



ORIGINAL ARTICLES

Exogenous application of calcium ameliorates salinity stress of mungbean

Haider Iqbal Khan¹, Totan Kumar Ghosh¹ and Md. Abdul Baset Mia^{1*}

¹Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

ARTICLE INFO

Keywords:

Amelioration, exogenous Ca, micromorphology, mungbean, salinity.

Received : 19 November 2023

Revised : 18 December 2023

Accepted : 27 December 2023

Published : 30 December 2023

Citation:

Khan, H. I., T. K. Ghosh and M. A. B. Mia. 2023. Exogenous application of calcium ameliorates salinity stress of mungbean. *Ann. Bangladesh Agric.* 27(2): 91-104.

ABSTRACT

An experiment was conducted to investigate the impact of exogenous Ca in alleviating salinity stress of mungbean. Two mungbean genotypes, BD 6895 and BD 6905, were used with four different Ca levels (5, 10, 15 and 20 mM) under 10 dSm⁻¹ salinity conditions in a hydroponic culture. The application of exogenous Ca positively influenced on overall plant growth and development. Although both genotypes exhibited similar plant heights, they gave the peak (56.65 and 48.6 cm for BD 6895 and BD 6905, respectively) at 10 mM Ca. Higher levels of exogenous Ca were associated with increased chlorophyll content. Maximum Chlorophyll (1.6 mg g⁻¹) was observed at 15 mM Ca treatment. Furthermore, exogenous Ca application reduced Malondialdehyde levels (at 15 mM Ca: 1.14 and 1.69 μ mole/g for BD 6895 and BD 6905, respectively). Both the genotypes exhibited a proline content pattern, demonstrating proline content upsurge with increased Ca application. Microscopic analysis revealed larger vascular areas with exogenous Ca (BD 6895: 402 μ m, BD 6905: 398.3 μ m) compared to smaller areas under salinity stress (BD 6895: 258.7 μ m, BD 6905: 248.4 μ m). Salinity stress induced changes in upper epidermis thickness, leaf tissue compactness, chloroplast breakdown and chlorosis in mungbean plants. However, exogenous Ca application counteracted these detrimental effects, enabling mungbean plants to thrive in saline conditions. In conclusion, the study highlights the positive influence of exogenous Ca (10-15 mM) in promoting mungbean growth and managing salt stress.

Introduction

Mungbean, a delectable pulse crop with a wide range of applications, holds great significance in Bangladesh's agricultural landscape. Its ability to thrive in diverse soil and environmental conditions and its short growth cycle grant it a distinct advantage (HanumanthaRao *et al.*, 2016). Despite a gradual rise in demand for these pulses over the years, the yield is significantly compromised by

various abiotic and biotic stresses. Among these stresses, salinity is a primary hindrance to mungbean growth and productivity. Salinity stress profoundly impacts plant growth and development, manifesting as osmotic stress, ionic stress due to specific ion imbalances, and dietary nutrient disparities (Parida and Das, 2005). High salt stress inflicts severe harm on plants, and moderate to low levels impede plant growth rates, resulting in observable changes in morphology, physiology and biochemical attributes (Hasegawa, 2013).

*Corresponding Author: Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706. Email: miabaset@bsmrau.edu.bd

<https://doi.org/10.3329/aba.v27i2.72530>

ISSN 1025-482X (Print)/2521-5477 (Online) © 2023 ABA. Published by BSMRAU. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/bync-nd/4.0/>)

Abiotic stresses, particularly salinity, intricately weave their deleterious effects into the mungbean crop's morphology, physiology, and yield. Salinity-induced alterations extend to micromorphological characteristics, which, in turn, lead to modifications in physiological traits (Khan *et al.*, 2022a). The plant's micromorphology, particularly its internal structure causing significant shifts in tissue constructions and systemic changes (Broderson and McElrone, 2013).

Introducing a high-yielding mungbean variety with salt tolerance has the potential to seamlessly integrate into the current cropping practices in saline soil, a landscape that is still evolving. Adapting this crop to the existing cropping patterns is of utmost importance. The Department of Crop Botany at BSMRAU has undertaken a comprehensive series of experiments, focusing on the assessment of fifty-two mungbean genotypes generously provided by the Plant Genetic Resources Centre (PGRC), the Bangladesh Agricultural Research Institute (BARI), Gazipur. These experiments explore the mungbean plant's feedback to saline conditions.

Along with the genetic potential, the exogenous application of Calcium (Ca) positively recovers of deleterious effects due to salinity (Sharma and Dhanda, 2015; Ahmad *et al.*, 2019; Feng *et al.*, 2023a). These reflected the shoot and the total dry matter accumulation and anatomical attributes, namely the epidermis, cortex, and stele, which are affected by salt stress. Physiological attributes like proline accumulation were increased with the increment of salinity, chlorophyll was retained in tolerant genotypes. Exogenous application of Ca is reported to ameliorate the deleterious effect of salinity even in the susceptible genotypes (Sharma and Dhanda, 2015; Feng *et al.*, 2023b).

Considering the above, the experiment was undertaken to investigate the effect of the exogenous application of Ca on the micromorphological and physiological attributes of the mung bean plant under salt-stress conditions.

Methodology

A glasshouse and laboratory experiment were conducted at the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. The effect of

Calcium (Ca) to manage the salinity in mungbean growth was observed in the experiment. It was laid out in a factorial completely randomized design with three replications. Two mungbean genotypes (BD 6895 and BD 6905) were factor one and Ca levels (control, 5, 10, 15, and 20 mM) under 10 dSm⁻¹ salinity level was factor two. The mungbean genotypes were screened and selected from our earlier experiment (Khan *et al.*, 2022b) where BD 6895 and BD 6905 was found salt tolerant and susceptible, respectively.

Plant growth: The mung bean seeds underwent sterilization using a 70% ethanol solution before sowing into 250 ml plastic pots, which were covered with small gravels. These plastic pots were positioned on cork sheets, resting on a plastic container filled with a nutrient solution. Seven days after germination, a NaCl solution at a specific concentration was administered to the plants. The plants were placed inside 10 L plastic containers containing a nutrient solution (Hoagland and Arnon, 1950). To ensure oxygenation and maintain the solution's uniformity, the hydroponic system was continuously aerated with an air pump.

Anatomical observation

Leaf and stem samples were harvested at 30 days of sowing. Cross-sections of 2 cm slices from both the leaf and stem were carefully selected and allowed to soften for one week in a glycerin mixture (70% glycerin and 30% water, in a 1:1 ratio). Subsequently, freehand cross-sections were performed using razor blades. These sections were stained with Safranin, followed by fast green, and then mounted with glycerin on glass slides, each covered with a coverslip. The slides were examined using a digital compound microscope, and photographs were captured at magnifications ranging from 10X to 40X. An image focus 4.0 software on a computer was utilized to measure the anatomical parameters.

