Original Research Article

CaO nanoparticles Seed Invigoration on Seed Germination and Seedling Growth of Rice var. TRY 3

ABSTRACT

Rice (*Oryza sativa* L.), a vital cereal and staple food crop in South East Asia. It faces significant challenges in cultivation worldwide, due to soil salinity. Seed enhancement techniques like priming has shown resilience against salt stress. In this study, the potential of nano-priming with calcium oxide nanoparticles (CaO NPs) to enhance rice seed germination and growth was investigated. CaO NPs were synthesized from organic waste (eggshells) using a sol-gel technique and characterized using Raman and UV-Vis spectroscopy. Paddy seeds were primed with varying concentrations of CaO NPs, and their germination and seedling growth were assessed. The results showed that nano-priming with 40 ppm of CaO NPs significantly improved germination, root and shoot length, dry matter production, and seedling vigour compared to the control group. However, higher concentrations of CaO NPs had a detrimental effect on germination. The study suggests that CaO NP seed priming has the potential to enhance rice seedling development, potentially through improved ion homeostasis, water uptake, and physiological responses.

1. INTRODUCTION

Rice (*Oryza sativa* L. 2n=24) important cereal and stable food crop in South East Asia. Worldwide China is the leading producer of rice followed by India. In India 43 million hectares are under rice cultivation with an average yield of 2.56 tons/ha. Tamilnadu ranks second in rice production next to Punjab, and accounts 2.2 million ha under rice production with an average productivity of 2.8 tonnes/ha. Water constraint, imbalanced fertilizer and increasing soil salinity/alkalinity are the major constraints for rice production. Worldwide, Particularly, Bangladesh, China and India have significant challenges in cultivation due to soil salinity. It is the second most prevalent issue after drought. The sensitivity of rice to salt, especially during the seedling and reproductive stages, greatly affects its growth and productivity (Lutts, Kinet and Bouharmont 1995, Khan and Abdullah 2003). Soil salinity above EC 4 dSm^{-1} is moderate for rice, while salinity exceeding $8dSm^{-1}$ is considered severe (Ghassemi et al., 1995). Salinity can lead to various morphological, physiological, and biochemical changes in rice plants, including severe cases of plant mortality (Gupta *et al.,* 2021). Despite efforts in classical breeding to enhance rice's resistance to salt stress, success has been limited, and most current rice varieties are unable to tolerate high levels of salinity (Rabbani *et al.,* 2013, Solis *et al.,* 2020).

Use of quality seeds (Jerlin et al. 2010) and quality enhancement technique such as seed priming (Mukiri et al. 2021) are viable techniques, aimed at altering the physiological and biochemical properties of seeds to enhance their tolerance against abiotic stress. It involves inducing extensive physiological reorganization through hydration and dehydration processes. Priming leads to various physicochemical changes that modify the protoplasmic characteristics and enhance the physiological activity of the embryo and associated structures. As a result, the seeds exhibit improved water absorption, facilitated by increased cell wall elasticity, and the development of a stronger and more efficient root system (Krishnasamy and Srimathi 2001). Seed priming with carbon nanoparticles (CNPs) and chitosan (CS) helps in enhancing seedling growth and preserving pigment levels. Further, there are scientific reports evidenced the effect of NPs priming on improved germination and seedling growth (Raja et al., 2018)

Nano-priming of paddy seeds with calcium oxide nanoparticles can result in several potential effects. Firstly, it has been observed that such priming can enhance the germination process, promoting faster and more efficient seed germination (Dhage et al., 2020). CaO NPs stimulate metabolic activity, leading to increased nutrient uptake and root development, ultimately resulting in enhanced seedling vigour (Alam, Ullah et al. 2021). Moreover, calcium oxide nanoparticle priming has the potential to enhance stress tolerance in paddy plants. Studies have demonstrated that nano-priming with calcium oxide nanoparticles can confer improved resistance to abiotic stresses such as drought and salinity (Zhang, Yu et al. 2018). By enhancing the plants' ability to withstand adverse environmental conditions, nano-priming can contribute to better survival rates and overall growth performance. This study aimed to reveal effect of CaO NPs on improved germination growth of rice.

