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calcium's role in enhancing wheat plasma membrane stability under salinity

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دور الكالسيوم في تعزيز ثباتية الغشاء البلازمي في القمح تحت ظروف الملوحة

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الملخص:
تهدف هذه الدراسة إلى تقييم واقع إدارة النفايات الطبية في بعض أقسام المركز الطبي بمدينة البيضاء، من خلال قياس كميات النفايات وتصنيفها إلى طبية وغير طبية، بالإضافة إلى تقييم كفاءة استخدام المحرقة ومدى ملاءمتها للأنواع المختلفة من النفايات. جُمعت البيانات ميدانياً على مدار 9 أسابيع باستخدام ميزان رقمي، وتم تحليلها إحصائياً باستخدام Excel وSPSS. أظهرت النتائج أن قسم الباطني نساء سجّل أعلى كمية نفايات (175.21 كجم)، تلاه قسم الأطفال، بينما كانت الكميات أقل في قسمَي الجراحة للرجال والنساء وكما تبين أن النفايات غير الطبية – مثل الورق والنايلون – كانت الأكثر انتشاراً، باستثناء قسم الجراحة نساء حيث غلبت النفايات الطبية. تعمل المحرقة من نوع KALFRISA بوقود الديزل وبسعة 7 كجم، وتُستخدم لحرق نفايات محددة، في حين تُستثنى منها بعض الأنواع مثل السوائل والقطع المعدنية وتُظهر الدراسة أن الفروقات بين الأقسام لم تكن معنوية إحصائياً ($P > 0.05$) وعليه توصي الدراسة بتوفير صناديق الأدوات الحادة في جميع الأقسام، وتحسين عمليات الفرز والتصنيف، وتوسعة الطاقة الاستيعابية للمحرقة، مع تعزيز برامج التدريب والرقابة.

الكلمات الدالة: الاجهاد الملحي، اشارة الكالسيوم، ثباتية الغشاء البلازمي، المرسال الثانوي، نبات القمح.

Abstract
Salinity is one of the most serious abiotic stresses limiting agricultural productivity worldwide, with wheat *Triticum aestivum* L. being particularly vulnerable. Calcium, beyond its recognized role in soil amelioration, plays a pivotal role at the cellular level in mitigating the harmful effects of salinity. Under salt stress, calcium functions as a secondary messenger that regulates ion transport and signaling, thereby maintaining ion homeostasis within the cytoplasm. It also stabilizes the plasma membrane by reducing electrolyte leakage, enhancing membrane integrity, and protecting cells from oxidative damage induced by excessive sodium ions. These protective mechanisms collectively contribute to improving the physiological performance and stress tolerance of wheat plants grown under saline conditions.

Understanding calcium's role in maintaining plasma membrane stability provides valuable insights into developing strategies for enhancing wheat resilience to salinity stress.

Keywords: Calcium signal, Plasma membrane stability, Salt stress, Secondary messenger, Wheat plant.

1. Introduction

Soil salinity is a global challenge that threatens sustainable agriculture, particularly in arid and semi-arid regions where evapotranspiration rates are high and irrigation practices often lead to the accumulation of soluble salts. It is estimated that more than 20% of the world's irrigated lands are affected by salinity, resulting in significant reductions in crop growth and yield. In Libya, salinity represents a major constraint to agricultural production due to the extensive presence of saline soils and the reliance on groundwater with high salt content.

Soil salinity is one of the major abiotic stresses limiting crop growth and productivity, particularly in arid and semi-arid regions where soil and water salinization is prevalent [4]. Sodium chloride (NaCl) is the primary source of this stress, causing osmotic stress, ion toxicity, and disturbances in cellular ionic homeostasis, which negatively affect plant development [15]. Wheat *Triticum aestivum* L. is particularly sensitive to salinity, as high NaCl concentrations reduce seed germination, impair root and shoot growth, and disrupt ion distribution within plant tissues [28].

The plasma membrane plays a crucial role in protecting plant cells under salt stress by regulating selective ion transport and maintaining cellular integrity. Salinity can disrupt membrane structure and function, leading to increased permeability, ion leakage, and cellular damage [14]. Calcium (Ca^{2+}) is an essential nutrient that stabilizes plasma membranes by strengthening interactions among phospholipids and membrane proteins and modulates stress signaling pathways that enhance plant tolerance to adverse environmental conditions [31]. Exogenous calcium has been shown to mitigate salt stress by improving membrane stability, maintaining ionic balance, and activating antioxidant defense mechanisms [22].

Calcium also acts as a secondary messenger in plant abiotic stress responses. Cytosolic Ca^{2+} concentrations increase under stress conditions, triggering various signaling cascades depending on the stress type. Calcium transporters in the plasma membrane and intracellular organelles help maintain optimal cytoplasmic calcium levels [8]; [27].

Agricultural gypsum ($CaSO_4 \cdot 2H_2O$) is a rich source of soluble calcium and is widely used to improve saline and sodic soils. It displaces toxic sodium ions, enhances soil aeration, porosity, and permeability, and promotes better nutrient uptake by plant roots [19]; [5]. Evaluating the interaction between calcium sulfate and NaCl in wheat seedlings under salt stress can provide insights into physiological mechanisms of salt tolerance and inform strategies for improving crop resilience and productivity.

Study problem:

Salinity is one of the most significant environmental stresses affecting the growth and productivity of wheat *Triticum aestivum* L. in many agricultural regions worldwide, including Libya. Salt stress disrupts ion homeostasis within plant cells and increases plasma membrane permeability, leading to electrolyte leakage and reduced physiological efficiency. With the increasing expansion of salt-affected lands, it has become essential to understand the cellular mechanisms that enable plants to adapt to these harsh conditions. Calcium is known for its vital role in stabilizing the plasma membrane structure and maintaining cellular integrity under environmental stresses. However, there remains a research gap regarding how calcium influences plasma membrane stability in wheat specifically under salinity stress, particularly from the

perspective of cellular physiology and molecular mechanisms related to ion homeostasis and reduction of electrolyte leakage.