Physiological attributes of salinity tolerance in mungbean

Estimation of proline content

The youngest fully extended leaf samples were used for proline estimation, following the method by Bates *et al.* (1973). The proline concentration was determined using a standard curve and calculated based on the fresh weight as follows:

Proline content ($\mu\text{mole g}^{-1}$ fresh wt.) = $\{\mu\text{g proline ml}^{-1} \times \text{vol. of extra. sol. (ml)} \times \text{toluene used (ml)}\} / \{115.13 \mu\text{g mole}^{-1} \text{ g sample}\}$

Estimation of chlorophyll content: The youngest fully extended leaf sample was used to estimate the Chlorophyll contents following Porra *et al.* (1989). The equation for measuring the chlorophyll a, b, and total chlorophyll is:

- Chlorophyll a (mg g^{-1} fresh weight) = $[12.21 (A663) - 2.81 (A646)] \times [V/1000 \times W]$
- Chlorophyll b (mg g^{-1} fresh weight) = $[20.13 (A646) - 5.03 (A663)] \times [V/1000 \times W]$
- Total Chlorophyll (mg g^{-1} fresh weight) = $[20.2 (A646) + 8.02 (A663)] \times [V/1000 \times W]$ Where, A = Absorbance at specific wavelengths, V = Volume of acetone used (mL) and W = Weight of fresh leaf sample in (g).

Determination of lipid peroxidation: The method by Tayebi-meigooni *et al.* (2012) was used to determine the Malon-dialdehyde (MDA) level. An extinction coefficient of $155 \text{ nm}^{-1} \text{ cm}^{-1}$ was adapted to calculate the MDA concentrations using the following formula:

MDA ($\mu\text{mole g}^{-1}$ fresh weight) = $[(A532 - A600)/155] \times 103 \text{ Dilution factor}$

Here, A is the absorbance at specific wavelengths.

Statistical software (Statistix10) was used to analyze the data and comparisons with P- values < 0.05 were considered significantly different by using honestly significant difference (HSD) values (Tukey's Test).

Results

Physiological attributes

Plant height

The plant height of BD6895 exhibited a greater vertical stature than genotype BD6905. Interestingly, both genotypes displayed a consistent increase in plant height with the introduction of exogenous Ca up to the concentration of 10 mM, after which a gradual decline was observed (Fig. 1). Notably, at the 10 dsm^{-1} , all levels of Ca application resulted in a similar plant height. It's worth emphasizing that BD6895, as the genetically taller genotype and found tolerant earlier (Khan *et al.*, 2022b), maintained its height across all levels of exogenous Ca application, underscoring its resilience and growth potential.

Remarkably, this trend was not exclusive to BD 6895; a similar pattern was also observed in the case of the shorter genotype, BD6905 found susceptible earlier (Khan *et al.*, 2022b). This intriguing parallelism suggests that the impact of exogenous Ca on plant height transcends genotype variations and underscores the importance of Ca in modulating plant stature.

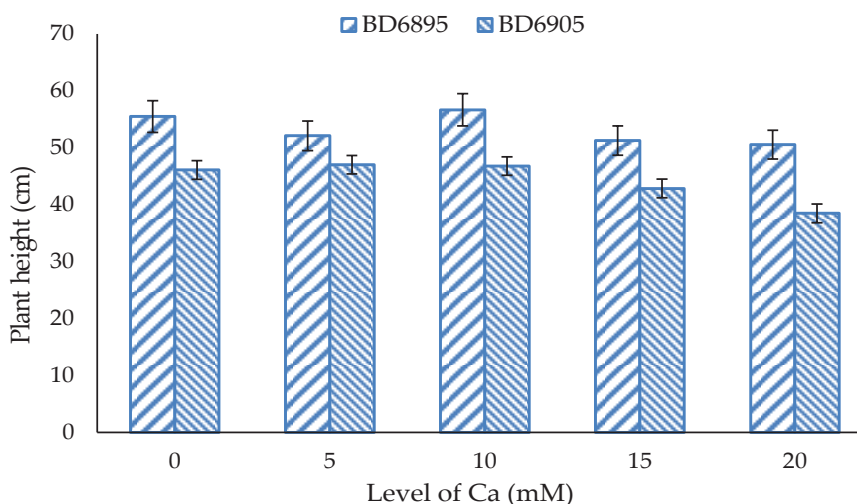


Fig. 1. Effect of Ca levels on plant height on mungbean under saline condition.

Root dry matter

As the levels of Ca increased, the root dry weights also increased. This indicates that there is a positive correlation between the amount of Ca applied and

dry weights, with genotype BD 6895 showing higher weights compared to BD 6905. A strong linear relationship exists between Ca levels and root dry weights for BD6905, as indicated by an R-squared the root dry weights (Fig. 2).

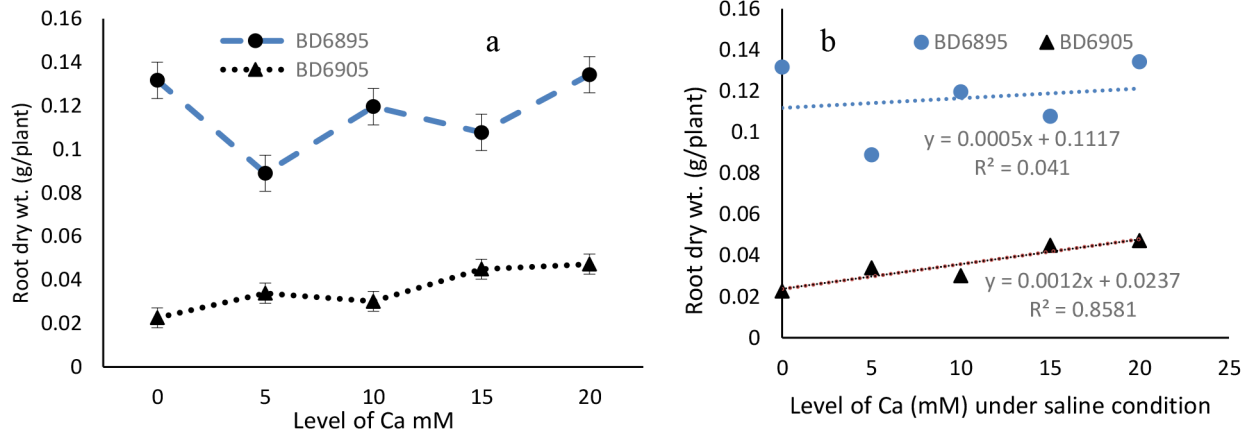


Fig. 2. Effect of different levels of Ca on root development of mungbean (a- line graph, b-regression curve).

The tolerant genotype BD 6895 exhibited higher values for root dry weights across all levels of Ca compared to the susceptible genotype BD 6905 (Fig. 2). This suggests that BD 6895 was to be more responsive to variations in Ca levels and tends to produce heavier roots. In the case of genotype BD 6905, the application of Ca has a positive relationship. A straight line is best fitted concerning to genotype BD 6905 ($R^2=0.86$), indicating a strong linear correlation between the increase in Ca levels and the root dry weights for BD6905. Increasing Ca levels positively impact root value of 0.86.

Shoot dry matter

The shoot dry matter of both genotypes, BD 6895 and BD 6905, changed with the increase in Ca (Fig. 3). This suggests that the presence of Ca influenced the growth and dry matter production in both genotypes. Genotype BD6895 showed a gradual weight reduction, but BD6905 exhibited the reverse. Genotype BD6895 exhibited a higher shoot dry matter than genotype BD6905.