2. MATERIAL AND METHODS

CaO nanoparticles were synthesized via sol-gel technique from organic waste (egg shells) (Habte et al., 2019). CaO NPs were characterized for, Raman and UV-Vis (Jaiswal et al., 2020). Paddy seeds of var. TRY 3 were surface sterilized with 1% sodium hypochlorite, and primed with CaO nanoparticles at 10ppm, 20ppm, 30ppm, 40ppm, 50ppm 60ppm, 70ppm, 80ppm, 90ppm and 100ppm solutions at 1:1 ratio (seed: solution) for 12hr, and seeds were shaded dried back to original moisture content. The CaO NPs primed seeds were evaluated for germination and seedling growth under controlled environment.

2.1. GEMINATION PERCENTAGE

The germination test for NPs primed paddy seeds was conducted following the ISTA guidelines, utilizing the roll towel method in germination chamber set at a constant temperature of 25±2°C and a relative humidity of 95±2 per cent. On the 14th day, the number of normal seedlings, abnormal seedlings, hard seeds, and dead seeds were recorded. The mean germination percentage was then calculated based on the number of normal seedlings.

2.2. SEEDLING LENGTH

During the final count, ten random normal seedlings were selected from each replication of treatments. The measurement taken was the length from the tip of the main leaves to the bottom of the primary root, which is referred to as the "seedling length." These measurements were recorded in centimeters. Mean values were then calculated based on the measurements of these ten normal seedlings for each treatment.

2.3. DRY MATTER PRODUCTION

Dry matter production was determined by selecting ten normal seedlings for measurement. After removing the cotyledon and seed coat, the seedlings were carefully folded and placed inside a paper cover. They were then subjected to shade drying for 24 hours followed by further drying in a hot air oven at 80 degrees Celsius for an additional 4 hours. After this, the seedlings were allowed to cool in a desiccator for 30 minutes. Using an electronic weighing balance, the weight of the seedlings dried in the hot air oven was recorded. This weight measurement was used to calculate the mean value, expressed in grams per 10 seedlings.

2.4. VIGOUR INDEX

The vigor index of the seedlings was determined following the procedure described by (Abdul-Baki and Anderson 1973). This index was calculated by multiplying the germination percentage with the total seedling length, which includes both the root and shoot lengths. The resulting vigor index values were calculated and presented as whole numbers.

Vigor Index = Germination (%) x Total Seedling Length (cm)

2.5. STATISTICAL ANALYSIS

The statistical analysis of the experimental data involved the use of analysis of variance (ANOVA) as a factorial combination of treatments. Mean values were then compared and separated based on the least significant difference (LSD) only if the F test of ANOVA for treatments showed significance at the 0.05 probability level. Prior to the analysis, the percentage data was transformed using the arcsine transformation. In cases where the F test was found to be non-significant, the notation "NS" was used to indicate this result.

3. RESULTS AND DISCUSSION

3.1. RAMAN SPECTROSCOPY OF ORGANIC WASTE (EGGSHELL) DERIVED CAO

The presence of oxide species was successfully detected by using raman spectroscopy technique. The characteristics bands at 281 cm ^{1},358 cm ^{1},675 cm ^{1}and 963 cm ^{1} conforming to a function of the calcium oxide content in the sample. The peaks at 281 cm ^{1}in the spectrum of eggshell-derived CaO confirmed the lattice vibration region. A function assigned to the Ca-O bonds is the sharp band at 358 cm ^{1}in the spectra of ES-CaO (Fig 1). According to a chemical perspective, the highly reactive calcium oxide formed from eggshell powder exposed to ambient air is responsible for the incidence of substantial peaks in the spectra for carbonate anion. The increased Ca^{2+} content of the equivalent carbonates that decomposed at high temperatures due to the calcination of powdered eggshell and produced highly reactive CaO can be used to explain the persistence of the Raman signal found in the ES-CaO spectrum. (Jaiswal et al, 2020)

