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Original Research

Calcium Chloride Supplementation Improves *in vivo* Salt Stress Tolerance of Drumstick (*Moringa oleifera* L.)

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Abstract

Salinity is becoming a bigger problem for agricultural production across the world. Calcium chloride (CaCl2) was used to mitigate the adverse impact of NaCl-induced oxidative stress in terms of growth parameters, chlorophyll, carotenoid, protein, proline, phenolic content (TPC), flavonoid content (TFC), DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) activity and malondialdehyde (MDA) accumulation in *Moringa oleifera* L. plants. There were 8 treatments in the experimentation including control, 100 mM NaCl, 200 mM NaCl, 300 mM NaCl, 100 mM NaCl + 3 mM $CaCl_2$, 200 mM NaCl + 3 mM $CaCl_2$ 300 mM NaCl + 3 mM $CaCl_2$ and 3 mM $CaCl_2$ alone. The results NaCl (100 mM) with 3 mM $CaCl_2$ revealed that the salt stress inhibited the growth parameters of fresh (7.23±0.2), dry weight (4.03±0.2), number of leaves (13.66±0.6), shoot (17.17±1.0) and root length (19.07±0.7) reduced of *M oleifera*. While NaCl (100mM) with $CaCl_2$ (3 mM) treating significantly promoted the growth parameters of *M oleifera* under salt stress. NaCl (100mM) with $CaCl_2$ (3 mM) treating plants showed increased carotenoids (0.956±0.03), chlorophyll (1.576±0.04), protein (15.67±0.3), proline (61.66±2.3), TPC (28.20±0.24), TFC, compared with untreated *M oleifera* under salt stress. NaCl (100 mM) with $CaCl_2$ (3 mM) treating increased the antioxidant activities (DPPH, ABTS) and antioxidant enzymes activities (POD, SOD, CAT, GR, MDA) compared with untreated *M oleifera* under salt stress. Therefore, the $CaCl_2$ can be productively used to enhancement the seedling establishment and growth of *M oleifera* grown under salt stress conditions. The molecular mechanisms of Ca^{2+} signalling induced by $CaCl_2$ treating in *M oleifera* under salt stress as seed priming agents on the final yield, even under field conditions should be the focus for future research.

Keywords: antioxidant enzymes activities, $CaCl_2$, NaCl, proline, pigments, phytochemicals

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Introduction

The frightening challenges facing today's world are population growth, food shortages, climate change, and water scarcity. The production of *M oleifera*, a widely cultivated nutritious crop and the primary food source for a sizable section of the population, has been severely hampered by water shortage or drought stress [1]. The various most critical abiotic environmental impacts are drought, heat, heavy metal, and temperatures stress [2, 3]. Since NaCl accumulation is the number one purpose of soil salinity, excessive salt concentrations, in particular sodium chloride (NaCl), have a massive deleterious effect on important plant physiological processes [4, 5]. Malnutrition, oxidative stress, osmotic stress, and ionic toxicity are the physiological strategies most affected by salt tolerance [5, 6].

Salt stress reasons stomatal closure and oxidative stress, which decreases photosynthetic activity, each of which produce reactive oxygen species (ROS) [7]. Through growing electrolyte leakage, membrane lipid peroxidation, extra ROS can damage chloroplasts, inhibit oxidation response, and reduce photosynthesis [8]. The malondialdehyde (MDA) range was used as a measure of lipid peroxidation, elevated MDA ranges imply moderate lipid peroxidation, main to a large accumulation of reactive oxygen species (ROS) that damage the membrane system [9]. Plants stimulate a spectrum of signaling pathways in an adaptive response to salt-induced ROS negative results [10]. Antioxidant enzymes from the ascorbate-glutathione cycle (ASC-GSH cycle) consist of ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) [11]. Through catalysis or collaboration, this mechanism can also help remove unbound oxygen radicals and hydrogen peroxide (H2O2) from cells [12]. Plants with excessive antioxidant enzyme activity have proven massive resistance to oxidative harm because of ROS [13]. Physiological stress is pressure that affects many physiological techniques related to ion and osmotic aggregation together with proline and soluble sugars [14]. Because it avoids water loss and ionic toxicity, the accumulation of these compounds is essential for osmotic adaptation [15]. Under salinity pressure, calcium ions have a great influence on plant physiological processes and enhance plant development and biochemical factors [16, 17]. Abiotic stress is only a few of the stresses studied by calcium [18]. Calcium was already provided effectively with ion selectivity absorption and salt tolerance in a number of situations [19]. Calcium increased antioxidant activity reduced osmotic change, lipid peroxidation, and cellular membrane improved according to several reports [20]. He also determined that mild calcium awareness decreased the oxidative damage caused by NaCl in the Cakile marine ocean, while excessive interest in calcium accelerated salt tolerance in *Nitraria tangutorum* [21]. These studies advocate that calcium have several effects on pressure tolerance that can be a variety depending on the species or calcium awareness used.

