Eco. Env. & Cons. 28 (November Suppl. Issue) : 2022; pp. (S254-S259)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i07s.042

Effects of zinc oxide nanoparticles for promoting seed germination of rice (*Oryza sativa* L.) under salinity stress

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(Received 16 March, 2021; Accepted 12 May, 2021)

ABSTRACT

Salinity stress had a direct impact on rice (*Oryza sativa* L.) growth and physiological features throughout the early stages of development. The current study aimed to investigate the influence of Zinc oxide nanoparticles (ZnO-NPs) in the rice germination stage during salinity stress because the germination and seedling stage is critical for the correct development and growth of plants. The different concentrations of NaCl and ZnO NPs (T_1-60 mM NaCl, T_2-80 mM NaCl, T_3-100 mM NaCl, T_4-60 mM NaCl+50 ppm ZnO NPs, T_5-80 mM NaCl+50 ppm ZnO NPs and T_6-100 mM NaCl+50 ppm ZnO NPs) improve the germination rate in rice during salinity stress. We conducted our experiment in a landrace of rice Kargi and salinity tolerance rice variety CSR 30. The current study's findings reveal that ZnO NPs have a significant effect in reducing salt stress during rice germination.

Key words: *Germination, Nanoparticles, Salinity, Kargi, CSR 30*

Introduction

The most significant abiotic factor that directly impacts plant growth and development is salinity (Hafsi *et al.*, 2010; Parida and Das, 2005). Shortage of water in semi-arid and arid regions with high temperatures can cause the salinity problem that affects staple crop production like rice etc. (Kumar *et al.*, 2021; Minhas *et al.*, 2017). Salinity stress affects various biological functions of plants, e.g., photosynthesis, transpiration rate, stomatal conductance, cellular metabolism, stomata etc., decreasing these functions can directly inhibit the growth of plants (Kordrostami and Rabiei, 2019). The significant effect of salinity stress is causing osmotic stress due to the higher accumulation of ions that also cause oxidative stress that limits the water availability for the plants, and water unavailability has affected the mobilization of stored reserves of seeds that prevent or inhibit their germination (Ibrahim, 2016). Seed germination is a critical period of plants during their initial life cycle stage. If this period gets affected by salinity, then delaying the early growth of seed decreases the germination of plants that affect the yield of crops (Hussain, *et al.*, 2018). Zinc is the most important element for plant growth and development, as it aids in the activation of over 300 enzymes. These enzymes participate in various biochemical activities, e.g., photosynthesis, respiration, metabolism of carbohydrates, membrane biosynthesis process (Manea *et al.,* 2019; Vojodi Mehrabani *et al.,* 2017). All of these metabolic activities that directly influence the growth and development, as well as seed germination and seedling development, can be halted by a zinc deficit. (Sharma *et al.,* 2021a; Srivastav *et al.,* 2021).

Rice is a widely consumed dominant food worldwide, with more than half of the world's population eating rice on a daily basis. Rice, like other significant cereal crops like maize and wheat, is a salt-sensitive cereal crop, and the sensitivity of rice crops varies from variety to variety (Hussain, Cao, Zhong, Zhu, Khaskheli, Fiaz, Zhang, Jin, *et al.,* 2018; Munns and Tester, 2008). Rice germination and seedling stage are the utmost vulnerable to salinity stress, which impacts their entire life cycle and productivity (Munns and Tester, 2008).

Treatment with various substrate types like nanoparticles (NPs) can enhanceseed germination by improving uptake of water andmobilization of stored nutrients reserves of seeds (Ibrahim, 2016; Srivastav *et al.,* 2021). Nanoparticles are synthesized by naturally or engineered processing methods with higher bioactive and biosafety properties, and the size of nanoparticles is below 100 nm with elevated surface to volume ratio (Baz *et al.,* 2020; Tripathi *et al.,* 2017; Zaytseva and Neumann, 2016). NPs are rapidly uptake by plants tissue. Because of their size easily enters into plant cells through various plant organs, e.g., root, shoot, epidermis, cuticle, hydathodes, stomata, root tips, root junctions, and stigma, wounded or other pore areas of the plant (Dietz and Herth, 2011). ZnO-NPs are the most prominent NPs widely used in plant science research, and ZnO-NPs are also utilised to reduce the effects of salt stress and boost the rate of seed germination. (Mahajan *et al.,* 2019; Mishra, 2020; Ragab *et al.,* 2022). Our study aims to investigate the response of a landrace and salt-tolerance rice genotype Kargi and CSR 30 under different concentrations of NaCl levels and ZnO-NPs during the germination and seedling phase.