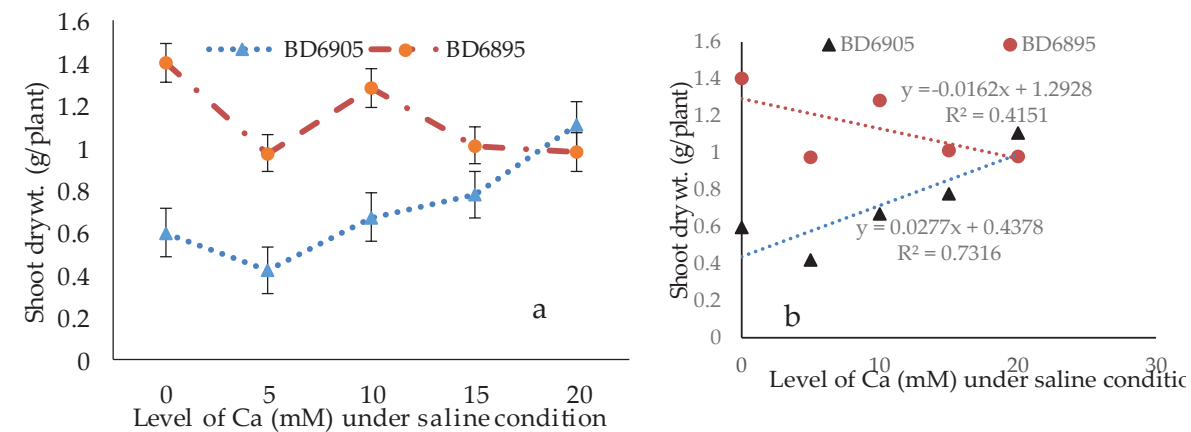


Fig. 3. Effect of different levels of Ca on shoot development of mungbean (a-line graph, b-regression curve).

This implies that BD 6905 produces dry matter in response to Ca more efficiently. Genotype BD 6905 showed a positive relationship with the increasing levels of Ca. For genotype BD 6905, a straight line best describes the relationship between shoot dry matter and Ca levels. This linearity suggests a consistent and predictable response of BD 6905 to changes in Ca concentration. In other words, there is a moderately strong correlation between Ca levels and shoot dry matter for this genotype. These varying levels of Ca in terms of their total dry matter (TDM) production under

salt stress is presented in Fig. 4. The total dry matter of genotype BD 6905 showed a progressive trend with the increasing level of Ca. In contrast to BD6905, the TDM of genotype BD6895 showed a declining trend with an increasing level of Ca. BD 6905 shows a positive and linear relationship between Ca levels and total dry matter, with an R² value of 0.75, suggesting a strong correlation with increasing Ca. BD 6895, on the other hand, exhibits a declining trend in total dry matter as Ca levels increase. This indicates findings suggest that increasing Ca levels

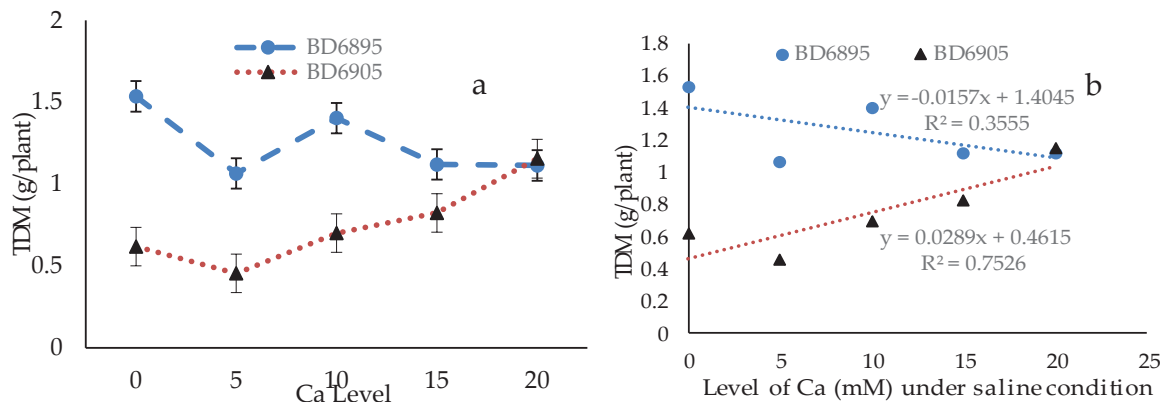


Fig. 4. Effect of different levels of Ca on Plant development of mungbean under saline conditions (a- line graph, b-regression curve)

positively influences the shoot dry matter of both genotypes, but genotype BD 6905 outperforms BD 6895 in terms of shoot dry matter production.

No statistically significant difference was observed between the two genotypes regarding their Chlorophyll content by exogenous application of Ca (Fig. 5).

Total dry matter

The response of genotypes BD 6905 and BD 6895 to that higher Ca levels may not be conducive to TDM production in BD 6895.

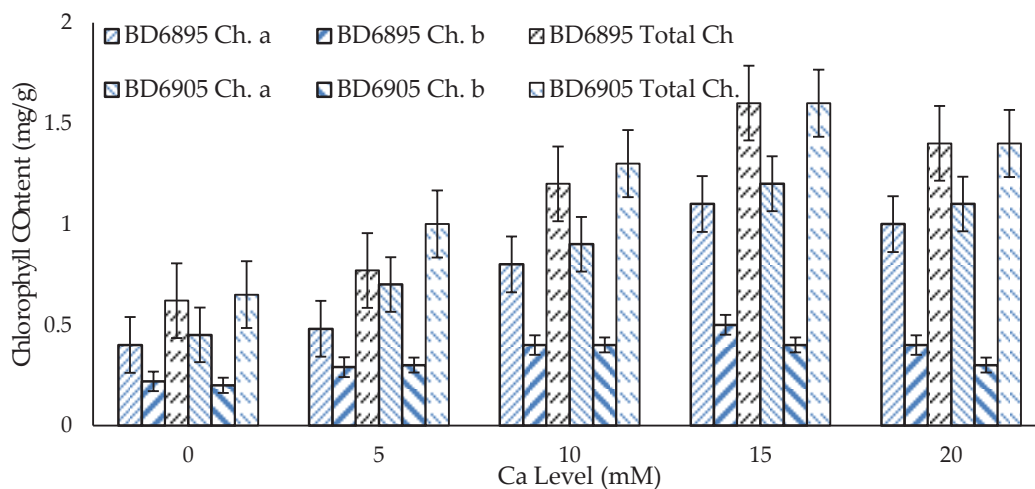
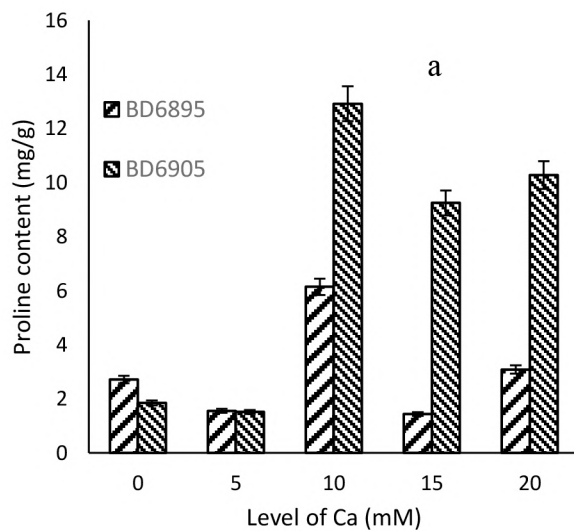


Fig. 5. Effect of different levels of Ca on chlorophyll development on mungbean under saline condition.

