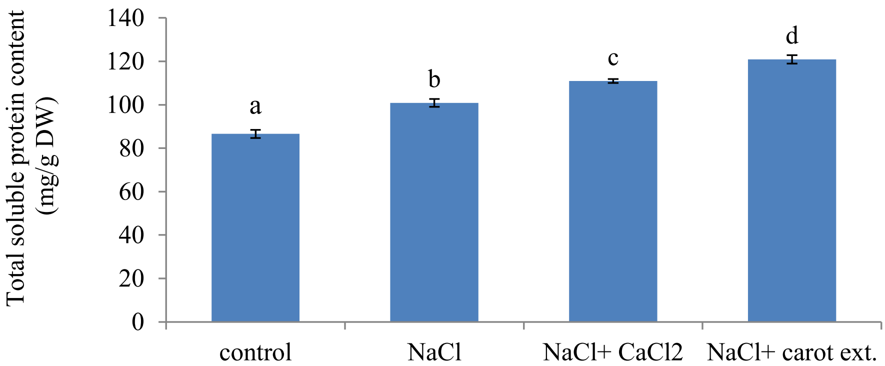

Fig. 9. Effect of seed priming with $CaCl_2$ or carrot root extract on total soluble protein content of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

Results in Figure 10 indicate that salinity resulted in a significant increase in total alkaloid content of leaves. Meanwhile, presoaking of lupine seeds in either $CaCl_2$ solution or carrot extract reduced it significantly by 26.3% and 35.9%, respectively, compared with the NaCl-stressed plants.

Malondialdehyde content

As shown in Fig. 11, MDA content increased significantly under salinity stress by 34.6%, relative to the control. Seed priming in carrot or $CaCl_2$, MDA content was remarkably reduced, compared to that of the stressed plants.

Antioxidants

The results in Figs. 12-14 reveal that salinity resulted in a highly significant increase of catalase and peroxidase activities and ascorbate content. The percentage of increases were 66.7%, 78.6% and 38.6%, respectively, compared to the control. Seed priming with $CaCl_2$ solution or carrot extract alleviated the harmful effects of salinity stress, resulting in remarkable reductions in the activities of peroxidase, catalase and ascorbate content, compared to salinity stressed plants.

Ultrastructural alterations

TEM images of lupine leaves in Fig. 15 showed a well-preserved plasma membrane closely adhering to the cell wall with clearly defined intercellular spaces in the control plants. The stress treatment of NaCl caused detachment of plasma membrane from cell wall, leading to development of apoplastic space between cell wall and plasma membrane (plasmolysis). In contrast, seed presoaking in either $CaCl_2$ solution or carrot root extract restored the original membrane structure and shape.

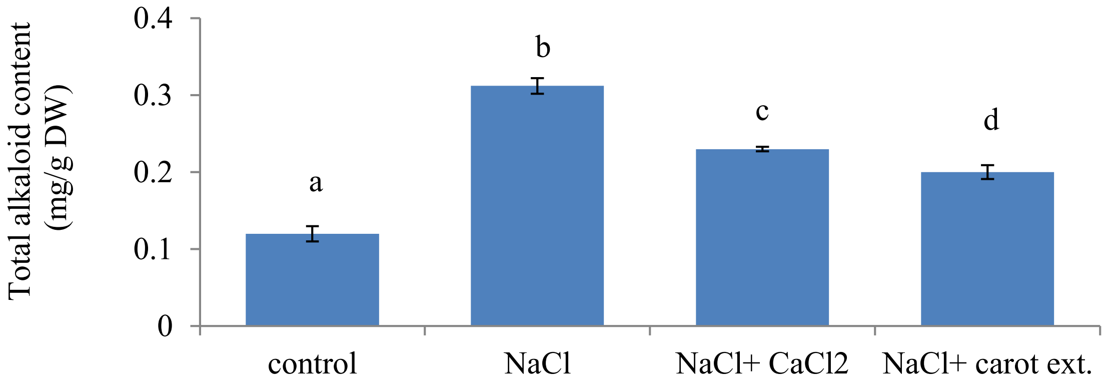

Fig. 10. Effect of seed priming with $CaCl_2$ or carrot root extract on total alkaloid content of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