As copresent cations (e.g., Na^{+}, Ca^{++}, and Mg^{++}) may play an important role in the rate of chloride ingress, different chloride based salts can influence the transport properties of cementitious materials in different ways [22]. Additionally, chemical reactions between salt and the hydrated cementitious binder are possible. While NaCl appears to cause only a minor change in transport properties, $CaCl_2$ and $MgCl_2$ can cause much more drastic changes than NaCl [23, 24, 25]. Chemical phases (e.g., Friedel's salt, Kuzel's salt, calcium oxychloride, magnesium oxychloride, brucite, or magnesium silicate hydrate) may form in a solution containing $CaCl_2$ and $MgCl_2$ [22]. In wheat under salt stress, studies of SA and $CaCl_2$ alone and in combination revealed that combined treatments reduced oxidative stress [26].

The drumstick tree (*Moringa oleifera* Lam.,) a multipurpose Moringaceae tree grown in India and Pakistan's sub-Himaylian region, is a tropical tree from the Moringaceae family [27, 28]. The therapeutic and nutritional benefits of moringa's essential minerals, amino acids, and vitamins [29]. Moringa leaves are mineral-rich and have a high antioxidant activity rate, making them salt-resistant [29]. The rate of increase in salinity is troubling. Salt is currently threatening 6.67 million hectares of agricultural land [30]. Calcium chloride ($CaCl_2$) has been shown to be the most effective priming agent for enhancing sorghum germination under salt stress (NaCl) [31], but its physiological effects unknown. To the best of our knowledge, no research on moringa's tolerance to salinity has been published. Moringa plant growth and production can be hampered by salinity in the soil. The objective of this study was to see how $CaCl_2$ influences the growth, physiological response, antioxidant enzymatic activity, and biochemical content of salt-stressed Moringa.

Materials and Methods

Moringa (*M. oleifera*) seeds were collected from Saudi Arabia. Moringa was grown in acid-washed sand-filled pots in a five-replication fully randomised design (CRD). The experiment was performed in the University of Tabuk greenhouse from August to October 2020. Temperature (32-36ºC) and relative humidity (47-56%) were measured during the experiment with a 14-hour average photoperiod. The usage of NaCl salt in Hoagland's solution, 4 salt levels were mounted at the 5 leaf levels: 2 (manipulate), 4, 8 and 12 dS m min^{-1}. Three plants were randomly selected on from each replication after 30 days of salt stress for growth, physiological and mineral evaluation. The suggested level of every parameter measured the usage of information from 15 plants (four plants from each replication).

Treatments and Samplings

The treatments were divided into 8 sections. They were, control, 100 mM NaCl, 200 mM NaCl, 300 mM NaCl, 100 mM NaCl + 3 mM $CaCl_2$, 200 mM NaCl + 3 mM $CaCl_2$, 300 mM NaCl + 3 mM $CaCl_2$ and 3 mM $CaCl_2$. Ten pots were used for each treatment. The pots were irrigated with the suitable treatment solutions before transplanting, and the electrical conductivity (EC) of the soil mixture was measured. The plants in the control group were irrigated with well water. Three plants were planted per pot, and the pots were watered to field capacity with deionized water for first 30 days after planting (DAP), with special care taken to avoid leaching. On 45, 60, and 75 DAP, the soil's original EC level was maintained by flushing each pot with the needed volume of corresponding treatment solution. To minimise spatial effects in the greenhouse, the position of each pot was randomised every four days. On 20 DAP, the seedlings were trimmed down to one in each pot. On 90 DAP, plants were uprooted at random and their growth, pigment content, antioxidant enzymatic activity and biochemical constituents were measured.