The chlorophyll content exhibited a progressive increase as the concentration of exogenously applied Ca rose, showing a positive correlation up to the 15 mM level. However, a gradual decline in chlorophyll content became evident beyond this threshold. This finding underscores the complex relationship between Ca levels and chlorophyll production, suggesting an optimal Ca concentration for maximizing chlorophyll content in these genotypes.



Interestingly, both genotypes followed a similar pattern characterized by a sudden surge in proline content at the 10 mM level of exogenous Ca application and slow reduction later.

Lipid peroxidation /Malon-dialdehyde (MDA)

The findings revealed a consistent decrease in MDA values for both genotypes as the exogenous application of Ca increased (Fig. 7). This declining trend in MDA levels exhibited a robust linear

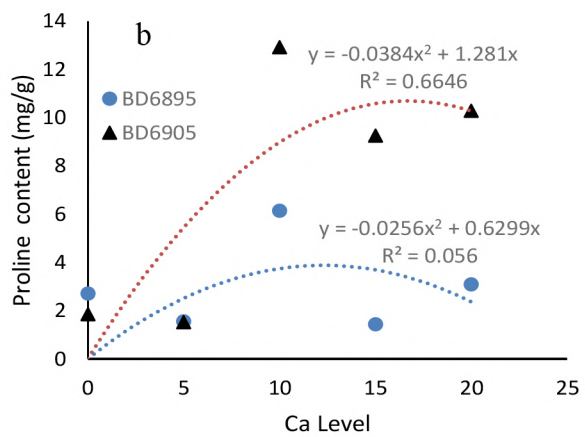


Fig. 6. Effect of different levels of Ca on proline content development on mungbean under saline condition (a- column chart, b-regression curve).

Content of proline

The application of exogenous Ca led to an initial rise in proline content, which was dose-dependent. Notably, genotype BD 6895 consistently exhibited lower proline levels than BD 6905 across all Ca levels, as depicted in Fig. 6. This increase in proline content at higher levels of exogenous Ca indicates the plant's enhanced ability to tolerate salinity-induced stress, underlining the potential role of proline as a stress-responsive compound. In both genotypes, the proline level increased with exogenous Ca application, indicating the amelioration effect in response to the stress that plants face.

relationship. Genotype BD 6905 displayed a higher MDA value when compared to genotype BD 6895, as illustrated in Fig. 6. This indicates the tolerant potential of BD 6895 over BD 6905. This distinction in MDA levels between the two genotypes underscores the variability in their response to exogenous Ca, with BD 6905 showing a relatively higher MDA value despite the overall declining trend observed in both genotypes.

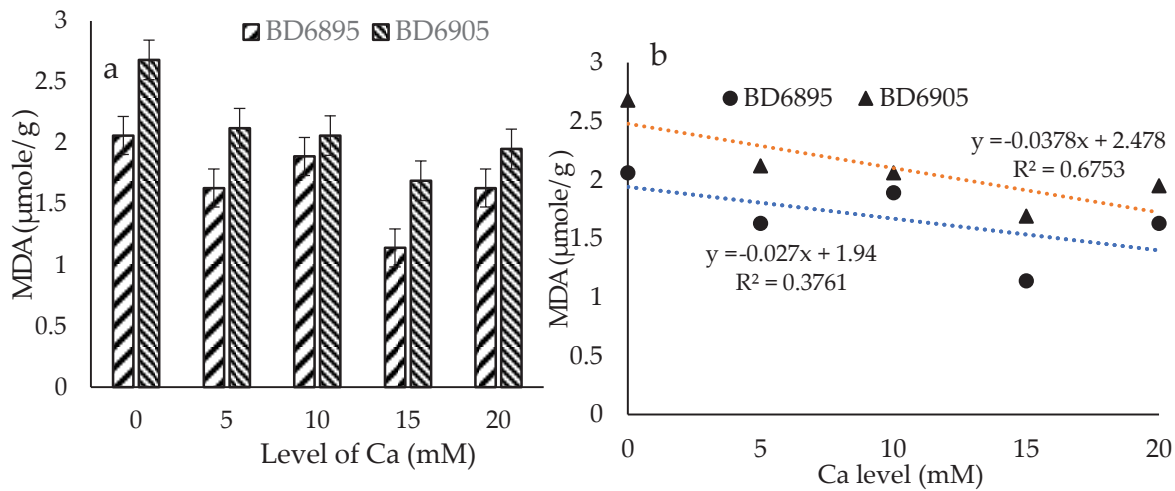


Fig. 7. Effect of different levels of Ca on Lipid peroxidation (MDA) of mungbean under saline condition (a- column chart, b-regression curve).

Anatomical attributes

Vascular bundle

The salinity stress pointedly affected mungbean plants vascular bundle strands (Xylem and Phloem area). With salinity, the vascular bundle strands

decreased, indicating that salinity stress had a damaging effect on the development of vascular structures. In the quest to overcome such stress, the amelioration effect of the exogenous application of Ca (10 mM) was vivid in the present experiment.

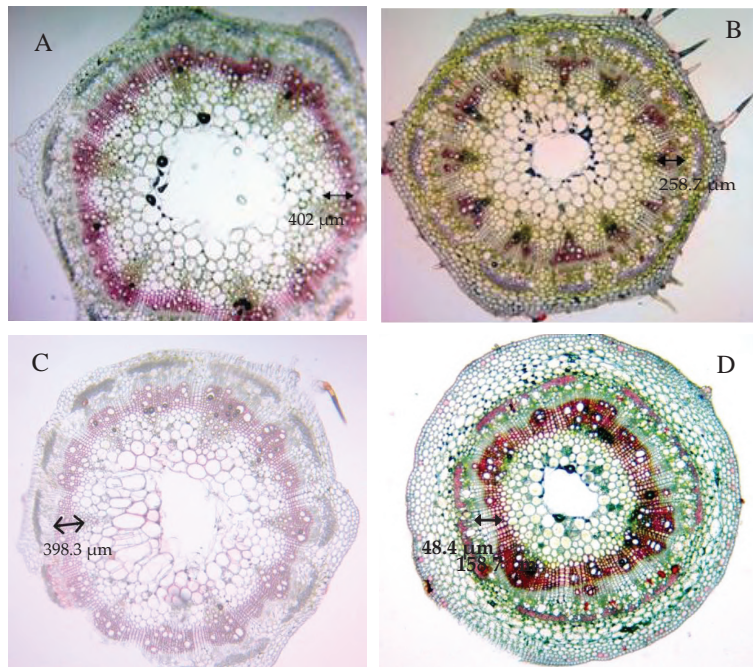


Fig. 8. Mungbean stem anatomical features under salt stress conditions (A: BD 6895 with Ca + salt; B: BD 6895 with salt; C BD 6905 with Ca + salt; D: BD 6905 under salt).