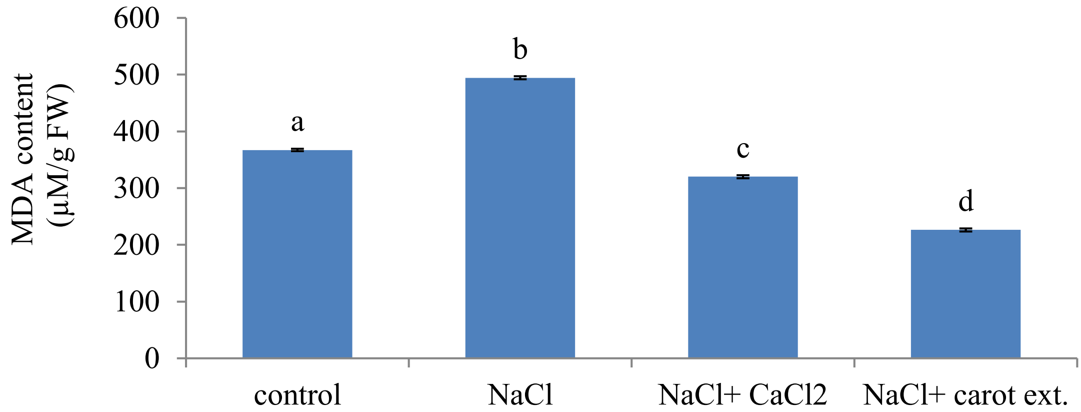

Fig. 11. Effect of seed priming with $CaCl_2$ or carrot root extract on malondialdehyde content of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

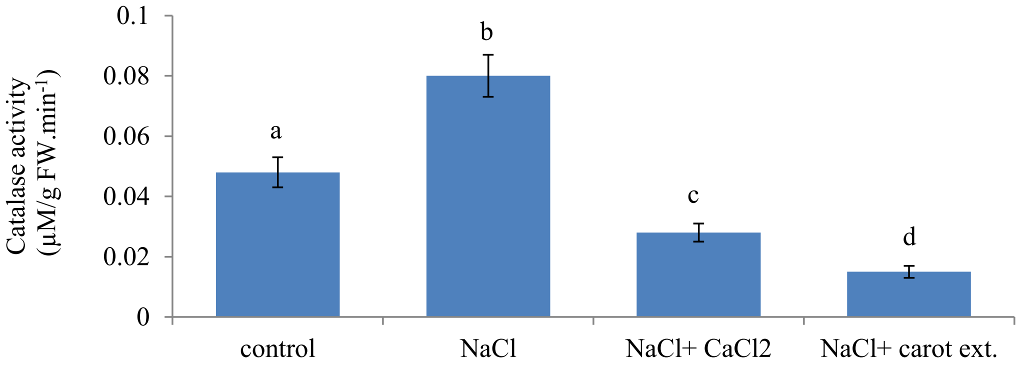

Fig. 12. Effect of seed priming with CaCl$_2$ or carrot root extract on catalase activity of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

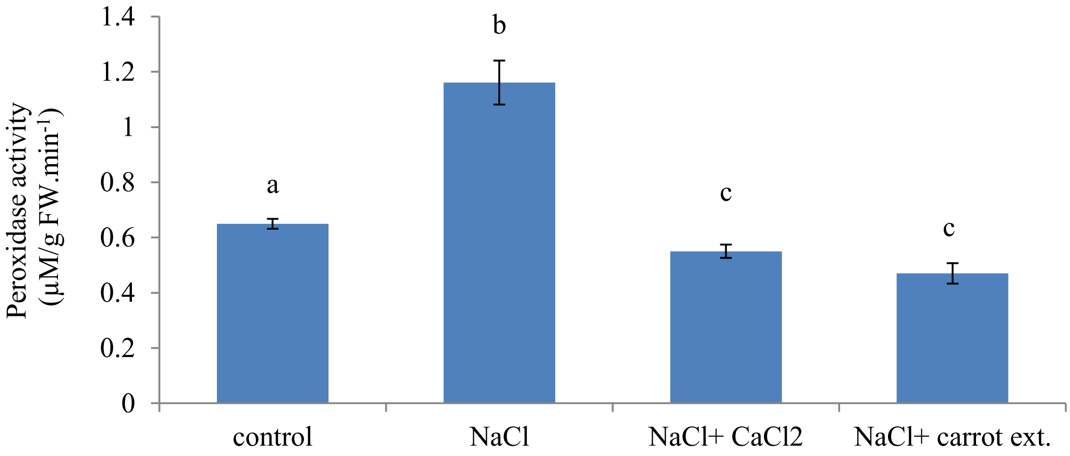

Fig. 13. Effect of seed priming with CaCl$_2$ or carrot root extract on peroxidase activity of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