Growth Parameters

Growth elements, including shoot length, root length, leaf and twig diversity adjusted to the plant, as well as roots and shoots with wet and dry weight, were analyzed after harvest using known statistical techniques. Fresh biomass from roots and sprouts was weighed immediately after harvest, then dried in the shade and then oven-dried at 70±2ºC until a constant dry matter weight was obtained for measurement changes.

Chlorophyll and Carotenoid Determination

Nagata and Yamashta [32] developed a protocol for determining chlorophyll and carotenoid content. In 10 ml of 80% acetone, 1 g of Moringa leaves were changed into soil and Whatman No.1 filter paper. UV spectrophotometer was used to test the absorbance at 663, 505 and 453 nm after the filtered extract was transferred to the cuvette (UV- Thermo Scientific Evolution 201, USA).

Total Phenolic Contents (TPC)

Moringa leaf TPC was decided consistent with the revised technique the usage of the Singleton and Rossi [33] technique. Folin Ciocalteu (2N) and Na_2CO_3 reagent were used as reagents. The standard absorbances of gallic acid (100, 150, 250 and 500 mg of L^{-1} gallic acid) and Moringa samples were measured at 760 nm the usage of a UV spectrophotometer (UV- Thermo Scientific Evolution 201, USA).

Total Flavonoid Contents (TFC)

TFC in Moringa leaves was measured using the Crozier et al. [34] method and TFC was determined the use of quercetin (1 mg/mL) as usual with the resource of using UV–Vis Spectrophotometer (Thermo Scientific Evolution 201).

Total Antioxidant Assays (DPPH and ABTS Radical Scavenging Activity)

Sieved leaves, then discarded (1 g) pounded with liquid nitrogen (LN2) in a mortar then hammered. The fresh powder was extracted collectively with four ml of 60% (v/v) acid methanol (methanol: HCl; 99:1) at 25±2ºC at room temperature, for two hours for antioxidant extraction. After the chlorophyll was extracted with chloroform at room temperature of 252ºC, the chlorophyll was centrifuged as soon as at 10,000 rpm at 4ºC for 10 minutes. The supernatant was used in keeping with the analysis of typical antioxidant strength.

The radical's capacity to remove ABTS (2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) come to be decided constant with Re et al. [35] calculated. According with Williams et al. [36] measured the potential to eliminate DPPH radicals (2,2-diphenyl-1-picrylhydrazyl).

Enzyme Extraction and Antioxidants Assay

In 2.5 ml of cold 50 mM phosphate buffer, fresh leaves (0.25 g) were homogenised and filtered (pH 7.8). The filtered mixture was centrifuged for 20 minutes at 4ºC and 15,000 rpm in a separate tube, the supernatant was removed and tested. Use an ultraviolet spectrophotometer (Thermo Scientific Evolution 201) to determine superoxide dismutase (SOD) (EC 1.15.1.1) at 560 nm using the method specified by Giannopolitis and Ries [37] and the malondialdehyde content (MDA) to be determined according to Kumazawa et al. [38]. The activities of catalase (CAT) (EC 1.11.1.6) and peroxidase (POD) (EC 1.11.1.7) were determined normal with the method of chance and Maehly [39]. The enzymatic activity of SOD was determined in step with Beauchamp and Fridovich [40]. The Bradford method is used to estimate the protein content of an enzyme.

Statistical Analysis

Analysis of Variance (ANOVA) was used to interpret data using SPSS version 20.0 (IBM Corporation). Means and standard errors (±) are used to represent data. The Duncan Multiple Distance Test (DMRT) was used to measure the middle section at the 5% significance level.

Table 1. Effect of different concentration and combination of NaCl and $CaCl_2$ stress under growth Moringa plant.