The areas of both xylem and phloem were significantly altered by the salinity stress (Fig. 8). Comparatively large vascular areas were detected with the exogenous Ca application (BD 6895: 402 μm and BD 6905:398.3 μm). In comparison, the smaller areas (BD 6895: 258.7 μm and BD 6905:248.4 μm) were found in the salinity stress condition (10 dSm^{-1}) for both plant genotypes. This suggests that salinity levels lead to a reduction in the xylem and phloem areas, and the application of exogenous Ca aids to overcome the salinity-induced stress.

Salinity stress has a noticeable impact on the anatomy of mung bean plants, particularly on their vascular bundles, xylem, phloem, and cell wall thickness. The findings suggest that salinity levels hinder the growth and development of vascular tissues in these plants. The application of exogenous Ca supports mungbean plants to overcome such chaotic conditions.

Leaf anatomy

The effects of salinity on the anatomy of mung bean plant leaves, and the positive influence of exogenous Ca (Ca) application (10 mM) are clear (Fig. 9). Under salinity stress (10 dSm^{-1}), the upper epidermis of leaves from both genotypes exhibited a tendency to become thicker. This could be an adaptive response to the stress, possibly to reduce water loss or protect against ion uptake. Salinity stress had a varying impact on different leaf tissues. Palisade tissue, which is columnar and rich in chloroplasts, was less affected than the spongy parenchyma tissue. Despite the changes, both tissues became more compact due to salinity stress.

The anatomical images revealed that at higher levels of salinity, there was a clear breakdown of chloroplasts in the palisade tissues, resulting in chlorosis. Chlorosis is a condition characterized by the loss of green color in plant tissues, often due to reduced chlorophyll content or functionality. In this context, it suggests that salinity negatively affects the photosynthetic capacity of the plants.

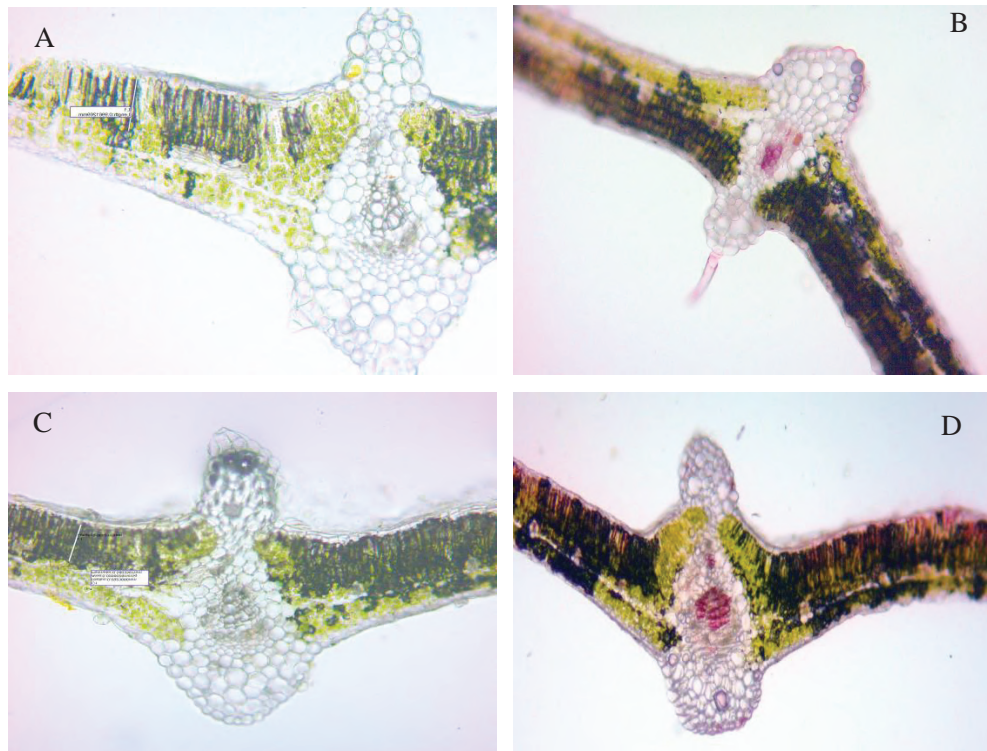


Fig. 9. Mungbean leaf anatomy under salt stress conditions (A: BD 6895 with Ca + salt; B: BD 6895 with salt; C BD 6905 with Ca + salt; D: BD 6905 under salt).

As anticipated, the application of exogenous Ca was found to ease the detrimental effects of salinity on the mung bean plants. This indicates that supplementing with Ca helps the plants thrive even under salinity stress. Ca is believed to play a role in various physiological processes in plants, including cell membrane stability and stress response mechanisms.

Salinity stress leads to changes in the upper epidermis thickness, compactness of leaf tissues, chloroplast breakdown, and chlorosis in mung bean plants. The exogenous application of Ca has a positive influence, helping the plants better cope with salinity stress.

Discussions

A positive correlation exists between the amount of Ca applied and the dry weights (stem, root, and total) of both genotypes under saline conditions. This indicates that Ca positively impacted overall plant growth and biomass production. The application of exogenous Ca served to inhibit ionic toxicity and alleviate osmotic stress (Islam *et al.*, 2023). As a result, the application of Ca significantly improved the plant's growth, development, and production attributes. Calcium ameliorates the membrane leakage of cells, especially the mesophyll tissue.

Generally, plant hindered growth is one of salinity's predominant effects. Several studies have shown a steady reduction in root and stem dry matter as salinity levels increase (Khan *et al.*, 2022b; HanumanthaRao *et al.*, 2016). Increased growth and vigor appraisal were observed in mungbean even under salt-stressed conditions with Ca (Sharma and Dhanda, 2015; Feng *et al.*, 2023 a). The decline in plant growth and biomass yield is a characteristic significance of salinity across various plant species. This reduction is due to stress-induced inhibition of cell elongation, cell division, and the obstructed uptake of nutrients by the plant. The imbalance in water potential between the apoplast and symplast caused by the salinity results in decreased turgor pressure, which subsequently leads to reduced plant growth (Islam *et al.*, 2023). It's worth noting that

the inhibition of crop growth under salt stress can be mitigated by the external application of Ca (Islam *et al.*, 2018). The exogenous application of Ca also enhances the uptake of essential nutrient elements, defending against the antagonistic stress effect on crop growth and yield (Ahmad *et al.*, 2015).

The reduction in plant height, as well as root and shoot length with increasing salinity, is reported (Khan *et al.*, 2022 b). Both genotypes showed increased plant height with the introduction of exogenous Ca up to a concentration of 10 mM. However, a gradual decline in plant height was observed after this threshold. This implies an optimal range of Ca concentration for maximizing plant height.

Although there was no noteworthy variation in chlorophyll content, the chlorophyll content increased as the concentration of exogenously applied Ca rose. This increase showed a positive correlation up to 15 mM, after which a gradual decline was observed. This indicates an optimal Ca concentration for maximizing chlorophyll content in these genotypes. Application of Ca in saline conditions increased chlorophyll and carotenoid content (Sharma and Dhanda, 2015). Seifikalhor *et al.* (2019) reported that the sharp upsurge in the intracellular Ca^{2+} in response to salinity resulted in a can help plants to recover the salinity-induced damage to plants.