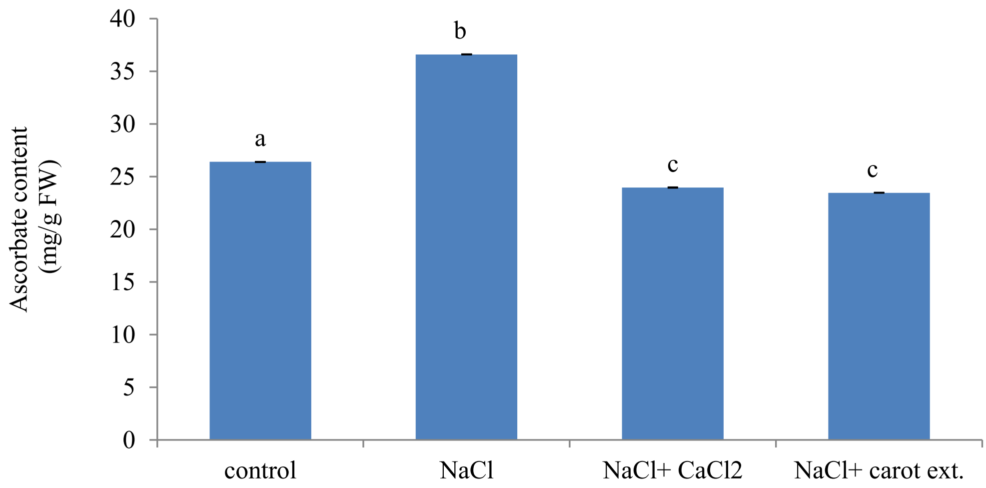

Fig. 14. Effect of seed priming with CaCl$_2$ or carrot root extract on ascorbate content of 30-day-old *Lupinus termis* plants grown under salinity stress [Values are means of three replicates ± SD, different letters indicate statistically significant differences (P≤ 0.01)].