Treatments	Fresh Weight g^{-1} plant	Dry Weight g^{-1} plant	Number of Leaves plant	Shoot Length cm^{-1}	Root Length cm^{-1}
Control	9.66±0.3[a]	5.77±0.3[a]	11.06±0.7[c]	27.07±1.1[a]	26.67±0.9[a]
Control (3 mM $CaCl_2$)	3.03±0.6[f]	0.97±0.1[f]	16.03±0.6[a]	25.76±1.3[b]	23.07±1.0[b]
100 mM NaCl	4.03±0.3[e]	2.17±0.3[d]	3.23±0.5[c]	7.57±1.1[e]	7.37±0.7[e]
200 mM NaCl	3.19±0.3[f]	1.17±0.3e	1.77±0.4[c]	5.37±1.0[e]	4.33±0.5[e]
300 mM NaCl	2.36±0.1[g]	0.97±0.1[f]	0.97±0.4[d]	1.57±0.8[f]	1.66±0.5[f]
100 mM NaCl+ 3 mM $CaCl_2$	7.23±0.2[b]	4.03±0.2b	13.66±0.6[b]	17.17±1.0[c]	19.07±0.7[c]
200 mM NaCl+ 3 mM $CaCl_2$	6.66±0.3[c]	3.07±0.3c	10.69±0.7[d]	12.27±1.2[d]	13.36±0.7[d]
300 mM NaCl+ 3 mM $CaCl_2$	5.66±0.1[d]	1.66±0.1e	8.83 ±0.5[e]	7.66±0.8[e]	10.07±0.6[d]

Results

Effect of NaCl and $CaCl_2$ Stress under Growth Parameters *Moringa oleifera* Stress

The data presented in Table 1 shows that shoot and root length, number of leaves, fresh weight and plant dry weight were significantly reduced in plants that were stressed with 300 mM NaCl (sodium chloride), 300 mM NaCl with 3 mM $CaCl_2$. Calcium chloride and 3 mM $CaCl_2$ were compared with untreated plants. $CaCl_2$ treatment significantly increased fresh weight, dry weight, number of leaves, shoot length, and root length by 5.66, 1.66, 8.83, 7.66, and 10.07 respectively, as compared to NaCl treatment (Table 1). It is observed that $CaCl_2$ could effectively improve *Moringa oleifera* under salt stress.

The data's were recorded after 21 days of stress treatment.

Data are Mean±standard error of three (3) independent replicates with 5 plants for each replicate of each treatment. Values followed by the same letters within a column are not significantly different at 5% level according to DMRT (Duncan's multiple range test).

Effect of NaCl and $CaCl_2$ on Leaf Chlorophyll, Carotenoids, Proline, Protein Content under Stress in Plant

The pigment chlorophyll decreased in NaCl-stressed plants, but increased when $CaCl_2$ was applied. The chlorophyll content increased in all plants with age (Table 2). In compared to NaCl-stressed plants, NaCl with $CaCl_2$ increased chlorophyll content. Plants treated with NaCl was a slight reduction in carotenoid content. The carotenoid content of $CaCl_2$ plants decreased as well, but it was still higher than that of NaCl-treated

Table 2. Effect of Moringa leaf extract (MLE) on total chlorophyll, carotenoids, proline, protein content in plants grown under NaCl and $CaCl_2$ stress.

Treatments	Carotenoids (mg g^{-1} FW)	Chlorophyll (mg^{-1} g FW)	Protein (in µg)	Proline (µg g^{-1} DW)
Control	0.766±0.03[b]	1.942±0.03[b]	19.66±0.4[b]	42.56±1.6[d]
Control (3 mM $CaCl_2$)	1.156±0.04[a]	2.877±0.06[a]	23.77±0.5[a]	49.27±1.5[d]
100 mM NaCl	0.596±0.02[c]	0.996±0.02[d]	11.06±0.3[d]	57.56±1.9[c]
200 mM NaCl	0.496±0.02[c]	0.876±0.02[d]	9.66±0.2[e]	57.06±1.8[c]
300 mM NaCl	0.386±0.02[d]	0.876±0.02[d]	7.56±0.2[f]	65.96±2.0[b]
100 mM NaCl+ 3 mM $CaCl_2$	0.956±0.03[b]	1.576±0.04[c]	15.67±0.3[g]	61.66±2.3[b]
200 mM NaCl+ 3 mM $CaCl_2$	0.756±0.02[b]	1.276±0.03[c]	13.56±0.3[c]	69.56±2.5[b]
300 mM NaCl+ 3 mM $CaCl_2$	0.656±0.02[c]	1.166±0.03[c]	11.67±0.2[d]	75.63±2.4[a]

Table 3. Effects on antioxidant phytochemicals and antioxidant capacity in Moringa leaf extract (MLE) plants grown under NaCl and $CaCl_2$.