Both genotypes displayed a consistent decrease in MDA values as the exogenous application of Ca increased. MDA is a marker of oxidative stress; lower values suggest reduced oxidative stress in the plants. BD6895 had higher MDA values than BD6905, indicating that it may have experienced slightly higher oxidative stress even with the Ca application. The presence of salinity had a notable effect on the increased accumulation of MDA (Khan *et al.*, 2022b). However, applying exogenous Ca significantly reduced the accumulation of MDA in plants exposed to salinity (Islam *et al.*, 2023). Salt stress leads to the rapid overproduction of ROS, causing cell lipid peroxidation and membrane damage, as evidenced by increased MDA content

(Mansour 2013; Bose *et al.*, 2014). In a study by Zhang and Yang (2022), NaCl treatment significantly raised ROS and MDA levels, while CaCl₂ supplementation reduced these levels, suggesting that exogenous Ca enhances membrane integrity and safeguards plant cells from ROS-induced oxidative harm (Tuna *et al.*, 2007; Tahjib-Ul-Arif *et al.*, 2018; Roy *et al.*, 2019). Feng *et al.* (2023 b) emphasized the vital role of Ca ions in osmotic regulation, ultimately reducing salinity stress.

Proline is an amino acid that can help plants cope with stress. Both genotypes exhibited a similar pattern characterized by a sudden increase in proline content at the 10 mM level of exogenous Ca application, followed by a slow reduction. This suggests that Ca levels influence proline production and may be part of the plant's stress response mechanism. Various osmolytes, such as proline, positively influence on physiological processes by aiding in the cellular equilibrium maintenance under salt stress through diffusion regulation (Yang and Guo, 2018; Khan *et al.*, 2022b). Ca was reported to be an essential signaling molecule involved in proline biosynthesis (Islam *et al.*, 2023). An augmented proline level is found with low proline oxidase activity and more synthesis of P-5-CR and γ -glutamyl kinase in roots and shoots. Several reports have shown the facilitated osmolyte synthesis by adding exogenous Ca in salinity-induced mungbean (Misra and Gupta, 2006). During the current experiment, it was observed that the proline content in leaves increased in response to salinity stress with the exogenous Ca application which indicates the mediation of salinity. This mirrors findings reported by Ghosh *et al.* (2015), which indicated a similar augmentation of proline in mungbean under saline conditions as survival technique. Additionally, Reddy *et al.* (2015) anticipated that the enhanced assembly of proline may mitigate the stress imposed by salinity, providing protection to photosynthetic and antioxidant enzyme activities.

To thrive in salt-stressed environments, plants must precisely regulate ion balance to maintain internal stability. The salt overly sensitive (SOS) stress signaling path is crucial part in bolstering

salt tolerance and ion equilibrium (Manishankar *et al.*, 2018; Xiao and Zhou, 2023). This pathway employs the SOS-controlling protein to transfer sodium ions (Na⁺) from a plant's roots to its shoots. Overexpressing this protein has proven to boost plant salt tolerance (Seifkalthor *et al.*, 2019). An increase in intracellular Ca ions (Ca²⁺) concentrations hastens their binding with the SOS protein, subsequently enabling the regulation of intracellular sodium levels. Following this interaction, the SOS protein is translocated to the plasma membrane and undergoes phosphorylation. This phosphorylated SOS protein plays a critical role in promoting the efflux of sodium ions (Na⁺), thus reducing the risk of sodium toxicity within plants (Han *et al.*, 2019).

Anatomical and morphological changes are major role in salinity stress, indicating the tolerance level (Silva *et al.*, 2021). Salinity gradually alters the internal structure of plants, leading to a reduction in both the xylem and phloem range in stems. This environmental stress triggers structural modifications in the stem xylem and phloem, consequential in a decrease in the vascular area and increased cell thickness with rising salinity levels compared to the control (Khan *et al.*, 2019). The application of diluted seawater also significantly diminishes stem dimensions, changing the tissue thickness of the xylem and phloem (Eisa *et al.*, 2017).

A decrease in the area of vascular cylinder (VC) and cortical parenchyma (CP) affecting the VC/CP ratio in mung beans under salt stress has been reported (Nikpour-Rashidabad *et al.*, 2019). Younis *et al.* (2014) noted a decreasing trend in the stem's xylem and phloem areas, indicating that as salinity levels increase, the growth of these cell types diminishes. Such deleterious effects of salt stress were found to be managed with the exogenous application of Ca. Regulating plant phenotype and photosynthetic apparatus protection has been reported in Soybeans (Feng *et al.*, 2021). Application of Ca in Mongolian pine was reported to Improve chlorophyll content and maintain other anatomical features and physiological metabolisms (Li *et al.*, 2022).

Under saline conditions, plant leaves tend to become thicker, more succulent, and exhibit lower specific leaf areas (Khan *et al.*, 2022a). An increase in epidermal cutin and a decrease in lamina thickness in mung beans has been reported by Boghdady (2009). The thickness of both the upper and lower epidermis in salt-treated plants leaves was greater than the control (Khan *et al.*, 2022a). Salt-tolerant plants have thicker leaves, which help maintain turgor and leaf water content (Nawaz *et al.*, 2014). Srivastava (2022) found a positive link between salt stress tolerance and thicker epidermal cells, enhancing water efficiency and the effectiveness of NaCl in the leaf's epidermis. Increasing sclerenchyma in higher salinity is also vital for maintaining organ rigidity, a key feature for salt resistance.

In brief, the total amelioration phenomena can be described as salt stress triggering an increase in cytosolic Ca ion concentration (Ca^{2+}) (Yang *et al.*, 2019). The interplay of ABA, Ca^{2+} , and ROS represents a complex signaling network that controls plant resistance to the challenges of salt stress. The role of Ca^{2+} signaling is pivotal in real-time responses to salinity stress. ABA efficiently aids plants in surviving salinity by joining the Ca ions (Ca^{2+}). This integration occurs through activating plasma membrane-bound channels for the external Ca^{2+} or releasing internal Ca^{2+} from the vacuole (Edel and Kudla, 2016). The harm caused to the plant cell wall by sodium ions (Na^+) is mediated by elevated Ca^{2+} (Szymańska *et al.*, 2019).

Overall, the findings suggest that the exogenous application of Ca has several positive phenomena on mungbean plant's growth and stress responses. The findings of the current experiment indicated that the optimum dose of exogenous Ca varied between 10 to 15m M. However, there are optimal concentration ranges for different parameters, and these can vary between genotypes, as evidenced by the differences between BD6895 and BD6905 in their response to Ca.

Conclusion

Exogenous Ca positively influenced plant growth and biomass production, mitigating ionic toxicity and osmotic stress. Consequently, it significantly improved plant growth and development. Both genotypes exhibited increased plant height up to a 10 mM Ca concentration, but a decline occurred beyond this threshold, indicating an optimal Ca range for maximizing plant height.