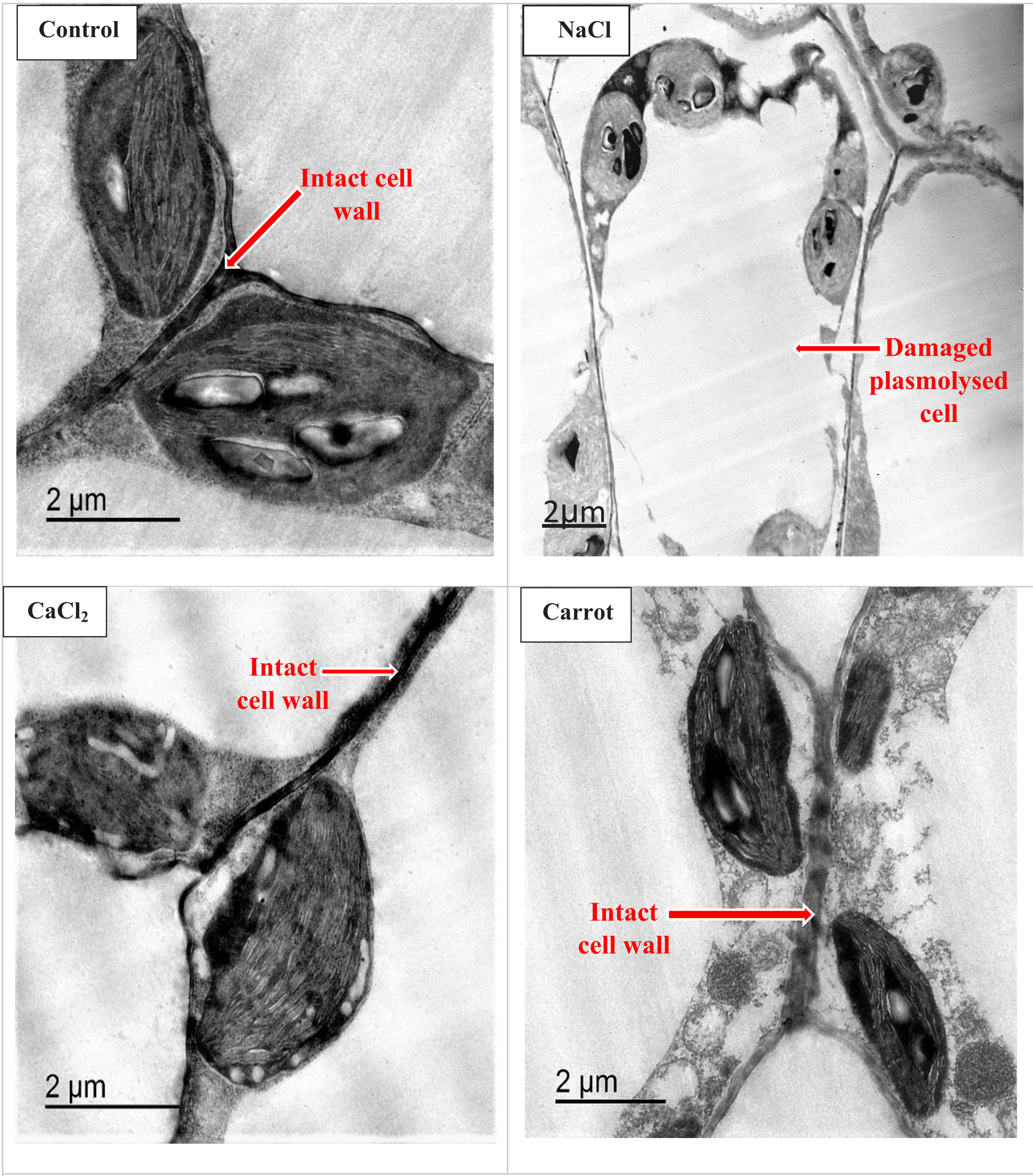

Fig. 15. Electron micrographs showing the effect of seed priming with $CaCl_2$ or carrot root extract on cell wall and intercellular space of leaflets of 30-day-old *Lupinus termis* plants grown under salinity stress. Control: Intact cell wall structure and clear intercellular space. NaCl treatment: Separation of the protoplasmic content away from cell wall. Primed seeds in $CaCl_2$: Normal cell wall structure and intercellular space. Primed seeds in carrot extract: Normal cell wall structure and intercellular space.

Figure 16 showed uniform chloroplasts in lupine leaves of control plants with an almost oval shape and few starch grains. Their thylakoid membranes were intact with organized grana. NaCl treatment induced a marked change in the chloroplast ultrastructure as some chloroplast membranes were degenerated leading to clearly disorganized grana. Priming with $CaCl_2$ solution caused a partial improvement in the chloroplast structure. Priming with carrot extract resulted in perfect alleviation of the disruptive effect of salinity on chloroplasts, which regained their uniformity and appeared with well-organized grana similar to those in the control specimens.