Treatments	TPC (GAE mg/g)	TFC (RE mg/g)	DPPH (IC50-µg/ml)	ABTS (µM TE/g)
Control	32.46±0.18^{b}	131.66±2.48^{f}	47.86±0.20^{a}	82.55±0.82a
Control (3 mM $CaCl_2$)	35.53±0.26^{a}	184.96±3.07^{d}	25.87±0.24^{f}	54.74±0.28c
100 mM NaCl	21.63±0.17^{e}	127.83 ±1.52e	31.46±0.15^{d}	60.31±0.34b
200 mM NaCl	19.49±0.38^{f}	131.40±2.18^{f}	33.43±0.24^{c}	61.62±0.28d
300 mM NaCl	17.26±0.33^{f}	153.16±2.02^{e}	35.43±0.16^{b}	63.52±0.57c
100 mM NaCl + 3 mM $CaCl_2$	28.20±0.24^{c}	345.79±1.37^{a}	23.56±0.27^{g}	52.35±0.62h
200 mM NaCl+ 3 mM $CaCl_2$	25.36±0.54^{d}	283.56±1.92^{b}	24.59±0.23^{f}	54.74±0.28c
300 mM NaCl+ 3 mM $CaCl_2$	23.53±0.37^{d}	211.79±1.83^{e}	26.86±0.24^{e}	56.38±0.53f

plants. In terms of increased carotenoid content, the combinations of NaCl and $CaCl_2$ showed a partial recovery (Table 2). Although it was lower than control plants, it was higher than NaCl-treated plants.

Compared to control plants, NaCl lowered protein content and $CaCl_2$ stress in *Moringa oleifera*. When compared to NaCl-stressed plants, the addition of $CaCl_2$ to NaCl treatments increased the protein content (Table 2). NaCl and $CaCl_2$ treatments increased the content of proline in plants when compared to the control. When comparing NaCl-stressed plants to NaCl-treated plants, $CaCl_2$ treatment decreased the proline content in plants (Table 2).

Data are Mean±standard error of three (3) independent replicates with 5 plants for each replicate of each treatment. Values followed by the same letters within a column are not significantly different at 5% level according to DMRT (Duncan's multiple range test).

Effects of $CaCl_2$ on Total Phenolic and Flavonoid Content in *Moringa oleifera* Leaves

The phenolic (TPC) and flavonoid (TFC) content of *M. oleifera* leaves dried extracts was quantified in Table 3. The TPC (28.20 GAE mg/g) and TFC (Table 3 345.79 RE mg/g) of leaf extract was higher content than the other extracts. This extract showed the positive effects of the total flavonoid contents on the activities of antioxidants.

Antioxidant Activity

The antioxidant activity was tested by evaluating the effects of leaf extracts on the free radical scavenging activities of DPPH and ABTS. At 100 mM with 3 mM $CaCl_2$ leaf extracts with the IC_{50} value of 23.56 µg ml^{-1} and ABTS value of 52.35 µM TE/g displayed the highest antioxidant activity as compared to other treatments (Table 3). The higher total flavonoid contents in *M. oleifera* leaf these effects may be related to under 100 mM NaCl with 3 mM $CaCl_2$ treatment.

TPC – Total phenolic content; FLC – flavonoids content; DPPH – 2,2-diphenyl-1-picryl hydrazyl radical scavenging activity; ABTS – 2,2′-azinobis (3-ethyl-benzothiozoline)-6-sulfonic acid disodium salt scavenging activity: GAE – Gallic Acid Equivalents; E – Rutin Equivalents.

Data are Mean±standard error of three (3) independent replicates with 5 plants for each replicate of each treatment. Values followed by the same letters within a column are not significantly different at 5% level according to DMRT (Duncan's multiple range test).

Effects on Antioxidant Systems under NaCl Stress

Membrane Damage

Salt stress significantly increased MDA content in the *M. oleifera* plants (Table 4). The MDA Content increased significantly by 125 and 129, when compared to the control and NaCl stress plants. This results indicating that $CaCl_2$ could effectively protect plasma membranes under salt stress.

Antioxidant Enzyme System

Salt stress significantly increased the activities of antioxidant enzymes in *M. oleifera* plants (Table 4). The activities of SOD, CAT, POD, and GRD in *M. oleifera* were significantly increased by 23.06, 44.16, 0.94, and 16.13, when compared to the control and NaCl stress plants. The quantitatively results of antioxidant enzyme activity agree (Table 4). These results suggested

Table 4. Effects on antioxidant enzyme activity of Moringa leaf extract (MLE) plants under NaCl and $CaCl_2$ stress.