Materials and Methods

NPs preparation

For this experiment commercial grade zinc oxide (ZnO) NP powder (particle size < 50 nm) was obtained from Sisco Research Laboratories (SRL) Pvt.Ltd, Maharashtra. 50 ppm solution of ZnO-NPs was prepared utilizing the procedure adopted from Rajput *et al.* (2021) with the help of sonicator.

Germination Experiment

Kargi landrace rice seed was collected from a farmer from village Pauha,post-Belasin, Machhlishahr, Jaunpur, Uttar Pradesh, India, and CSR 30 salt tolerance rice genotype was obtained from Basmati Export Development Foundation, Meerut, India. The seeds of both genotypes were sterilized in 0.01% $HgCl_2$(2 min) and washed with distilled water. We performed a Petridish experiment for this used Tarsons 90 (diameter) X 14 (H) mm catalog number-460090.10 seeds. Both rice genotypes were transferred on Whatman No. 2 filter paper and watered with varying concentrations of NaCl and ZnO-NPs solution (All treatments were performed in triplicate) and transferred into growth chamber at 28 ± 1 °C with relative humidity 75-80%, and 14h/8h photoperiod. To the end of germination, the plants were watched for 9 days. The treatment of NaCl and ZnO-NPs are followedbelow:

T_0: Control
T_1: 60 mM NaCl
T_2: 80 mM NaCl
T_3: 100 mM NaCl
T_4: ZnO NPs (50 ppm) + 60 mM NaCl
T_5: ZnO NPs (50 ppm) + 80 mM NaCl
T_6: ZnO NPs (50 ppm) + 100 mM NaCl

Germination Evaluation

We evaluated the following parameters:

Germination percentage (GP)

GP = (Number of normally germinated seeds ÷ Total number of seeds sown) ×100 (1)
GP was calculated according to Kandil *et al.* (2012).

Germination index (GI)

$GI = \Sigma (Gt/Tt)$ (2)
wherethe mean number of germinated seeds on day t, and Tt is the number of days (Hakim *et al.,* 2010; Sharief, 2012).

Relative seed germination Rate (RGR)

RGR=(SC\SS)×100 (3)

Where SC is the number of seeds germinated in control, and SS is the number of germinated seeds in treatment (*Germination and Growth Characteristics of Mungbean Seeds (Vigna Radiata L.) Affected by Synthesized Zinc Oxide Nanoparticles - Inpressco*, n.d.).

Statistical Analysis

The experiment was carried out in a completely randomized design (CRD)with three replicates of each treatment. The statistical analysis was performed using MS Excel. A p-value ≤ 0.05 (or ≤ 0.01) in a Student's t-test was used to evaluate statistical significance(Hoshmand, 1994).

Results

Germination percentage (GP)

Kargi and CSR 30 germination response was evaluated under salinity stress and ZnO-NPs treatments. The germination of Kargi and CSR 30 under NaCl treatments T1, T2, T3, was reduced (31.82, 40.91, 50, 26.19, 35.71 and 45.24%) compared with their controls (Fig. 1). On the contrary, the ZnO-NPs and NaCl treatments T4, T5, T6 of Kargi and CSR 30 shows less effect on germination (22.73, 31.82, 36.36, 16.67, 25 and 30.95%), respectively their controls and NaCl treatments T1, T2, T3 (Fig. 1). Kargi shows higher GP in normal conditions than CSR 30 and CSR 30 slightly higher GP in saline conditions.

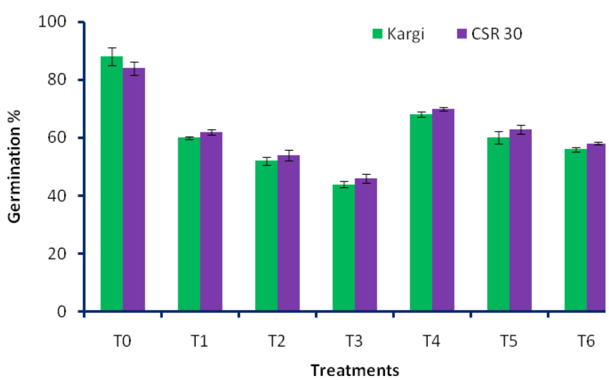

Fig. 1. Impact of ZnO-NPs, NaClin control, and saline conditions in the GP of Kargi and CSR 30 seeds after 9 days of treatments. The results are the averages of three replicates. The least significant value (LSD) among the treatments at p ≤ 0.01 is represented by the error bars.