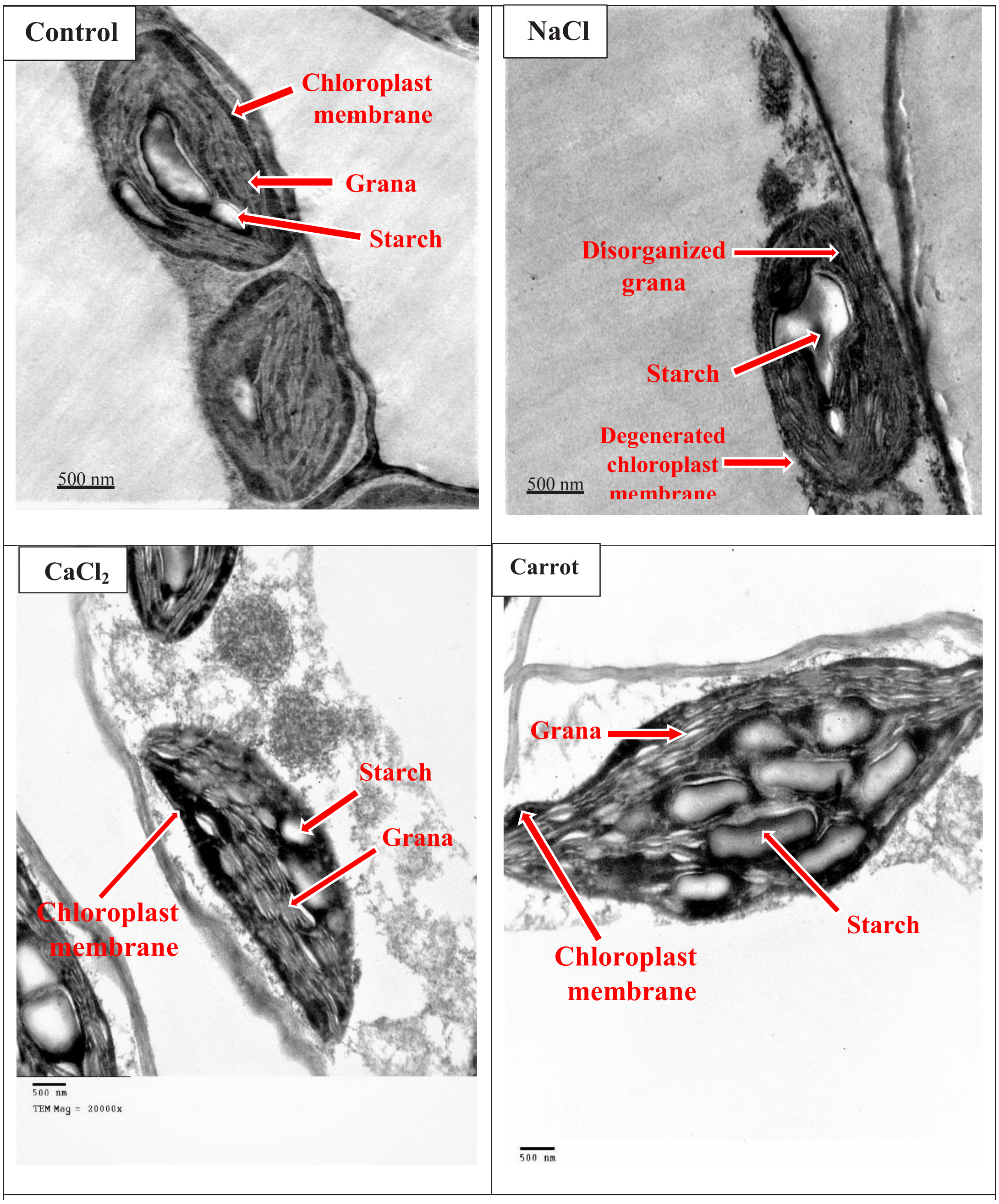

Fig. 16. Electron micrographs showing the effect of seed priming with CaCl$_2$ or carrot root extract on chloroplast of leaves of 30-day-old *Lupinus termis* plants grown under salinity stress. Control: Organized grana and intact thylakoid membranes. NaCl treatment: Degeneration of the chloroplast membranes and a complete disorganization of grana. Primed seeds in CaCl$_2$ and Primed seeds in carrot extract: The chloroplast retained its uniformity with well- organized grana.

Figure 17 showed that, in the control, the nuclei of some lupine leaf cells were with intact nucleoli and contents, but in salt-stressed plants the nucleoli seem to have disintegrated and the nucleus had an indefinite structure. Priming of lupine seeds with CaCl$_2$ solution or carrot extract maintained the nucleus structure where it appeared with a well-defined nucleolus similar to that of the control plants.

Generally, all results indicated that seed priming with carrot extract was more efficient in mitigating the harmful effects of salinity than with CaCl$_2$.