Based on the above, the research problem arises from the need to:

Determine the extent to which calcium can enhance plasma membrane stability in wheat under salinity.

Elucidate the physiological and molecular mechanisms through which calcium protects plant cells from salinity-induced damage.

Provide scientific data that can contribute to developing agronomic strategies to improve wheat salinity tolerance and increase productivity in salt-affected soils.

Aims of study:

Testing the effect of different concentrations of sodium chloride on germination percentage,

Studying the effect of different concentrations of sodium chloride on the fresh and dry weight of wheat *Triticum eastivum L*.

Studying the effect of different concentrations of sodium chloride on the stability of the plasma membrane of root tip cells of wheat *Triticum eastivum L*.

Testing the effect of the interaction between calcium sulfate and sodium chloride on the above-mentioned parameters and its role in protecting wheat plants from salt stress.

Materials and Methods:

1. Experimental Design:

The experiment was designed in a similar manner, using a dispersed method. Calcium sulfate was the least significant difference between the variable concentrations control and three different concentrations of sodium chloride, in addition to an interaction between three different concentrations of sodium chloride and three different concentrations of calcium chloride. We used three concentrations for each of the aforementioned concentrations, and all experimental conditions were standardized: irrigation temperature, lighting. We conducted the study within the Department of Botany, Faculty of Science, Sebha University, in the fall of 2022. The study was conducted from seed germination to the seedling growth stage, taking 37 days.

2. Plant Materials:

3. Wheat *Triticum aestivum* L. seeds cultivar ACSAD 71.

4. Chemicals:

Sodium chloride NaCl, calcium sulfate hydrate $CaSo_4.7H_2O$, hydrochloric acid HCl, Clorox, ethanol, acetone, distilled water.

Equipment used: Petri dishes, pots, sand, filter papers, oven, incubator, balance, steam autoclave, electric conductivity meter and other necessary glass wares for the tests.:

The solutions used in this study were prepared as described in the following tables [2]:

Table 1: Preparation of different concentrations of sodium chloride:

Sodium chloride concentration mM	**Final volume of solution / 1L distilled water**
0.00	1000 mL
25	1000 mL
50	1000 mL
100	1000 mL

Table 2.2 Preparation of the reaction between calcium sulfate and sodium chloride.

Calcium Sulfate Concentration mM/L	Sodium Chloride Concentration mM/L	Final Volume Completed with Nutrient Solution
2	25	1000 mL
6	25	1000 mL
12	25	1000 mL
2	50	1000 mL
6	50	1000 mL
12	50	1000 mL
2	100	1000 mL
6	100	1000 mL
12	100	1000 mL

Table 2: Preparation of Hoglund's solution 1/5 excluding the use of calcium nitrate.

Substance	Symbol	Concentration	Category
Potassium nitrate	KNO_3	2.0 mM	Macronutrients
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	0.4 mM	
Ammonium dihydrogen phosphate	$NH_4H_2PO_4$	0.4 mM	
Ammonium molybdate	$(NH_4)_6Mo_7O_2 \cdot H_2O$	0.004 ppm	Micronutrients
Copper sulfate	$CuSO_4 \cdot 5H_2O$	0.004 ppm	
Zinc sulfate	$ZnSO_4 \cdot 7H_2O$	0.01 ppm	
Boric acid	H_3BO_3	0.1 ppm	
Manganese sulfate	$MnSO_4$	0.1 ppm	

5. Seed germination test:

Seeds for this experiment were selected to be of uniform size and without impurities. They were sterilized by placing them in 3% Clorox solution for 5 minutes. They were then run under running water for 15 minutes to remove the Clorox. The seeds were further washed in distilled water to remove excess Clorox. The sterilized seeds were then spread in each 9 cm diameter Petri dish lined with two size 11 filter papers. Three replicates of every treatment were done, and in each replicate, ten seeds were used in the experiment. Through the given parameters, 5 ml of test solutions and distilled water, which served as the
control, were added to the respective replicates at the same time, after which the Petri dishes were transferred into an incubator adjusted to 23± 2◦C.

$$\text{Germination Percentage \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seed}} \times 100$$

6. Seedling Growth Test:

Seeded sterilization and the screening were carried out as described for seed germination test. After sterilization, the samplings were planted in sent in the same size of the plastic pots containing the same level of the measured clean sand. The sand was screened and thoroughly

washed by 1% hydrochloric acid, free from impurities and salt. Five seeds were planted in each pot [5].

7. Measuring Fresh and Dry Weight:

The fresh weight of the shoot and root system g was measured using a sensitive balance. The seedlings were then placed in an oven at 85°C for 72 hours, and the dry weight g was measured using a sensitive balance.

8. Estimation of the Plasma Membrane Stability of Root Tip Cells:

The stability of the plasma membrane of root tip cells was estimated by estimating the leakage of electrolytes from the root tips according to the method of [6]. Five 1-cm-long root tips were taken from the plant and placed in tightly capped glass tubes containing 25 ml of distilled water at 10°C. The samples were left for 24 hours with continuous shaking. The samples were then placed in a steam sterilizer at 120°C. The electrical conductivity was calculated using an electrical conductivity meter at 25°C.