Treatments	Superoxide dismutase (SOD Unit g^{-1} FW)	Catalase (μMH_2O_2 red.mg^{-1} protein min^{-1})	Peroxidase (POD Unit g^{-1} FW)	Glutathione reductase (GR Unit g^{-1} FW)	Lipid peroxidation (MDA n mole TBARS g^{-1} DW)
Control	17.06±0.6^{g}	43.46.2^{c}	0.87±0.03^{f}	14.16±0.4^{d}	132±23^{c}
Control (3 mM $CaCl_2$)	20.64±0.5^{f}	41.46.1e	0.93±0.03^{e}	12.56±0.3^{e}	108±37^{e}
100 mM NaCl	37.36±0.6^{a}	45.56.3^{b}	1.91±0.05^{a}	17.69±0.4^{b}	151±13^{b}
200 mM NaCl	33.76±0.7^{b}	43.06.3^{c}	1.76±0.05^{a}	19.73±0.5^{a}	156±54^{b}
300 mM NaCl	34.26±0.7^{c}	47.56.2^{a}	1.03±0.04^{c}	20.96±0.4^{a}	159±24^{a}
100 mM NaCl+ 3 mM $CaCl_2$	20.56±0.6^{f}	46.56.5^{b}	1.83±0.03^{b}	17.29±0.5^{b}	127±43^{d}
200 mM NaCl + 3 mM $CaCl_2$	21.13±0.6^{e}	42.06.2^{d}	1.25±0.04^{d}	15.06±0.5^{d}	129±47^{d}
300 mM NaCl + 3 mM $CaCl_2$	23.06±0.6^{d}	44.16.4^{d}	0.94±0.04^{e}	16.13±0.7^{c}	125±53^{d}

that $CaCl_2$ further enhance antioxidant enzyme activity under salt stress.

Data are Mean±standard error of three (3) independent replicates with 5 plants for each replicate of each treatment. Values followed by the same letters within a column are not significantly different at 5% level according to DMRT (Duncan's multiple range test).

Discussion

Abiotic stress such as salinity is involved in the development of reactive oxygen species (ROS). To maintain metabolic function under stress and to prevent oxidative injury, a balance is required between the formation and depletion of ROS. Calcium (Ca), an integral factor, is involved in many aspects of plant metabolism, including membrane stabilization, signal transduction, and regulation of enzyme activity [41]. It helps in the reversal of a harmful salinity on plants by adding Ca^{2+} to the medium [42]. The study aim was to learn more about the physiological processes that determine salt tolerance, and how $CaCl_2$ can trigger these processes to counteract salt-induced photosynthesis reductions [43]. Plants prefer to increase defence mechanisms of growth and development when they are stressed [44]. Plant life are ready with antioxidant protection systems and possible osmotic regulation to counter NaCl-induced oxidative stress. The application of 5- and 10-mM calcium to plants exposed to NaCl decreases the range of carotenoids, proline, chlorophyll, and MDA while increasing the activity of antioxidant enzymes such as SOD, CAT, POD, and GR, as well as various phytochemicals and overall phenol content material [45, 46].

Plant growth and development are influenced by physiological and molecular changes in plant tissues and cells caused by abiotic stress [47, 48]. The shoot and root length, fresh and dry weight and number of plant leaves were reduced at a concentration of 300 mM NaCl [49]. As compared to plants treated with different concentrations of calcium and sodium chloride, as well as plants not treated with calcium chloride, the plant's growth potential is increased with 100 mM NaCl at 3 mM $CaCl_2$. However, plants treated with NaCl alone and without $CaCl_2$, the plants react positively to combined stress by increasing proline and protein content while simultaneously decreasing leaf chlorophyll and carotenoids [8, 23]. As shown by the growth of G. gracilis seedlings under alkali salts stress, salinity can inhibit root growth by altering the natural water potential, increasing ion toxicity, or causing an ion imbalance [49]. $CaCl_2$ treatment significantly increased the growth parameters of *V. unguiculata* plants, according to Taffouo et al. [50]. $CaCl_2$ was already shown to play a key role in plant response to abiotic stress [51], as well as plant growth and development, including seed germination, in previous studies [52]. Salt stress reduced plant growth and development, according to this study. $CaCl_2$ treatments increased germination under salt stress to different degrees, and the effect was significantly better than hydro-priming, suggesting that Ca^{2+} may play a key role in this process. Rashid et al (2022) reported plant growth length, grain yield, fresh and dry biomass were also significantly enhanced [53].