Germination index (GI)

Salinity stress might also help to lower GI symptoms. (Fig 2). The greater GI fall was noticed for saline conditions in treatments T1, T2, T3 of Kargi and CSR 30 genotypes followed by their controls, and the least reductions were obtained in T4, T5, T6 treatments of ZnO-NPs and NaCl in comparison to their control and T1, T2, T3 (Fig 2). But compared to Kargi, the CSR 30 shows a slight GI increment in all treatments (T1, T2, T3, T4, T5, T6) (Fig. 2).

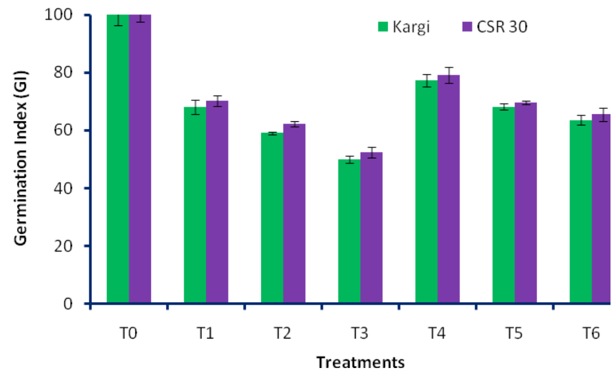

Fig. 2. Effect of ZnO-NPs, NaClin control, and saline conditions in the GI of Kargi and CSR 30 seeds after 9 days of treatments. The results are the averages of three replicates. The least significant value (LSD) among the treatments at p ≤ 0.01 is represented by the error bars

Relative seed germination rate (RGR)

Relative seed germination rate is affected by salinity stress. In saline NaCl medium (T1, T2, T3) decrease the RGR in Kargi (32.23, 41.20 and 50.17%) and CSR (30, 39 and 40%) rice genotypes compared with their controls. But in saline NaCl and ZnO-NPs treatment (T4, T5, T6), the rate of RGR is less affected in Kargi

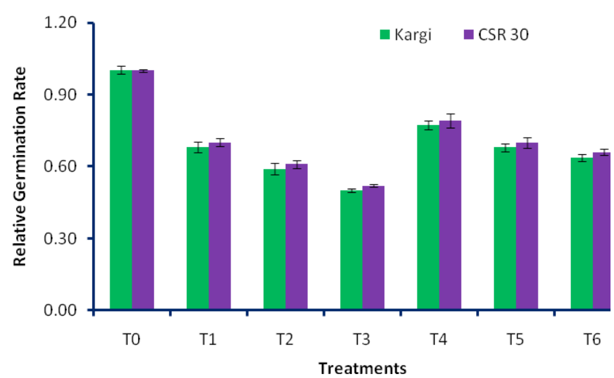

Fig. 3. Effect of ZnO-NPs, NaClin control, and saline conditions in the RGR of Kargi and CSR 30 seeds after 9 days of treatments.The results are the averages of three replicates. The least significant value (LSD) among the treatments at p ≤ 0.01 is represented by the error bars.

(23.09, 32.23 and 36.45%) and CSR 30 (21, 30 and 34%) respectively to their controls. The ZnO-NPs medium T4, T5, T6 showed less affected RGR than saline medium T1, T2, T3 of Kargi and CSR 30 rice genotypes. Our experimental result demonstrated that with a rise in the the degree of salinity stress, GP, GI, and RGR attributes of Kargi and CSR 30 genotypes were decreased. Application of ZnO-NPs plays a protective role due to which less impact of salinity stress has been seen on the GP, GI, and RGR parameters of Kargi and CSR 30 genotypes.