The relative damage rate was calculated using the following equation:

Relative Injury Percentage % =1-[1-(T1/T2)/1-(C1/C2)] ×100

T1 = the electrical conductivity of the treatment before being placed in the steam sterilizer.
T2 = the electrical conductivity of the treatment after being placed in the steam sterilizer.
C1 = the electrical conductivity of the control treatment before being placed in the steam sterilizer.
C2 = the electrical conductivity of the control treatment after being placed in the steam sterilizer.

9. Statistical Analysis:

The data included in this study were obtained and then statistically analyzed using the SPSS program using analysis of variance ANOVA according to the Latin square design to test the significance of the difference between the treatments using the least significant difference LSD test at 0.5%.

Results and discussion:

10. The effect of different concentrations of sodium chloride on wheat seed germination:

11. Seed germination

The effect of different concentrations of sodium chloride NaCl on the germination percentage of wheat seeds is shown in Figure 1. The statistical analysis of variance One-Way ANOVA indicates that there are significant differences in the final germination percentage among the different NaCl concentrations, with the differences being highly significant. In addition, Tukey's test shows that there are significant differences between the mean values of the different treatments during the germination period. This test reveals the differences between the mean germination percentage of seeds under the control treatment and those germinated under the higher NaCl concentrations. It is observed from Figure 1. that the mean germination percentage under the 100 mM NaCl concentration was very low, while it increased under the 25 mM treatment, showing a value very close to the mean germination percentage under the control. As for the 50 mM concentration, there were significant differences between it and the other concentrations. Final germination percentage of wheat seeds was significantly reduced with increasing concentrations ($F = 35.6$, $P < 0.001$). The differences were statistically significant between the seeds germinated under control conditions and those treated with various sodium chloride levels.

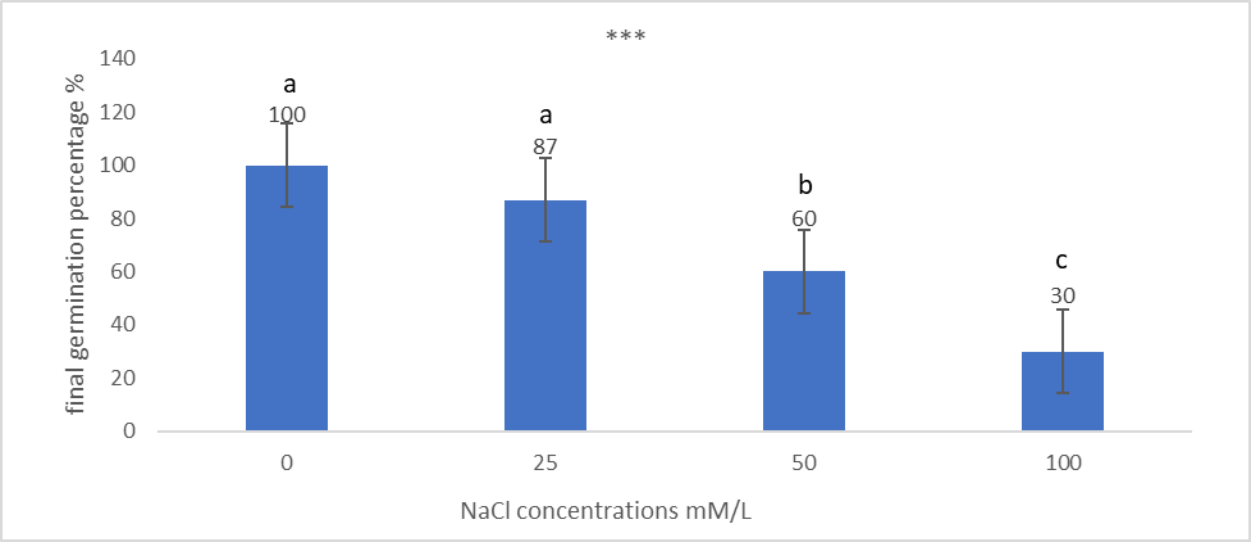

Fig 1: Effect of different sodium chloride concentrations on seed germination percentage.

*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$
+ = Not significant Bars I = SEMean Similar letters = Not significant
Different letters = Significant

The results showed a significant effect of different sodium chloride concentrations on the germination percentage of wheat seeds, with a clear decline in germination as salt concentration increased. These findings are consistent with recent studies in the field of salt stress on plants. For example, a recent study by [32] demonstrated that increasing NaCl concentration suppresses germination processes due to increased osmotic pressure and accumulation of toxic sodium ions that disrupt ion homeostasis in cells. Moreover, a study by [18] revealed that the effect of salinity on wheat germination is also linked to increased accumulation of reactive oxygen species (ROS), which cause damage to cellular components and inhibit the activity of vital enzymes necessary for germination. Tukey's analysis confirmed significant differences between treatments, indicating a clear impact of salinity levels on germination efficiency, with notably lower germination percentages at higher concentrations ($F = 35.6$, $P < 0.001$). These results align with findings by [26], who showed that salt stress negatively affects membrane stability and vital functions in wheat seeds. Based on these studies, it can be concluded that salinity is a critical stress factor that hampers wheat seed germination through osmotic stress, ionic toxicity, and oxidative stress. This highlights the need for developing salt-tolerant wheat varieties or environmental solutions to mitigate these effects.

12. Fresh and dry weight parameters:

The effect of different concentrations of sodium chloride NaCl on fresh and dry weight parameters of wheat seedling is shown in Figure. 2. One-way ANOVA revealed highly significant differences in seedling fresh weight among the different sodium chloride concentrations ($F=25.43$; $P<0.001$), as well as in dry weight ($F=18.67$; $P<0.001$). Tukey's pairwise comparison test showed that the fresh weight under the control treatment 0.0 mM NaCl was the highest 1.70 g and significantly different from all other concentrations. Fresh weight decreased progressively with increasing salinity, reaching 0.48 g at 100 mM. For dry weight, the

control 0.0 mM and 25 mM treatments were statistically similar 0.58 and 0.50 g , whereas values decreased significantly at 50 mM 0.38 g and 100 mM 0.22 g.