3.2. UV-VIS SPECTROSCOPY OF ORGANIC WASTE (EGGSHELL) DERIVED CAO

With wavelengths ranging from 200 to 800 nm, the UV-visible absorbance and reflectance spectra of powdered CaO made from chicken eggshells were captured. It was determined by the perceived spectrum that the CaO made from eggshell has enough transmission throughout the IR and visible spectrum. This result is in accordance with (Jaiswal et al., 2020, Marquis et al., 2015) (Fig.2.).

3.3 GEMINATION PERCENTAGE

Germination and growth attributes were observed between hydroprimed seeds and nanoprimed seeds. Seeds were primed with various concentrations of CaO nanoparticles and observed for germination and growth parameters along with a control group. The results demonstrated that, on average, seeds primed with 40ppm nanoparticle concentration exhibited the highest germination rate of 98 percent, whereas the control group only showed 86 percent germination (Fig.3.). Nanopriming with 40ppm CaO NP shows 13.9 per cent increase in germination.

The observed increase in germination can be attributed to the effective regulation of ion homeostasis (Na^+/K^+), as proposed by (Fu, Khan et al. 2023). Nanopriming seems to facilitate increased water uptake by penetrating the seed coat and creating more channels for water entry (Khodakovskaya, Dervishi et al. 2009). This accelerated water uptake enhances α-amylase activity, which, in turn, increases the amount of soluble sugars, thus promoting respiratory metabolism for seed germination, as suggested by (Sami, Yusuf et al. 2016). Additionally, the elevated level of Ca^{2+} triggered by nanopriming synthesizes polyamines, which have been shown to improve seed germination, as discussed by (Mazhar, Ishtiaq et al. 2023).

However, treatment with higher concentration of CaO NP showed reduction in germination percentage. Treatment with 100ppm CaO NP reduced germination by 4.65 per cent compared to control. Elevated calcium levels within seeds can disrupt the balance of ions, thereby influencing the water imbibition process. The presence of excessive calcium can result in the formation of insoluble calcium pectate, which negatively affects water uptake and impedes the expansion of cell walls crucial for seed germination (Bewley, Black et al. 1994). This phenomenon may explain the gradual decrease in germination observed as the concentration of CaO nanoparticles increases.

3.4 SEEDLING LENGTH

CaO nanoparticle at 40ppm recorded significantly higher root length (14.80). Priming with 40ppm CaO NP recorded approx. 38 per cent increase in root length as compared to control (Fig.4.).

Seed priming using CaO NP had a notable impact on the shoot length. Seeds treated with 40 ppm of CaO NP demonstrated a considerable increase in shoot length (20.19), surpassing the control group by 12 percent (Fig.4.). This, in turn, lowers the root osmotic potential, hindering water absorption and transportation to the shoots. As a consequence, embryo expansion and seedling emergence are adversely affected (Aroca, Porcel and Ruiz-Lozano 2012). In sorghum, there was a drastic increase in shoot length when Ca^{2+} was supplied to seeds (Mulaudzi, Hendricks et al. 2020). As the concentration of CaO increases, there is a consistent reduction in the length of seedlings. For instance, at a CaO concentration of 100 ppm, the seedling shoot length measured 17.12 cm, the shoot length decreased by 15 per cent compared to the shoot length observed at 40 ppm CaO concentration. In sorghum, at higher levels of Ca^{2+} there was a linear decrease in seedling growth (Mulaudzi, Hendricks et al. 2020).

3.5 DRY MATTER PRODUCTION

Seed priming with CaO NPs, as increased the dry matter production of seedlings. CaO at 40ppm has highest dry matter production of 1.27g/10 seedlings, as compared to control which as dry matter production of 0.29 g/10 seedlings. (Fig.4.)