Discussion

The main goal of this research was to determine the impact of ZnO-NPs on salinity stress on landrace Kargi and salt-tolerant rice genotype CSR 30 during the germination and seedling phases.Previously we had mentioned that the effect of salinity in plants depends on species to species. As a result, the early stages of plant development, such as germination and seedling, are important periods that decide the future of the plant's vegetative and reproductive growth (Bybordi and Tabatabaei, 2009; Dehnavi *et al.,* 2020; El-Badri *et al.,* 2021). Figs 1-3 showsthe reduction in GP, GI, RGR of Kargi and CSR 30 because the excessive intake and storage of Na^+ and Cl^- which resulted in ion toxicity and osmotic stress. This further results in nutritional imbalance and oxidative stress, as well as a reduction in mineral and water mobilization("Ecophysiol. High Salin. Toler. Plants," 2006; Zhu, 2016).Germination parameters such as GP, GI, and RGR declined in the treatments T1, T2, T3 of Kargi and CSR 30 (Fig. 1, 2, and 3) because of a change in seed imbibition of water due to a fall in osmotic potential, water absorption is delayed, resulting in diminished germination. (Dehnavi *et al.,* 2020; Misra and Gupta, 2005). Because the higher accumulation of Na^+ ions in treatments T1, T2, T3 causes osmotic and pseudo-drought stress, which reduces water absorption and alters enzyme processes. This changes nucleic acid metabolism and hormonal imbalance, lessening the need for storing seed stores. (Dantas *et al.,*2007; Gomes-Filho *et al.,* 2008; Ismail *et al.,* 2022; Misra and Gupta, 2005; Promila and Kumar, 2000; Ryu and Cho, 2015).

Ajouri *et al*. (2004) and Muhammad *et al.,* (2015) stated about the use of ZnO-NPs can enhanveseed germination and seedling growth, same findings shown in our study for growth parameter's GP, GI, and RGR of treatments T4, T5, T6 of Kargi and CSR 30 rice genotypes (Figs. 1-3). Sharma *et al.* (2021a) described that ZnO-NPs could improve the á-amylase production, antioxidant level, and water uptake, leading to an enhanced seed germination rate than normal and saline conditions in rice (Fig. 4).

ZnO-NPs penetrated the seeds coat of rice, promoted the water uptake, increased metabolic activity, frequent hydrolysis of starch, and upregulated the antioxidant machinery that scavenged the reactive oxygen species (Afzal *et al.,* 2022; H, 2017; Sharma *et al.,* 2021b).

Conclusion

In this study, we have demonstrated that a considerable application of ZnO-NPs can alleviate the effect of salinity throughout the germination and seedling stages. Through this experiment, we have also classified the differences between salt-tolerant variety CSR 30 and landrace Kargi.In saline conditions, the germination parameters GP, GI, RGR are reduced in Kargi and CSR 30 but compared to Kargi, CSR 30 shows a slightly better response to the above germination parameters. ZnO-NPs improve the seed germination parameters GP, GI, RGR rate in Kargi and CSR 30 and CSR 30 here also show slightly higher response than Kargi for germination parameters GP, GI, RGR.

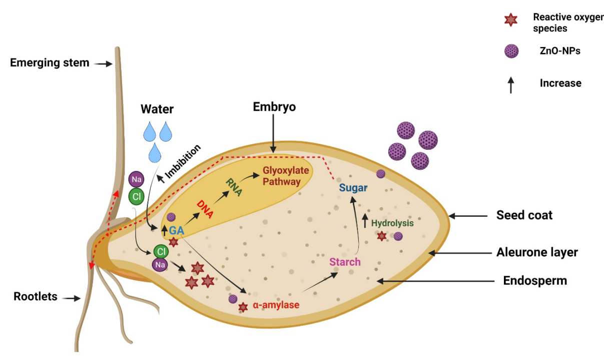

Fig. 4. Diagrammatic representation of the repercussions of NaCl and ZnO-NPs on seed germination of rice seed. The ZnO-NPs follow the following mechanisms. (1) Penetrating the seed coat and increasing the uptake of water, (2) ZnO-NPs and amylase complex upregulated the hydrolysis process of starch, (3) activating the antioxidant machinery reduced the level of ROS (4) increasing the mobility of glucose and minerals for enhancing the germination of rice seeds (H, 2017).

ZnO-NPs alleviate the effect of salinity by reducing the level of ROS that produces NaCl uptake by the seeds. Entry of NaCl through the imbibition process can create ionic toxicity and osmotic stressthat produced ROS that reduced the water uptake resulting in a slow breakdown of seed reserves like starch that reduced the mobilization of sugar and other important minerals that help in the development and germination of seeds (Fig. 4), ZnO-NPs can improve elimination of all these by activating the antioxidant machinery that reduces the level of ROS (Chanu Thounaojam *et al.,* 2021; Itroutwar *et al.,* 2020; Rajput *et al.,* 2018).

Acknowledgments

This study was supported by the Department of Agriculture Biotechnology, Collage of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut 250110, India, under the Ph.D. program.

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