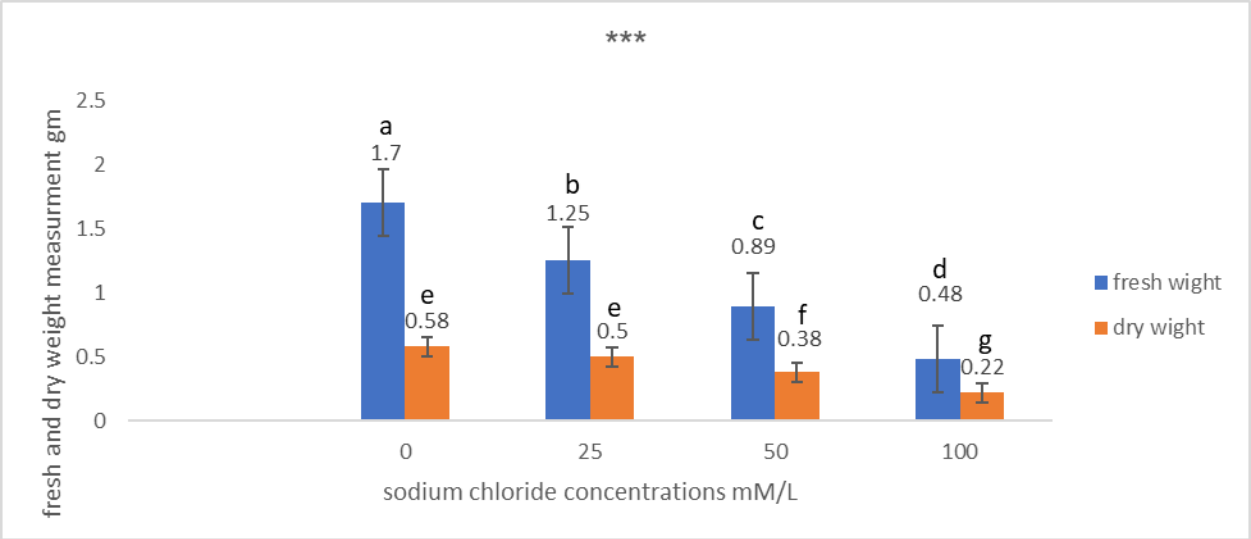

Fig 2: Effect of different sodium chloride concentrations on fresh and dry weight parameters of wheat seedling.

*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$
+ = Not significant Bars I = SEMean Similar letters = Not significant
Different letters = Significant

The results presented in Figure 2 indicate that increasing sodium chloride (NaCl) concentration in the growth medium led to a marked reduction in mean seedling length, with progressive declines observed from 0 to 100 mM NaCl. This pattern reflects the well-established inhibitory effect of salinity stress on vegetative growth in wheat (*Triticum aestivum*), likely due to osmotic stress, ion toxicity, and associated metabolic disruptions. Conversely, the application of calcium sulfate ($CaSO_4$) at concentrations of 2, 6, and 12 mM significantly improved seedling length compared to the corresponding NaCl treatments without calcium supplementation. Notably, the 6 mM $CaSO_4$ treatment resulted in the greatest improvement, followed by 12 mM, while the 2 mM treatment exhibited a comparatively modest effect. These findings suggest that calcium supplementation can partially mitigate salinity-induced growth reduction, potentially by enhancing ion homeostasis, stabilizing cell membranes, and reducing sodium uptake [7], [23]. Similar trends have been reported in recent studies, where calcium amendments improved wheat growth parameters under saline conditions. For example, [2] demonstrated that $CaSO_4$ application alleviated salt-induced growth inhibition by maintaining higher K^+/Na^+ ratios and enhancing antioxidant enzyme activities. Likewise, [9] reported that calcium supplementation under salinity stress improved chlorophyll content and biomass accumulation in wheat seedlings. Taken together, these findings support the hypothesis that exogenous calcium plays a critical role in modulating wheat's physiological responses to salinity, making it a promising agronomic strategy fo sustaining crop performance in salt-affected soils.

13. Plasma membrane stability of root tip cells parameters:

The effect of different concentrations of sodium chloride NaCl on plasma membrane stability of root tip cells is shown in Figure 3. One-way ANOVA was conducted to evaluate the effect of four salinity levels (0, 25, 50, and 100 mM NaCl) on the relative damage percentage in plants. The results revealed highly significant differences among treatments $F = 95.6$, $P < 0.001$ with the relative damage percentage increasing markedly as salinity concentration increased. Based on the Tukey's post-hoc test, the 0 mM treatment recorded the lowest significant damage percentage 1% followed by the 25 mM treatment 11% which differed significantly from the remaining treatments. The 50 mM treatment 25% showed moderate damage, which was significantly lower than the highest salinity treatment (100 mM), which recorded the highest damage percentage (77%).