3.6 VIGOUR INDEX OF SEEDLING

Seedling vigour has been observed to increase by 38.5 per cent compared to control. Seedling vigour for control was 2473 while for 40ppm nanoparticle concentration it was 3426 (Fig.3.). The increase in vigour can be attributed to higher and faster germination, and better seedling growth. Calcium is known to affect cell division and growth, which may have attributed to higher seedling growth, thus recorded increase in vigour (Javadi, Khomari and Sofalian 2016).

4. CONCLUSION

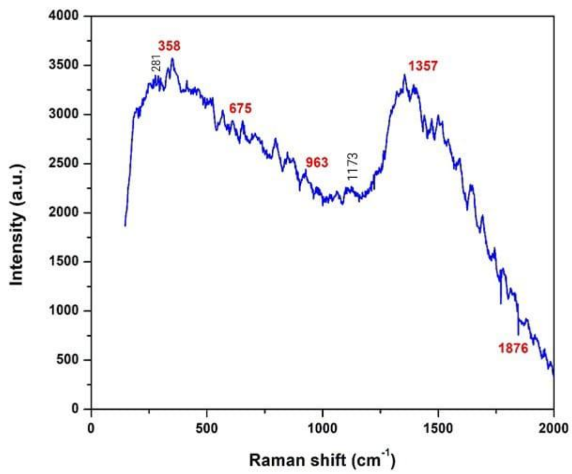
The study presented here focused on seed nanopriming as a potential enhancement technique for improving the germination and growth parameters of rice seedlings. Employing calcium oxide nanoparticles (CaO NP) for priming exhibited promising results. The germination, root and shoot growth, and overall vigour of rice seedlings were positively influenced by CaO NP priming, particularly at a concentration of 40 ppm. This nanoparticle-mediated seed priming strategy holds significant potential to enhance ion homeostasis, facilitate water uptake, and trigger beneficial physiological responses, leading to improved germination and seedling development.

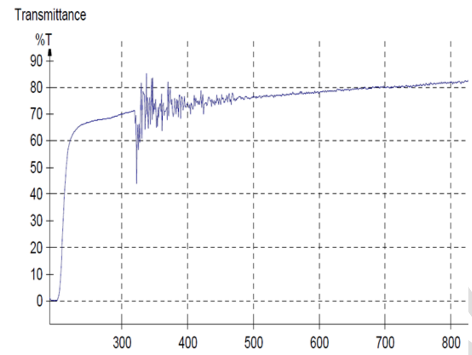
By harnessing the capabilities of nanotechnology and seed priming techniques, there is a potential avenue for enhancing the growth and yield of rice. Nonetheless, while the results are promising, additional studies and field trials are essential to validate the effectiveness of CaO NP seed priming across diverse rice varieties and real-world agricultural conditions. Moreover, a comprehensive understanding of the underlying molecular mechanisms driving the observed improvements in germination and growth attributes is necessary to fine-tune and optimize this technique. With concerted efforts in research and application, seed priming using nanotechnology could potentially revolutionize rice cultivation and ultimately contributing to global food security and sustainable agriculture.

Table 1: Effect of seed nano priming with different concentrations of CaO nanoparticles on germination percentage and growth parameters of paddy.

Priming treatments	**Germination per cent**	**Root length**	**Shoot length**	**Dry matter production**	**Vigour index**
Control	86(70)	10.74	18	0.29	2473
Hydropriming	88(71)	10.99	18.03	0.30	2533
10ppm	88(71)	11.003	18.07	0.36	2562
20ppm	92(78)	13.33	18.55	0.45	2937
30ppm	94(80)	13.96	19.72	0.69	3164
40ppm	98(84)	14.80	20.19	1.27	3426
50ppm	94(75)	13.15	19.54	0.46	3074
60ppm	92(71)	12.64	18.85	0.42	2897
70ppm	90(73)	12.48	18.72	0.41	2810
80ppm	88(72)	12.32	18.31	0.41	2695
90ppm	88(72)	12.11	17.5	0.36	2609
100ppm	82(66)	11.77	17.12	0.36	2367
Mean	**90**(74)	**12.44**	**18.55**	**0.48**	**2796**
SEd	2.1554	0.3243	0.4810	0.0143	73.2730
CD(0.05)	4.4485	0.6693	0.9927	0.0295	151.2284

Data in the parenthesis () denotes arc sine transformed values.