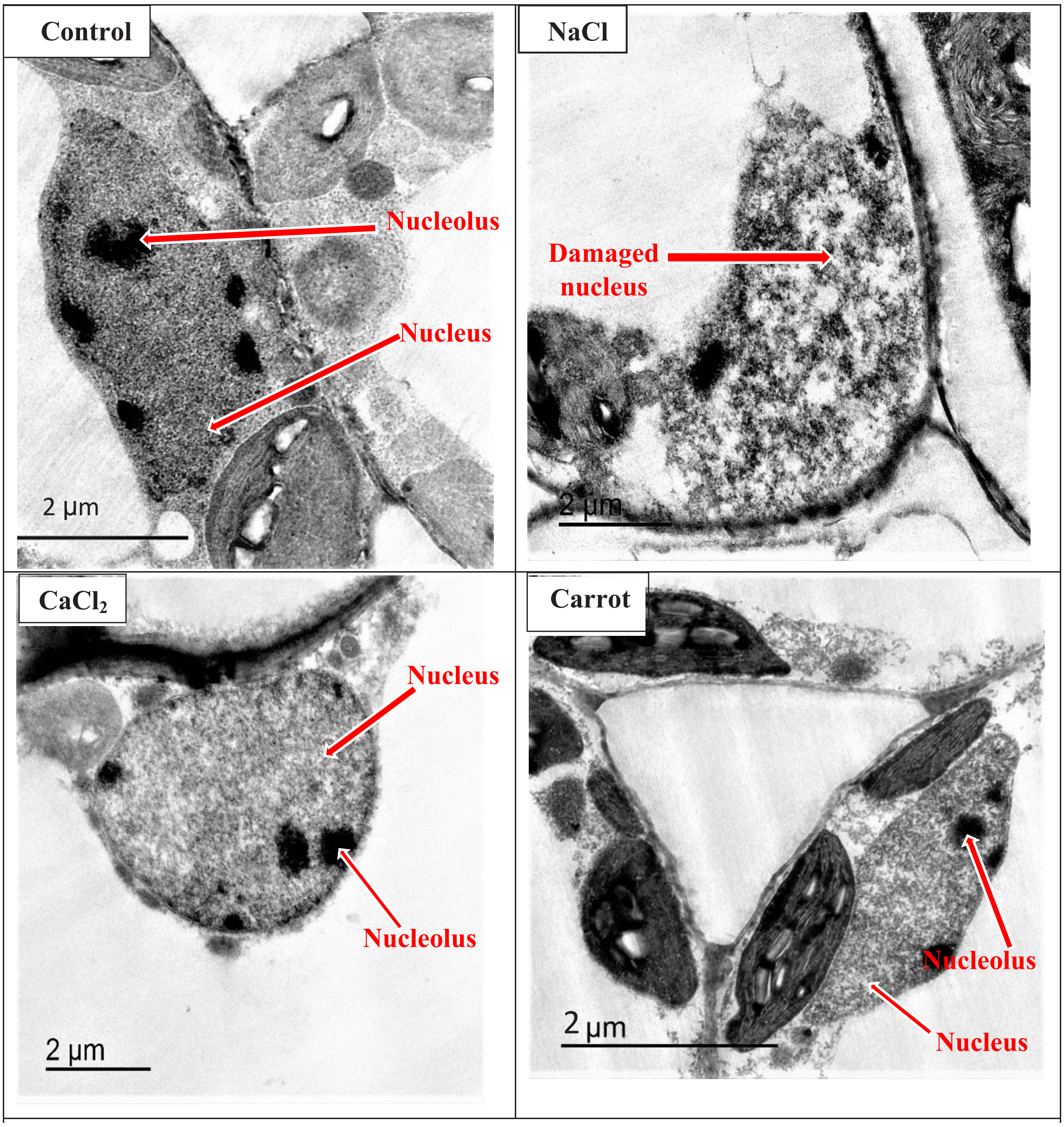

Fig. 17. Electron micrographs showing the effect of seed priming with $CaCl_2$ or carrot root extract on nucleus of 30-day-old *Lupinus termis* plants grown under salinity stress. Control: Clear chromatin bodies and nucleolus. NaCl treatment: Indefinite structure of nucleus. Primed seeds in $CaCl_2$ and Primed seeds in carrot extract: Nucleus with intact contents.

Discussion

Results of this study showed that salinity stress caused major metabolic and structural disturbances in lupine plants. All the measured growth parameters and water content were reduced with salinity stress and this was in harmony with the results of Nasri et al. (2017) on flax seedlings. This reduction in plant growth may be due to the osmotic stress caused by salinity which leads in turn to the inhibition of cell growth directly or indirectly through abscisic acid (Jouyban, 2012). The decline in growth could also be attributed to either the decrease in the uptake of mineral nutrients such as K^+, Ca^{2+} and Mn^{2+}, the changes in enzyme activity which consequently affects protein synthesis, the decrease in the level of growth hormones (Iqbal & Ashraf, 2010), the limitation in water absorption, or the decrease in metabolic activities (Akladious & Hanafy, 2018). Many plants go through osmotic regulation once they are exposed to salt stress by increasing the negativity of the osmotic potential of the leaf sap and this is often the simplest way by which they tolerate the harmful result of accumulation of salt in their cells (Abdul Qados, 2010). Therefore, the reduction in water content under salinity is also thought to be a defensive mechanism by which lupine plants tolerate salinity stress.

Salinity stress caused a decrease in the photosynthetic pigments of lupine plants which is concomitant with the results of Babar et al. (2014) on fenugreek. This decrease is attributed to the disruption of the ultrastructure of the chloroplast as a result of salinity stress (Fig. 17). Other subcellular damages caused by NaCl include separation of the plasma membrane from cell wall, degeneration of the chloroplast membranes, disorganization of grana and changes in nuclear and nucleolar integrities (Fig. 17). Navarro et al. (2007) and Bejaoui et al. (2016) reported similar observations. Various interpretations of the salinity-induced alterations in cellular membranes and organelles were put forward by numerous authors. Thus, disruption of thylakoids was explained by the overproduction of superoxide ions O_2^{-} (Hernandez et al., 1995), the degradation of Rubisco (Krupinska, 2006), and the osmotic imbalance between stroma and cytoplasm which may cause chloroplast swelling in salt-treated plants (Naeem et al., 2012).