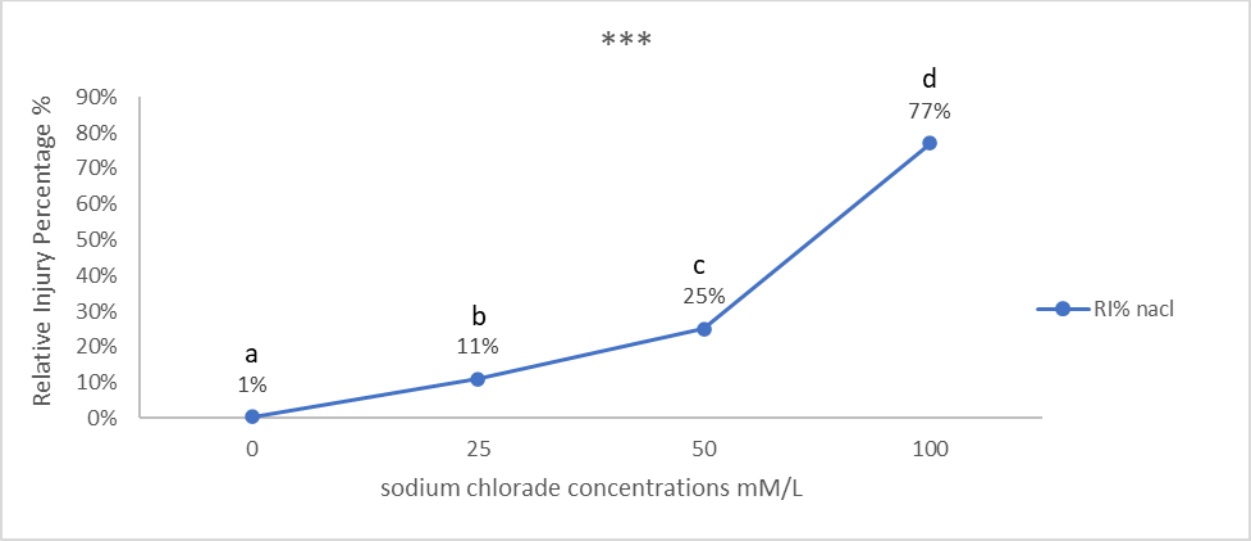

Fig. 3: Effect of different sodium chloride concentrations on plasma membrane stability of root tip cells.

*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$

+ = Not significant Bars I = SEMean Similar letters = Not significant

Different letters = Significant

The study results showed a marked increase in the relative damage percentage of plants with rising sodium chloride concentrations from 0 to 100 mM, where the percentage increased from 1% to 77%. These findings are consistent with numerous recent studies addressing the effect of salinity on wheat plants. For example, a study conducted on multiple wheat cultivars demonstrated that exposure to 100 mM NaCl led to a significant reduction in plant and root dry mass, indicating a clear negative effect of salinity on growth [29]. Another study showed that exposing different wheat varieties to concentrations of up to 200 mM resulted in the accumulation of sodium and chloride ions, along with a decrease in chlorophyll content, reflecting oxidative stress and deterioration of physiological performance [16]. These results are further supported by a comparative study between wheat cultivars with different salt tolerance levels, which showed that tolerant cultivars had a better ability to regulate calcium and potassium balance compared to sensitive ones, helping to reduce salinity-induced damage [10]. In addition, maintaining an appropriate ionic balance plays a vital role in wheat's ability to tolerate salinity, which is consistent with the observations in our study that recorded a substantial increase in relative damage at the highest concentration [20]. Based on these studies, the observed increase in relative damage in your experiment can be interpreted as a result of toxic ion accumulation,

osmotic stress, and oxidative stress-factors that contribute to membrane damage and reduced efficiency of the plant's metabolic processes

14. The effect of interaction between different concentrations of calcium sulfate and sodium chloride salt on seed germination and wheat seedling growth:

15. Seed germination percentage:

Figure 4. showed the interaction effect between calcium sulfate concentrations and sodium chloride concentrations on wheat seed germination percentage. Two-way ANOVA, conducted to assess the interaction between $CaSO_4$ concentration 2, 6, and 12 mM and NaCl levels 0, 25, 50, and 100 mM revealed highly significant differences among treatments $P < 0.001$; $F = 18.45$ in germination response. This indicates that variations in salinity and $CaSO_4$ concentration exert a substantial impact on germination. At 0 mM NaCl, no significant differences were observed among the three calcium concentrations, with germination percentages being nearly complete 100%. While at the concentration of 25 mM NaCl germination percentages showed a slight reduction 93–100% with minimal but significant differences between the lowest and highest values, suggesting the initial onset of salinity effects. At 50 mM NaCl, a clear decline in germination 83–90% was observed, with more pronounced statistical variation among treatments, indicating that 12 mM Ca^{2+} supplementation improved germination relative to lower concentrations. But at 100 mM NaCl germination percentages declined markedly 65–79% with significant differences among concentrations, and Ca12 showing the best relative performance.