UV-visible spectra of CaO prepared from waste eggshells transmittance

Fig.1 Raman spectroscopy analysis of CaO NPs **Fig.2 UV- visible spectra analysis of CaO NPs**

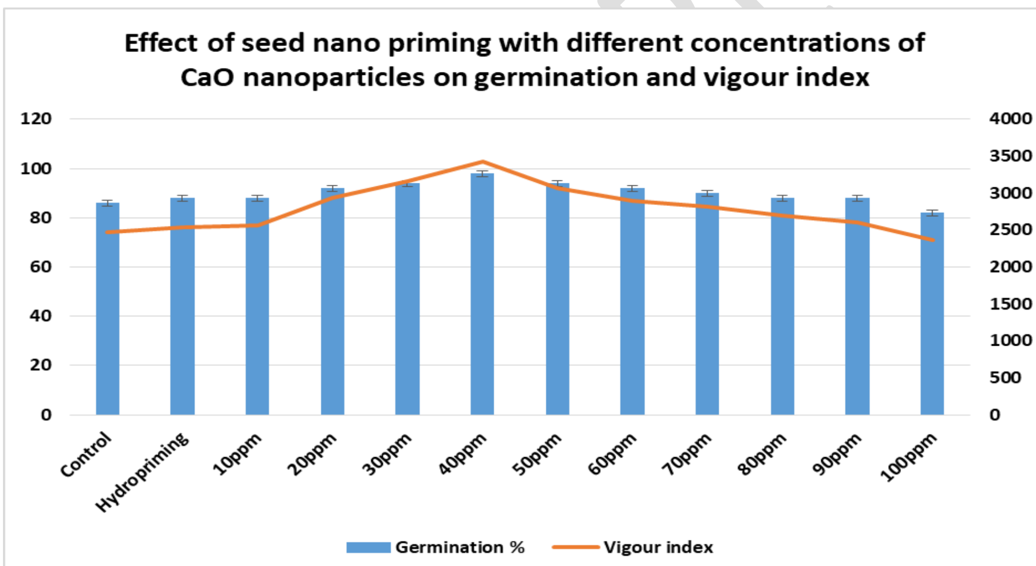


Fig.3. Effect of seed nano priming with different concentrations of CaO nanoparticles on germination and vigour index