While no significant differences in chlorophyll content were observed between genotypes, chlorophyll content increased with higher exogenous Ca concentrations. Concurrently, MDA values, a marker of oxidative stress, decreased with increasing exogenous Ca, indicating reduced oxidative stress in the plants. Salinity resulted MDA accumulation, but exogenous Ca alleviated it. Proline content patterns were similar between genotypes, highlighting calcium's role in stress response.

Salinity caused smaller vascular areas under salinity stress (BD 6895: 258.7 μm , BD 6905: 248.4 μm), chloroplast breakdown and chlorosis, negatively impacting photosynthetic capacity. Calcium countered salinity effects, enabling mung bean plants to thrive with larger vascular areas (BD 6895: 402 μm , BD 6905: 398.3 μm).

Nevertheless, the adverse effects were counteracted by the application of exogenous Ca, allowing mung bean plants to flourish in saline conditions. The results of the present experiment revealed that the most effective dosage of exogenous Ca ranged from 10 to 15 mM. In summary, the research underscores the beneficial impact of exogenous Ca in fostering mung bean growth and effectively managing salt stress.

Acknowledgment

The authors wish to express their gratitude to the Research Management Wing (RMW) at Bangabandhu Sheikh Mujibur Rahman Agricultural University in Gazipur, Bangladesh, for their financial support in facilitating the experiment.

References

- Ahmad, R., S. Hussain, M.A. Anjum, M.F. Khalid, M. Saqib, I. Zakir, A. Hassan, S. Fahad and S. Ahmad. 2019. Oxidative Stress and Antioxidant Defense Mechanisms in Plants under Salt Stress. In: Hasanuzzaman. M., K. Hakeem, K. Nahar and H. Alharby (ed.) *Plant Abiotic Stress Tolerance*. Springer, Cham. (https://doi.org/10.1007/978-3-030-06118-0_8)
- Ahmad, P., M. Sarwat, N.A. Bhat, M.R. Wani, A.G.Kazi and L.S.P. Tran. 2015. Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. *PLoS ONE*. 10(1): e0114571. (doi: 10.1371/journal.pone.0114571)
- Bates, L., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. 39: 205-207 (<https://doi.org/10.1007/BF00018060>)
- Boghdady, M. S. 2009. Physiological and Anatomical Studies on Mung Bean Plant Under Salinity Conditions. Ph. D. Thesis, pp 222, Faculty of Agriculture, Zagazig University, Egypt
- Bose, J., A. Rodrigo-Moreno and S. Shabala. 2014. ROS homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* 65(5): 1241-1257. (<https://doi.org/10.1093/jxb/ert430>)
- Broderson, C.R. and A.J. McElrone. 2013. Maintenance of xylem network transport capacity: a review of embolism repair in vascular plants. *Front. Plant Sci.* 4:108. (<https://doi.org/10.3389/fpls.2013.00108>)
- Edel, K. H. and J. Kudla. 2016. Integration of calcium and ABA signaling. *Curr. Opin. Plant Biol.* 33:83–91. (doi: 10.1016/j.pbi.2016.06.010)
- Eisa, S., M.A. Eid, E.H. El-Samad, S. Hussin, A.A. Abdel-Ati, S.H. Ali, H. Alsayed, M.E. Lotfy, A. Masoud, A.M. El-Naggar and M. Ebrahim .2017. *Chenopodium quinoa* Willd. A new cash crop halophyte for saline regions of Egypt. *Australian J. Crop Sci.* 11(3):343-351. (doi:10.21475/ajcs.17.11.03.pne316.)
- Feng, D., Q. Gao, J. Liu, J. Tang, Z. Hua and X. Sun. 2023 a. Categories of exogenous substances and their effect on alleviation of plant salt stress. *Eur. J. Agron.* 142: 126656. (doi: 10.1016/j.eja.2022.126656)
- Feng, D., X. Wang, J. Gao, C. Zhang, H. Liu, P. Liu and X. Sun. 2023 b. Exogenous calcium: Its mechanisms and research advances involved in plant stress tolerance. *Front. Plant Sci.* 14:1143963. (doi: 10.3389/fpls.2023.1143963)
- Feng, N., M. Yu, Y. Li, D. Jin and D. Zheng. 2021. Prohexadione-calcium alleviates saline-alkali stress in soybean seedlings by improving the photosynthesis and up-regulating antioxidant defense. *Ecotoxicology Environ. Saf.* 220: 112369.(doi:10.1016/j.ecoenv.2021.112366)
- Ghosh, S., S. Mitra and A. Paul. 2015. Physiochemical Studies of Sodium Chloride on Mungbean (*Vigna radiata* L. Wilczek) and Its Possible Recovery with Spermine and Gibberellic Acid. *Sci. World J.* 1-8. (<https://doi.org/10.1155/2015/858016>)
- Han, J. P., P. Köster, M.M. Drerup, M. Scholz, S. Li, K.H. Edel, K.Hashimoto, K. Kuchitsu, M.Hippler and J.Kudla. 2019. Fine-tuning of RBOHF activity is achieved by differential phosphorylation and Ca²⁺ binding. *New Phytol.* 221 (4): 1935–1949. (doi: 10.1111/nph.15543)
- HanumanthaRao, B., R.M. Nair and H. Nayyar. 2016. Salinity and High Temperature Tolerance in Mungbean [*Vigna radiata* (L.) Wilczek] from a Physiological Perspective. *Front. Plant Sci.* 29(7):957. (doi: 10.3389/fpls.2016.00957.)
- Hasegawa, P.M. 2013. Sodium (Na⁺) homeostasis and salt tolerance of plants. *Environ. Exp. Bot.* 92:19–31. (10.1016/j.envexpbot.2013.03.001)
- Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil, California Agricultural Experiment