The decrease in photosynthetic pigments may be due to a probable degradation of chlorophyll which results from the salt-induced deficiency of elements, including Mg, K and Fe. It is recognized that Mg and Fe play a pivotal role in the synthesis of those pigments (Jaleel et al., 2008), whereas K^+ ions play a role in enzyme activation, protein synthesis, osmoregulation, stimulating photosynthesis and maintaining cell turgor pressure (Hasanuzzaman et al., 2018). The decrease in pigment content may be because salt stress induces the synthesis of abscisic acid which causes the closure of stomata once it is transported to the guard cells. This stomatal closure results in a decrease of CO_2 attainability in the leaves and inhibition of carbon fixation, exposing chloroplasts to excessive excitation energy which consequently increases the oxidative stress and the generation of ROS (Parihar et al., 2015).

Osmoprotective compounds play a key role in mitigating salinity stress either via osmotic adjustment or by conferring some desiccation resistance to plant cells (Slama et al., 2015). The present results showed a highly significant increase in total sugars and total soluble protein content under salinity stress, which are in accordance with the results of Kapoor & Srivastava (2010). These accumulated storage reserves contribute to support basal metabolism under salinity (Amirjani, 2011). The accumulated protein might play a protecting role of the cell under stress by equalizing the osmotic strength of the cytosol with that of the vacuole and the external surroundings (Tekle & Alemu, 2016). Additionally, the present results indicated that salinity enhanced the aggregation of total alkaloids as reported by Jaleel et al. (2007). This increase could be associated with the inhibition caused by salinity within the transamination reactions and thus, the glutamic acid (the precursor of ornithine) is accumulated and transformed to other nitrogenous compounds like ornithine, which is further transformed to the tropane alkaloid (Ahmed et al., 1989).

The present results indicated that salinity led to a significant increase in the MDA, ascorbate and the activities of catalase and peroxidase. As membranes are chiefly composed of lipids and proteins, they are damaged under salinity and their damage induces the conversion of electrons in transport chains from the normal pathways to the oxygen-reducing ones, thus resulting in the overproduction of ROS which oxidize biomolecules including lipids, proteins, nucleic acids and carbohydrates (Bejaoui et al., 2016). The increment in ascorbate content of lupine leaves, as a method of defense mechanism, may be due to its role as an antioxidant and ROS scavenger. The ascorbate is also necessary for the synthesis of collagen which increases the tolerance of plants to oxidative stresses (Darvishan et al., 2013). The recorded induction of catalase and peroxidase activities indicated the presence of high levels of H_2O_2 owing to salinity stress (Yousuf et al., 2015).

Calcium (Ca) is a signaling molecule and it plays an essential role in response to abiotic stresses in plants (Cha-um et al., 2012). Our results revealed that priming with $CaCl_2$ alleviated most of the adverse effects of salinity on growth and photosynthetic pigments of lupine plants, which may be due to the capacity of Ca to retard and limit the entry of Na^+ into plant cells (Cha-um et al., 2012). Other functions of Ca in plant metabolism include: (i) Its role as a secondary messenger within the cytokinin-mediated chlorophyll biosynthetic pathway, in addition to its direct interaction with light through this pathway (Yousuf et al., 2015), which consequently leads to the increase in photosynthetic pigments, (ii) Its vital role in preventing the harm caused by cellular dehydration through balancing the osmotic strength of cytoplasm, and (iii) It plays a role in the improvement of chloroplast structure resulting in the improvement of pigments (Xu et al., 2013). Such roles would ultimately reflect on ameliorating the deleterious effects of salinity stress in plants.

On priming lupine seeds with $CaCl_2$, the total soluble sugars and alkaloid contents were reduced relative to the unprimed stressed plants, which may be due to reduced production of ROS and an improvement in plant tolerance of salinity. In contrast, protein content was augmented, which can be attributed to the ability of Ca to exhibit the best potential of its role in triggering protein synthesis (Amuthavalli et al., 2012). The present results showed that $CaCl_2$ led to depletion of the MDA and ascorbate contents as well as inhibition of catalase and peroxidase activities, compared to salt stressed plants. These declines may be because these primed plants modified their defense strategy against salinity stress by increasing total soluble protein content rather than increasing the ascorbate content (Kasim et al., 2017). The suppressed activities of catalase and peroxidase detected in the case of seed priming with $CaCl_2$ seems to suggest that these enzymes have a role in reducing ROS level and indicating that their activities are dependent upon Ca availability to the plant. The reduction in MDA content is also associated with the aggregation of ROS scavenging molecules, including proline and antioxidants which limit lipid peroxidation associated with the membrane damage under salinity stress (Qureshi et al., 2013).