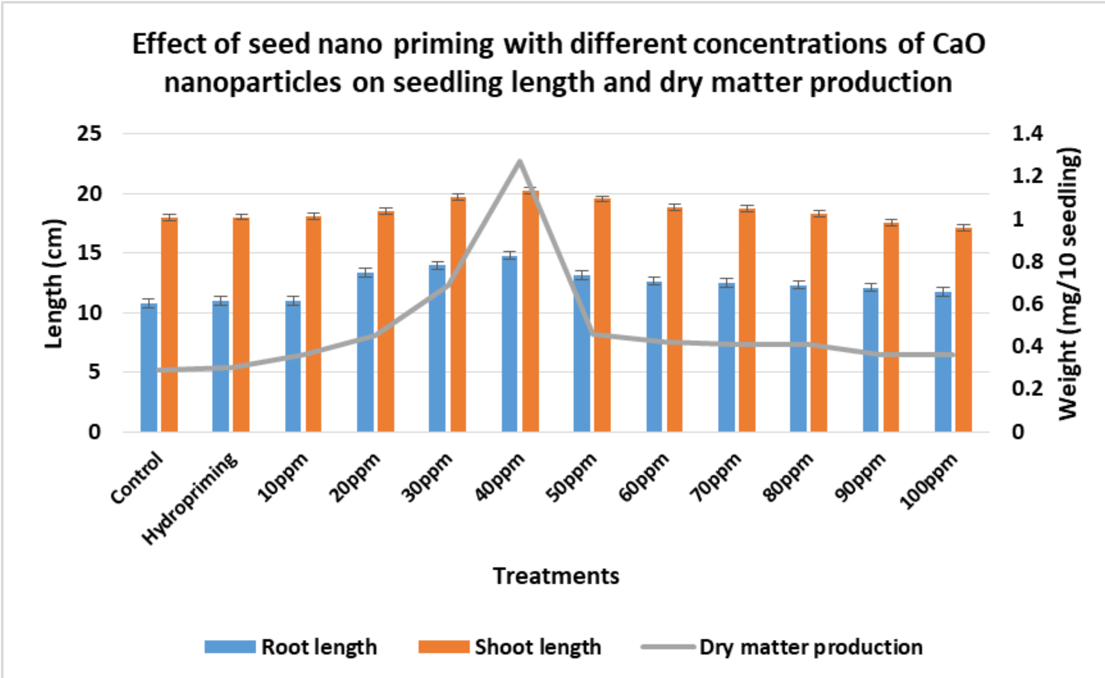


Fig.4. Effect of seed nano priming with different concentrations of CaO nanoparticles on seedling length and dry matter production

References

1. Abdul-Baki, A. A. and J. D. Anderson (1973). "Vigor determination in soybean seed by multiple criteria 1." Crop science **13**(6): 630-633.
2. Alam, A., Ullah, H., Cha-um, S., Tisarum, R., & Datta, A. (2021). Effect of seed priming with potassium nitrate on growth, fruit yield, quality and water productivity of cantaloupe under water-deficit stress. Scientia Horticulturae, 288, 110354.
3. Aroca, R., R. Porcel and J. M. Ruiz-Lozano (2012). "Regulation of root water uptake under abiotic stress conditions." Journal of Experimental Botany **63**(1): 43-57.
4. Bewley, J. D., et al. (1994). "Seeds: germination, structure, and composition." Seeds: physiology of development and germination: 1-33.
5. Dhage, S., & Biradar, D. P. (2020). Nanofertilizers: perspective to enhance growth, yield and NUE of crops. Indian Journal Pure and Applied Biosciences, 8(6), 339-49.
6. Fu, C., Khan, M. N., Yan, J., Hong, X., Zhao, F., Chen, L., & Wu, H. (2023). Mechanisms of nanomaterials for improving plant salt tolerance. *Crop and Environment*.
7. Ghassemi, F., Jakeman, A. J., Nix, H. A. 1995. Salinisation of land and water resources: 429 human causes, extent, management and case studies. CABI Publishing, Wallingford, p 526
8. Gupta, S., Schillaci, M., Walker, R., Smith, P. M., Watt, M., & Roessner, U. (2021). Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. Plant and Soil, 461, 219-244.
9. Habte, L., Shiferaw, N., Mulatu, D., Thenepalli, T., Chilakala, R., & Ahn, J. W. (2019). Synthesis of nano-calcium oxide from waste eggshell by sol-gel method. Sustainability, 11(11), 3196.
10. Jaiswal, K. K., Dutta, S., Pohrmen, C. B., Verma, R., Kumar, A., & Ramaswamy, A. P. (2021). Bio-waste chicken eggshell-derived calcium oxide for photocatalytic application in methylene blue dye degradation under natural sunlight irradiation. *Inorganic and Nano-Metal Chemistry*, *51*(7), 995-1004.

11. Javadi, A., S. Khomari and O. Sofalian (2016). "Seed vigor and boron and calcium nutrition influence oilseed rape germinability and seedling growth under salt stress." Journal of Plant Nutrition**39**(12): 1688-1696.