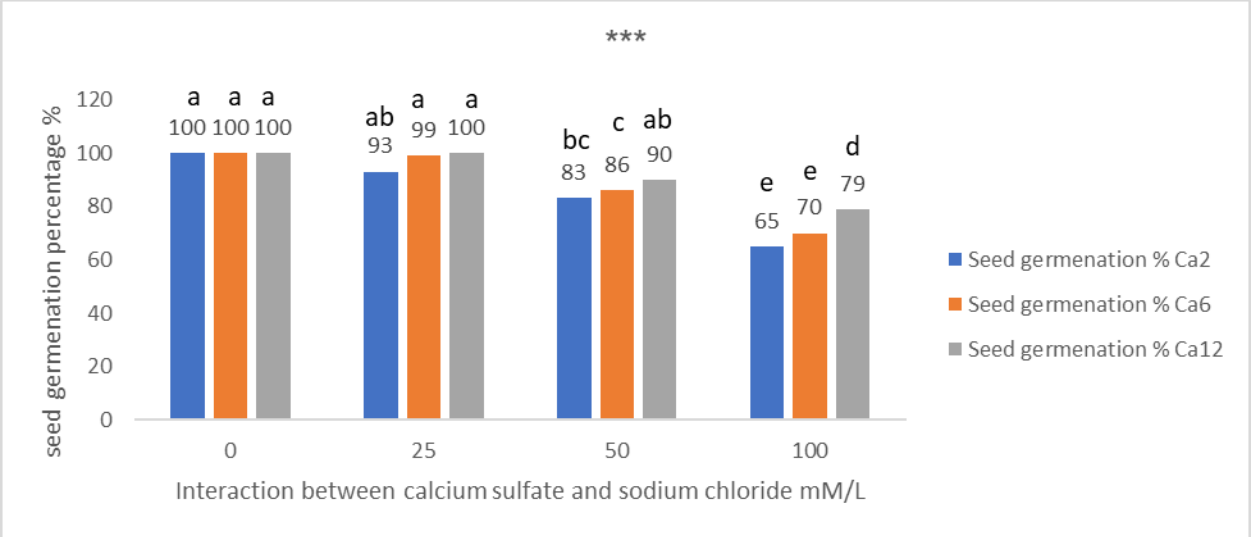

Fig. 4: Effect of interaction between different concentrations of calcium sulfate and sodium chloride salt on germination percentage of wheat seeds.

*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$

+ = Not significant Bars I = SEMean Similar letters = Not significant

Different letters = Significant

16. Fresh and dry weight parameters:

The present study demonstrates that exogenous application of calcium—particularly at 12 mM Ca^{2+}—effectively ameliorates the deleterious effects of high salinity 50–100 mM NaCl on wheat seed germination. This outcome likely results from multiple synergistic mechanisms. Notably, similar findings were observed by [21] who reported that calcium chloride ($CaCl_2$) seed priming under high salinity 200 mM NaCl maintained ionic equilibrium by reducing Na^+ accumulation and enhancing carbohydrate and protein mobilization, thus promoting germination vigor and

uniformity. Furthermore, this aligns with earlier reports by [25] whereby exogenous calcium mitigated NaCl stress through bolstering ion homeostasis e.g., sustaining K^+, Ca^{2+}, Mg^{2+} levels and preserving membrane integrity in wheat seedlings. Collectively, these insights support that calcium supplementation alleviates salt-induced germination stress through restoring ionic balance by limiting Na^+ uptake and preserving essential cation levels; enhancing mobilization of seed reserves via enzymatic metabolism; and activating antioxidant defenses and osmolyte accumulation. These mechanisms coherently explain the superior performance of 12 mM Ca^{2+} under high salinity in our study. To improve practical applicability.

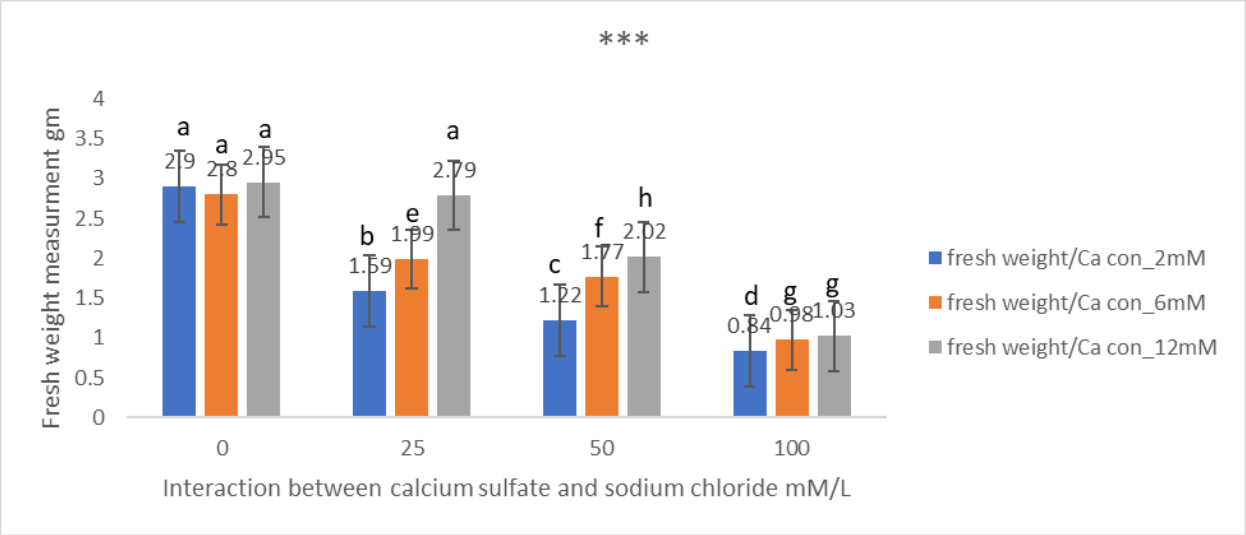

Fig. 5: Effect of interaction between different concentrations of calcium sulfate and sodium chloride salt on fresh weight of wheat seedling.
*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$
+ = Not significant Bars I = SEMean Similar letters = Not significant
Different letters = Significant