On seed priming with carrot extract, the restoration of the control values of all growth parameters, photosynthetic pigments, total soluble sugars and total soluble proteins or even surpassing them may be due to its contents of ascorbic acid, auxins, gibberellic acids, kinetin and cytokinins (as benzyl adenine), and some minerals (as K, P, Mg and Zn) as reported by Kasim et al. (2017). These ingredients are known to enhance the growth and increase cell division and cell enlargement (Ahmed et al., 2014). The rise in protein content may be due to the components of carrot root extract, which regulate the expression of salt-stress inducible proteins and induce *de-novo* synthesis of specific polypeptides known to play a crucial role in salt resistance (Abbas & Akladious, 2013). Additionally, Zinc plays a role in the improvement of growth because it is an essential component of numerous proteins in plants as it is a co-enzyme for production of many amino acids (Broadley et al., 2007). The mineral content of carrot root extract includes Mg, Ca, Cu, K and P. Mg is an essential element for chlorophyll synthesis and Ca can prevent cell membrane injury under stressful environmental conditions. Cu, P and K are necessary for the biosynthesis and translocation of carbohydrates and play a principal role in their metabolism (Abdul Qados, 2014). The presence of various vitamins such as vitamin C, B1, B2, B6, D and E (tocopherol) in carrot plays an essential role in the reduction of cell damage caused by free radicals and avoid pigment oxidation (Abbas & Akladious, 2013). Therefore, it seems that the constituents of carrot root extract have a significant role in maintaining foliar ultrastructure by keeping normal cell wall structure, integrity of membranes, chloroplast uniformity with well-organized grana and nucleus structure with well-defined nucleoli.

Conclusion

In the light of the present results, priming of *Lupinus termis* seeds with carrot root aqueous extract (100%) or $CaCl_2$ solution (10mM) is capable of alleviating most of the detrimental effects of NaCl salinity. Carrot root extract was relatively more effective than $CaCl_2$ in combating salinity stress. Therefore, it is recommended to apply seed priming with carrot root extract to lupine crops cultivated in soils with stressful levels of salinity. It has the added advantages of being locally available in abundance, cheap and safe to human health and the environment.

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التأثير الفسيولوجي لإستحثاث البذور باستخدام كلوريد الكالسيوم ومستخلص جذور الجزر على نباتات الترمس النامية تحت تأثير إجهاد الملوحة

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تم استحثاث بذور الترمس (صنف جميزة R2) بواسطة النقع لمدة ست ساعات في المستخلص المائي لجذور الجزر (100%) أو محلول كلوريد الكالسيوم (10مللى مول)، ثم تمت زراعتها وتركت تنمو لمدة ثلاثين يوما على التربة الطينية-الرملية (1:2 وزن/ وزن). وقد تم تعريض البادرات ذات عمر سبعة أيام والنامية من البذور المستحثة وغير المستحثة لإجهاد الملوحة بإستخدام كلوريد الصوديوم (150 مللي مول). وقد تسبب إجهاد الملوحة في نقص كل من الأطوال والأوزان الطازجة والجافة للجذور والسيقان، والمحتوى المائي للورقة وكذلك أصباغ البناء الضوئي. وقد أحدثت الملوحة ارتفاعا في كل من السكريات الذائبة، البروتينات، القلويدات، المالونداي الدهيد (MDA)، نشاط إنزيمي الكاتاليز والبيروكسيديز وحمض الأسكوربيك. وقد أدى استحثاث بذور الترمس باستخدام محلول كلوريد الكالسيوم أو مستخلص جذور الجزر إلى عكس كل النقص والزيادة السابق ذكرهما. وقد أوضحت نتائج الفحص بالميكروسكوب الإلكتروني أن الملوحة قد تسببت في انفصال الغشاء البلازمي عن الجدار الخلوي، وتمزق أغشية البلاستيدات الخضراء، وعدم انتظام صفائح الجرانا، واختفاء بعض النويات وكذلك ظهور بعض الأنوية الشاذة. وقد نتج عن استحثاث البذور الحفاظ على كل من التركيب السليم للجدار الخلوي، وسلامة أغشية البلاستيدات الخضراء، والترتيب المنتظم لصفائح الجرانا، والتركيب ذات النويات الواضحة للنواة وذلك بالمقارنة بمعاملة الكنترول.