The quantity of the total phenolic and flavonoid content in the *Moringa olifera* leaf extracts is higher (TPC (28.20 GAE mg/g) and TFC (345.79 RE mg/g) compared to the other extracts. The free radical scavenging activity was analyzed by performing DPPH and ABTS with the leaf extracts of different concentrations of stress treatments [54, 55]. At 100 mM NaCl and 3 mM $CaCl_2$ the IC_{50} value of 23.56 µg ml^{-1} and ABTS value of 52.35 µM TE/g. At the concentration

of NaCl and $CaCl_2$ the overall flavonoid content gets upregulated and thereby increase in the antioxidant capacity [56, 57]. Similarly, as compared to controls, $CaCl_2$ treatment improved DPPH radical scavenging activity in NaCl-stressed sunflower and red bean plants [58, 59].

Salinity treatment increased the proline content of all parts of the plants to a larger level in soybean [60] and sorghum [61]. Calcium treatment for NaCl-stressed plants, when compared to NaCl-stressed plants, the accumulation of proline content was significantly reduced [62]. NaCl-stressed plants, on either side, had a higher proline content than the control. MDA, a product of lipid peroxidation, is an indicator of membrane damage that leads to electrolyte leakage in high-salinity conditions [63]. MDA is commonly used to identify oxidative damage in fatty acids [64], and its accumulation in plants such as cotton [65], sugar beet [66], cowpea [67], maize [68], and rice [69]. The present study MDA Content increased significantly, when compared to the control and NaCl stress plants. When compared to plants treated solely with salinity, NaCl-treated plants supplemented with SA or $CaCl_2$ showed a significant decrease in MDA content in red bean leaf [59, 70]. It is hypothesized that using $CaCl_2$ leads to the induction of antioxidant enzyme activity, resulting in a long-term decrease in ROS and lipid peroxidation.

Plants adapt to salt stress not only through osmotic regulation and ion balance adjustments, but also through an antioxidant enzyme defence system that removes the reactive oxygen species (ROS) produced by salt stress [71]. Many researches has found that $CaCl_2$ treatments increased plant salt tolerance by enhancing the antioxidant enzyme system [72]. The antioxidant enzyme activities of SOD, CAT, POD, and GRD in *M. oleifera* were significantly increased, when compared to controls. However, the salt with $CaCl_2$ combination increases the activity of catalase (CAT), peroxidase (POD) and glutathione reductase (GR) [73, 74]. The $CaCl_2$ enhance the antioxidant enzymes SOD, POD, CAT and GR activity in salt-stressed *M. oleifera*. The higher antioxidant enzyme activity at the salt content is due to the greater clearance of ROS [75, 76, 77]. Rashid et al (2022) reported grain quality parameters such as protein, antioxidant enzyme activity, K and Ca were also significantly enhanced [53]. As $CaCl_2$ was combined with NaCl, the activity of antioxidant enzyme was increased when compared to NaCl-stressed plants. The current study findings suggests that adding $CaCl_2$ to *M. oleifera*. improved their tolerance to NaCl. Which could explain that plants grow best in saline conditions.

Conclusion

In conclusion, $CaCl_2$ treatment increased the content of osmotic adjustment substances in the *M. oleifera* plants under salt stress. In the growth of *M. oleifera* plants, combining $CaCl_2$ with NaCl treatments had a variety of effects on physiological parameters, total chlorophyll, carotenoids proteins, proline, phytochemicals, antioxidant activity and antioxidant enzyme activity. Under salt stress, $CaCl_2$ priming increased K^+/Na^+, especially Ca^{2+}/Na^+, which regulated ion balance. Ca^{2+} shown to improve plant growth in NaCl-stressed conditions by modulating overall metabolism. Therefore, the $CaCl_2$ can be productively used to enhancement the seedling establishment and growth of *M oleifera* grown under salt stress conditions. The molecular mechanisms of Ca^{2+} signalling induced by $CaCl_2$ treating in *M oleifera* under salt stress impact as seed priming agents on the final yield, even under field conditions should be the focus for future research.

Conflict of Interest

The authors declare that they have no conflict of interest.

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