12. Jerlin, R., Menaka, C., Raja, K., Moorthy, K. R., & Tamilkumar, P. (2010). Standardization of sieve size for grading of olitorius jute seeds. *Asian Journal of Agricultural Research*, *4*(1), 15-19.

13. Khan, M. A. and Z. Abdullah (2003). "Salinity–sodicity induced changes in reproductive physiology of rice (Oryza sativa) under dense soil conditions." Environmental and Experimental Botany**49**(2): 145-157.

14. Khodakovskaya, M., Dervishi, E., Mahmood, M., Xu, Y., Li, Z., Watanabe, F., & Biris, A. S. (2009). Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS nano*, *3*(10), 3221-3227.

15. Krishnasamy, V. and P. Srimathi (2001). "Seed Management of rainfed agriculture." Land and use planning and watershed management in Rainfed Agriculture (ed.) Balusamy, M., CR Chinnamuthu and A. Velayutham. Centre of Advanced studies, Department of Agronomy, Tamilnadu Agricultural University, Coimbatore: 140.

16. Lutts, S., J. Kinet and J. Bouharmont (1995). "Changes in plant response to NaCl during development of rice (Oryza sativa L.) varieties differing in salinity resistance." Journal of Experimental Botany**46**(12): 1843-1852.

17. Marquis, G., Ramasamy, B., Banwarilal, S., & Munusamy, A. P. (2016). Evaluation of antibacterial activity of plant mediated CaO nanoparticles using Cissus quadrangularis extract. *Journal of Photochemistry and Photobiology B: Biology*, *155*, 28-33.

18. Mazhar, M. W., Ishtiaq, M., Maqbool, M., Atiq Hussain, S., Casini, R., Abd-ElGawad, A. M., & Elansary, H. O. (2023). Seed Nano-Priming with Calcium Oxide Maintains the Redox State by Boosting the Antioxidant Defense System in Water-Stressed Carom (Trachyspermum ammi L.) Plants to Confer Drought Tolerance. *Nanomaterials*, *13*(9), 1453.

19. Mukiri, C., Raja, K., Senthilkumar, M., Subramanian, K. S., Govindaraju, K., Pradeep, D., & Ranjan, S. (2021). Immobilization of beneficial microbe Methylobacterium aminovorans in electrospun nanofibre as potential seed coatings for improving germination and growth of groundnut Arachis hypogaea. Plant Growth Regulation, 1-9.
20. Mulaudzi, T., Hendricks, K., Mabiya, T., Muthevhuli, M., Ajayi, R. F., Mayedwa, N.,. & Iwuoha, E. (2020). Calcium improves germination and growth of Sorghum bicolor seedlings under salt stress. *Plants*, *9*(6), 730.
21. Rabbani, G., Rahman, A., & Mainuddin, K. (2013). Salinity-induced loss and damage to farming households in coastal Bangladesh. International Journal of Global Warming, 5(4), 400-415.
22. Raja, K., Sowmya, R., Sudhagar, R., Sathya, M.P., Govindaraju, K. and Subramanian, K.S. (2019). Biogenic ZnO and Cu nanoparticles to improve seed germination quality in blackgram(Vigna mungo). Materials Letters, 235, 164-167.
23. Sami, F., Yusuf, M., Faizan, M., Faraz, A., & Hayat, S. (2016). Role of sugars under abiotic stress. *Plant Physiology and Biochemistry*, *109*, 54-61
24. .Solis, C. A., Yong, M. T., Vinarao, R., Jena, K., Holford, P., Shabala, L., ... & Chen, Z. H. (2020). Back to the wild: on a quest for donors toward salinity tolerant rice. Frontiers in Plant Science, 11, 323.
25. Zhang, W., et al. (2018). "Silicon promotes growth and root yield of Glycyrrhiza uralensis under salt and drought stresses through enhancing osmotic adjustment and regulating antioxidant metabolism." Crop Protection**107**: 1-11.