- Station, Circular-347.
- Islam, M.M., M.H. Faruqe, M.S. Rana, M. Akter and M.A. Karim. 2018. Screening of rice (*Oryza sativa* L.) genotypes at reproductive stage for their tolerance to salinity. *Agriculturists* 16: 55–67. (<https://doi.org/10.3329/agric.v16i1.375350>)
- Islam, M.M., K. Jahan, A. Sen, T.A. Urmi, M.M. Haque, H.M. Ali, M.H. Siddiqui and Y. Murata. 2023. Exogenous Application of Calcium Ameliorates Salinity Stress Tolerance of Tomato (*Solanum lycopersicum* L.) and Enhances Fruit Quality. *Antioxidants*. 12:558. (<https://doi.org/10.3390/antiox12030558>)
- Khan, H.I., T.K. Ghosh and M.A. Baset Mia. 2022 a. Altered anatomical appraisal of mung bean (*Vigna radiata* (L.) Wilczek) under salinity stress. *Bangladesh J. Sci. Ind. Res.* 57(3): 139-148. (<https://doi.org/10.3329/bjsir.v57i3.62016>)
- Khan M.A.H., M.A.B Mia, J.U. Ahmed, M.A. Karim And M.M.H. Saikat. 2019. Morpho-anatomical appraisal of some pulse crops under salinity stress. *Bangladesh J. Agril. Res.* 44(3): 439-451. (<https://doi.org/10.3329/bjar.v44i3.43477>)
- Khan, H. I., Khan, M.A.H., T.K. Ghosh and M.A.B. Mia. 2022 b. Evaluation of pant characteristics and physiological performance of mungbean (*Vigna radiata* (L.) Wilczek) genotypes under salt stress. *J. Asiat. Soc. Bangladesh, Sci.* 48(1-2): 97–110. (<https://doi.org/10.3329/jasbs.v48i1-2.64517>)
- Li, H., S. Huang, C. Ren, X. Weng, S. Zhang, L. Liu and J. Pei. 2022. Optimal exogenous calcium alleviates the damage of snow-melting agent to *Salix matsudana* seedlings. *Front. Plant Sci.* 13:928092. (doi: 10.3389/fpls.2022.928092)
- Manishankar, P., N. Wang, P. Köster, A.A. Alatar and J. Kudla. 2018. Calcium signaling during salt stress and in the regulation of ion homeostasis. *J. Exp. Bot.* 69(17): 4215–4226. (<https://doi.org/10.1093/jxb/ery201>)
- Mansour, M.M.F. 2013. Plasma membrane permeability as an indicator of salt tolerance in plants. *Biol. Plant.* 57(1): 1-10.
- Misra, N. and A.K. Gupta. 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. *J. Plant Physiol.* 163 (1):11-18. (<https://doi.org/10.1016/j.jplph.2005.02.011>)
- Nawaz, T., M. Hameed, M. Ashraf, M.S.A. Ahmad, R. Batool and S. Fatima. 2014. Anatomical and physiological adaptations in aquatic ecotypes of *Cyperus alopecuroides* Rottb. under saline and waterlogged conditions. *Aquat. Bot.* 116:60-68. (doi: 10.1016/j.aquabot.2014.01.001)
- Nikpour-Rashidabad, N., A. Tavasolee, S. Torabian and S. Farhangi-Abri. 2019. The effect of biochar on the physiological, morphological and anatomical characteristics of mung bean roots after exposure to salt stress. *Arch. Biol. Sci.* 71(2):321-327. (<http://www.serbiosoc.org.rs/arch/index.php/abs/article/view/3491>)
- Parida, A.K. and A.B. Das. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Safe.* 60(3):324–349. (doi: 10.1016/j.ecoenv.2004.06.010)
- Porra, R.J., W.A. Thompson and P.E. Kriedemann. 1989. Determination of Accurate Extinction Coefficients and Simultaneous Equations for Assaying Chlorophylls a and b Extracted with Four Different Solvents: Verification of the Concentration of Chlorophyll Standards by Atomic Absorption Spectroscopy. *Biochim. Biophys. Acta.* 975: 384-394. (doi: [10.1016/S0005-2728\(89\)80347-0](https://doi.org/10.1016/S0005-2728(89)80347-0))
- Reddy, P.S., G. Jogeswar, G.K. Rasineni, M. Maheswari, A.R. Reddy and R.K. Varshney. 2015. Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. *Plant Physiol. Biochem.* 94: 104–113. (doi: 10.1016/j.plaphy.2015.05.014)
- Roy, P. R., M. Tahjib-Ul-Arif, M.A.S. Polash,

- M.Z. Hossen and M.A. Hossain. 2019.. Physiological mechanisms of exogenous calcium on alleviating salinity-induced stress in rice (*Oryza sativa* L.). *Physiol. Mol. Biol. Plants*. 25(3): 611-624
- Seifikalhor, M., S. Aliniaefard, A. Shomali, N. Azad, B. Hassani, O. Lastochkina and T. Li. 2019. Calcium signaling and salt tolerance are diversely entwined in plants. *Plant Signal Behav.*14(11):1665455. (doi: 10.1080/15592324.2019.1665455)
- Sharma, A. and S. Dhanda. 2015. Application of Calcium Chloride to Mitigate Salt Stress In *Vigna radiata* L. Cultivars. *Int. j. curr. microbiol. appl. sci.* 4 (2): 764-769
- Silva, B.R.S., B.L. Batista and A.K.S. Lobato. 2021. Anatomical changes in stem and root of soybean plants submitted to salt stress. *Plant Biol.*23(1):57-65. (https://doi.org/10.1111/plb.13176)
- Srivastava, S. 2022. Morpho-Anatomical Adaptation against Salinity. In: Kimatu, J.N. (eds): *Plant Defense Mechanisms*. IntechOpen. (http://dx.doi.org/10.5772/intechopen.95214)
- Szymańska, K. P., L. Polkowska-Kowalczyk, M. Lichočka, J. Maszkowska and G. Dobrowolska. 2019. SNF1-related protein kinases SnRK2.4 and SnRK2.10 modulate ROS homeostasis in plant response to salt stress. *Int. J. Mol. Sci.* 20 (1): 143. (doi: 10.3390/ijms20010143)
- Tahjib-Ul-Arif, M., P.R. Roy, A.A.M. Sohag, S. Afrin and M.M. Rady. 2018. Exogenous calcium supplementation improves salinity tolerance in; a salt-susceptible high-yielding cultivar. *J. Crop Sci. Biotechnol.* 21(4): 383-394. (https://doi.org/10.1007/s12892-018-0098-0)
- Tayebi-meigooni A., Y. Awang, M. Mahmood, A. Selamat and Z. Wahab. 2012. Leaf water status, proline content, lipid peroxidation and accumulation of hydrogen peroxide in salinized Chinese kale (*Brassica alboglabra*). *J. Food Agric. Environ.* 10(2): 371-374. (https://doi.org/10.1234/4.2012.2990)
- Tuna, A.L., C. Kaya, M. Ashraf, H. Altunlu, I. Yokas and B. Yagmur. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environ. Exp. Bot.* 59(2):173-178. (https://doi.org/10.1016/j.envexpbot.2005.12.007)
- Xiao, F. and H. Zhou. 2023 Plant salt response: Perception, signaling, and tolerance. *Front. Plant Sci.* 13:1053699. (doi: 10.3389/fpls.2022.1053699)
- Yang, Y. and Y. Guo. 2018. Unraveling salt stress signaling in plants. *J. Integr. Plant. Biol.* 60(9):796-804. (doi: 10.1111/jipb.12689.)
- Yang, Y., Y. Wu, L. Ma, Z. Yang, Q. Dong, Q. Li, X. Ni, J. Kudla, C. Song and Y. Guo. 2019. The Ca²⁺ sensor SCaBP3/CBL7 modulates plasma membrane h⁺-ATPase activity and promotes alkali tolerance in arabidopsis. *Plant Cell.* 31 (6): 1367–1384. (doi: 10.1105/tpc.18.00568)
- Younis, A., A. Riaz, I. Ahmed, M.I. Siddique, U. Tariq, M. Hameed and M. Nadeem. 2014. Anatomical changes induced by NaCl stress in root and stem of *Gazania harlequin* L. *Agric. Commun.* 2(3): 8-14.
- Zhang, T. and H. Yang. 2022. Physiological and Biochemical Mechanisms of Exogenous Calcium Chloride on Alleviating Salt Stress in Two Tartary Buckwheat (*Fagopyrum tataricum*) Varieties Differing in Salinity Tolerance. *Phyton-Int J Exp Bot.* 91(8):1643–1658. (https://doi.org/10.32604/phyton.2022.019572)