Figure (6) showed the effect of interaction between different concentrations of calcium sulfate and sodium chloride salt on dry weight of wheat seedling. A Two-Way ANOVA was performed to examine the effects of NaCl concentration (0, 25, 50, 100 mM), $CaSO_4$ concentration (2, 6, 12 mM), and their interaction on the dry weight of wheat seedlings. The analysis revealed highly significant effects of salinity (NaCl) ($F = 56.24$, $P < 0.001$) and $CaSO_4$ supplementation ($F = 8.73$, $P < 0.01$), as well as a significant interaction effect (NaCl × $CaSO_4$) ($F = 5.96$, $P < 0.01$).
Under 0 mM NaCl, dry weight values ranged between 1.79–1.84 g, with no significant differences among $CaSO_4$ treatments. At 25 mM NaCl, dry weight decreased significantly to 0.95 g at 2 mM $CaSO_4$, while higher $CaSO_4$ levels improved biomass (1.20–1.40 g), with 12 mM $CaSO_4$ showing the highest recovery, comparable to control values.
At 50 mM NaCl, biomass declined markedly (0.67–0.79 g), with no significant differences among $CaSO_4$ treatments.
At 100 mM NaCl, the lowest dry weight values were observed (0.40–0.50 g), again without significant differences among $CaSO_4$ treatments.
The results indicate that the supplementation of calcium sulfate, particularly at 12 mM, helped to alleviate the negative impact of moderate salinity (25 mM NaCl) on dry weight, while its effect diminished at higher salinity levels (50 and 100 mM NaCl). This pattern agrees with the literature, which suggests that the effectiveness of calcium depends on both the intensity of stress and its concentration; calcium improves growth under moderate salinity but has limited ability to

counteract severe stress [1] ; [30] Ion balance and membrane protection Ca^{2+} contributes to maintaining a higher K^+/Na^+ ratio and stabilizing cell membranes, which ensures efficient nutrient uptake and metabolic activity. In wheat, the application of $CaSO_4 \cdot 2H_2O$ at 3–6 mM under 50 mM NaCl improved dry weight and water status while enhancing ion balance efficiency [30]. In salt-stressed wheat seedlings [32] reported that exogenous $CaCl_2$ reprogrammed starch metabolic pathways, providing an additional source of carbon and energy for growth. This aligns with the recovery of dry weight observed in the present study at moderate salinity 25 mM NaCl with 12 mM $CaSO_4$.[17] and [1] demonstrated that external calcium increases chlorophyll pigment content and activates antioxidant enzymes thereby reducing reactive oxygen species accumulation and improving stress tolerance. However, under severe salinity (50–100 mM NaCl), osmotic and ionic toxicity exceed the compensatory capacity of calcium alone, which explains the current results. These findings are consistent with recent reviews on wheat salinity tolerance, highlighting calcium as a key mineral element that enhances salt stress resilience and improves growth under moderate stress, while its effectiveness diminishes under severe salinity conditions [34] ; [13] ; [12].

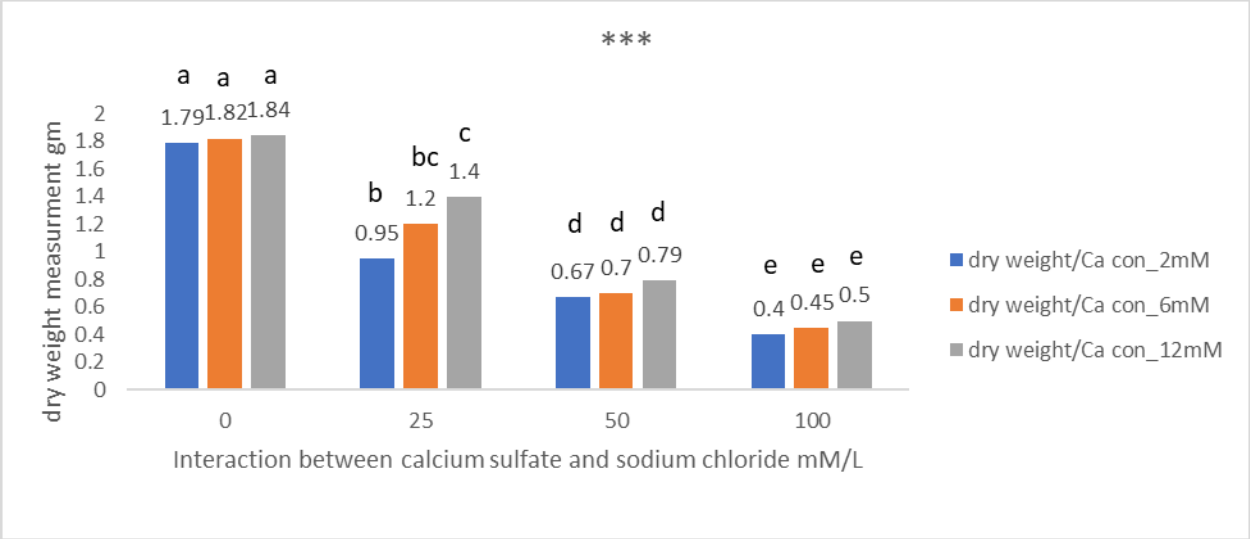

Fig. 6: Effect of interaction between different concentrations of calcium sulfate and sodium chloride salt on dry weight of wheat seedling.
*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$
+ = Not significant Bars I = SEMean Similar letters = Not significant
Different letters = Significant

17. Plasma membrane stability of root tip cells parameters:

The effect of the interaction between different concentrations of calcium sulfate and sodium chloride on plasma membrane stability of root tip cells is shown in Figure 7. Statistical analysis (Two-way ANOVA) revealed a significant interaction effect ($NaCl \times CaSO_4$; $F = 12$, $P < 0.01$). The results indicate that salinity significantly increases relative injury in wheat seedlings. The relative injury (RI%) increased progressively with rising NaCl concentrations, reflecting the osmotic and ionic stresses imposed on plant cells, consistent with previous findings [3]; [11]. Calcium supplementation ($CaSO_4$) demonstrated a protective effect against salt-induced injury. At all tested NaCl concentrations, increasing $CaSO_4$ levels reduced RI%, indicating that calcium mitigates the detrimental effects of salinity. This is in agreement with studies reporting that Ca^{2+}

stabilizes cell membranes, enhances selective ion uptake, and improves stress tolerance in wheat seedlings [11]. The interaction between NaCl and $CaSO_4$ was significant. The protective effect of calcium was more pronounced at moderate salinity levels (25–50 mM NaCl) but less effective at high salinity (100 mM NaCl). This suggests that under severe salt stress, the capacity of seedlings to utilize calcium for stress mitigation is reduced, which aligns with previous reports [3].

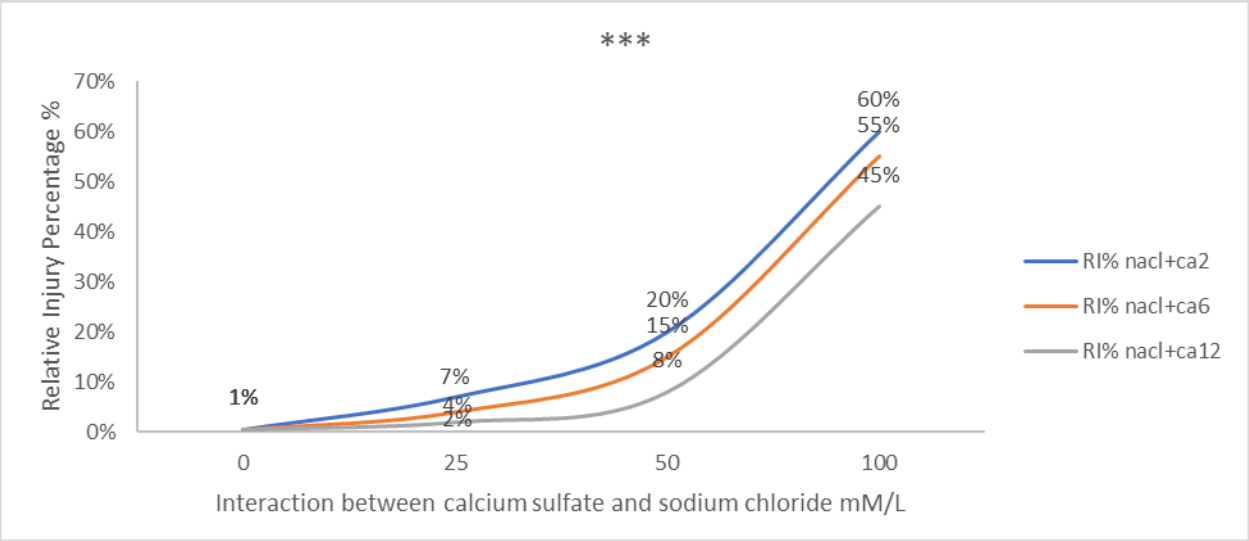

Fig. 7: Effect of interaction between different concentrations of calcium sulfate and sodium chloride salt on plasma membrane stability of root tip cells.
*** = Significant at $P < 0.001$ ** = Significant at $P < 0.01$ * = Significant at $P < 0.05$
+ = Not significant Bars I = SEMean Similar letters = Not significant
Different letters = Significant

Conclusions:

Germination under salinity: The results showed that increasing NaCl concentration caused a significant decrease in wheat seed germination, with a very low percentage at 100 mM, while it remained close to the control at 25 mM. This confirms the osmotic and ionic stress effects of salinity on germination processes (Figure 1).

Plant growth parameters: The fresh and dry weight of seedlings decreased clearly with increasing salinity, with the highest weight recorded at 0 mM NaCl and progressively decreasing with higher salt levels, reflecting the negative impact of salt stress on root and shoot growth (Figure 2).

Plasma membrane stability: Membrane stability tests showed that the relative injury of the plasma membrane increased significantly with rising NaCl concentration, reaching 77% at 100 mM, while it was lowest in the control (1%), confirming that salinity increases membrane permeability and causes electrolyte leakage (Figure 3).

Effect of calcium: The interactions between $CaSO_4$ and NaCl showed that calcium addition, especially at 12 mM, reduced the decline in germination, increased fresh and dry weight, and markedly decreased relative plasma membrane injury, particularly under moderate salinity (25–50 mM NaCl). This highlights the role of calcium in:

Enhancing plasma membrane stability and reducing electrolyte leakage.

Maintaining ionic homeostasis within the cells.

Mitigating the negative physiological impact of salinity (Figures 4 –7).

Overall conclusion: The results demonstrate that calcium acts as a key protective factor under salt stress, enhancing plasma membrane stability and improving wheat physiological performance, with its effect being more pronounced under moderate salinity compared to high salinity.

Recommendations

Use of calcium under moderate salinity: It is recommended to apply calcium sulfate ($CaSO_4 \cdot 7H_2O$) at a concentration of 12 mM, especially under moderate salinity conditions (25–50 mM NaCl), as the results showed significant improvements in germination percentage, fresh and dry weight, and a reduction in relative plasma membrane injury.

Salinity management in fields: Wheat plants should be exposed to lower NaCl concentrations <50 mM because higher concentrations lead to severe reductions in germination and growth and increase membrane damage. This includes monitoring irrigation water quality and managing salt-affected soils.

Enhancing membrane stability: It is recommended to supplement plants with suitable calcium sources to improve plasma membrane stability, maintain ion balance, and reduce leakage of essential ions such as K^+ and Mg^{2+}, thereby enhancing wheat tolerance to salinity.

Complementary strategies: Calcium application can be combined with other agronomic practices, such as fertilization adjustments or selecting salt-tolerant wheat varieties, especially in soils with moderate to high salinity.

Further studies: Further research is recommended to evaluate the effects of calcium on different wheat cultivars and under various salinity levels, with a focus on molecular and physiological mechanisms of calcium in plasma membrane stabilization and regulation of salt-responsive genes.

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