International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(1): 435-439
www.biochemjournal.com
Received: 01-10-2023
Accepted: 05-11-2023

TK Nagarathna
Professor of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Corresponding Author:
TK Nagarathna
Professor of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Enhancing rice resilience to zinc deficiency: Insights from leaf and seed zinc accumulation, glutathione reductase activity and hydrogen peroxide

TK Nagarathna

DOI: https://doi.org/10.33545/26174693.2024.v8.i1Sg.366

Abstract
The experiment was conducted to investigate the responses of two rice genotypes, IR 74 (sensitive) and RIL 46 (efficient), to zinc deficiency. Grown in both field and controlled conditions, the experiment aimed to understand the physiological and biochemical factors influencing their responses for developing zinc-efficient rice varieties. Under normal zinc conditions, both genotypes exhibited superior root systems, but zinc deficiency hindered root growth, more so in IR 74. RIL 46 showed enhanced zinc uptake and translocation under both conditions, maintaining higher zinc content in grains. Glutathione Reductase (GR) enzyme activity in leaves revealed a strategic response in RIL 46 to mitigate oxidative damage under zinc deficiency. Hydrogen peroxide accumulation in roots indicated a heightened oxidative stress response in the sensitive line. In conclusion, RIL 46 demonstrated genetic resilience to zinc deficiency, emphasizing the importance of zinc accumulation, GR activity and hydrogen peroxide levels as indicators for selecting resilient rice varieties in zinc-limiting environments.

Keywords: Glutathione reductase, hydrogen peroxide accumulation, zinc deficiency, zinc-efficient varieties, zinc stress adaptation

Introduction
Zinc (Zn) is an essential micronutrient for plants, plays a crucial role in various physiological and biochemical processes. Zinc is involved in a wide range of biological functions in plants and humans including serving as a cofactor for numerous enzymes, maintaining the structure of proteins and playing a role in gene expression (Costa *et al*., 2023 & Roohani *et al*., 2013) [4, 5]. The World Health Organization (WHO) estimates that around two billion people worldwide suffer from Zn deficiency. Zinc deficiency is indeed a significant global health issue, particularly in developing countries. One of the primary reasons for zinc deficiency in many populations is the consumption of diets that are low in zinc (Hussain *et al*., 2022) [6], particularly in regions where people rely heavily on plant-based foods. And also due to inadequate Zn availability in soils which reduces yield and nutrient content in grains (Saleem *et al*., 2022) [7].

One of the best strategies is to focus on identifying and developing plant varieties that are efficient in taking up and utilizing zinc from the soil and produce Zn dense grains by selecting the parental lines with desirable traits through breeding programmes (Khan *et al*., 2022) [8].

Rice is a major staple food for a significant portion of the world's population, particularly in Asia where it serves as a primary source of calories. However, rice is known to be deficient in certain micronutrients, including zinc (Gindri *et al*., 2020) [9]. Identifying Zn efficient rice genotypes by understanding their physiological, biochemical and molecular mechanism is a best option combat the Zn deficiency both in plants and humans.

The response of rice plants to zinc deficiency can vary based on genetic, physiological and biochemical factors. Among biochemical aspects, glutathione reductase plays a crucial role in enhancing plant tolerance to abiotic stresses. Similarly, plants overproduce reactive oxygen species like hydrogen peroxide (H_2O_2) during abiotic stresses and hence, regulation of Glutathione Reductase and H_2O_2 is vital for imparting stress tolerance in plants (Hasanuzzaman *et al*., 2017) [10].

An experiment was conducted in two contrasting rice genotypes to investigate their varied responses to zinc deficiency. The goal was to study the physiological and biochemical factors influencing these responses, providing valuable insights for the development of zinc-efficient rice varieties through breeding programs further to improve crop productivity and nutritional quality under zinc-limiting conditions.

Materials and Methods

For the experiment, two contrasting Zn deficient efficient rice varieties, IR 74 (sensitive) and RIL 46 (tolerant) developed at IRRI, Philippines were grown. One set was grown in the field condition and other set was grown in phytotron facility (controlled environment). In both the conditions one set of plants were maintained with Zn and another set was without Zn (Fig. 1).

In the experiment, the "+Zn" plot received 15 kg per hectare of zinc in the form of $ZnSO_4{\cdot}7H_2O$ in addition to recommended dose of nitrogen, phosphorous and potassium, while the "-Zn" plot received no zinc.

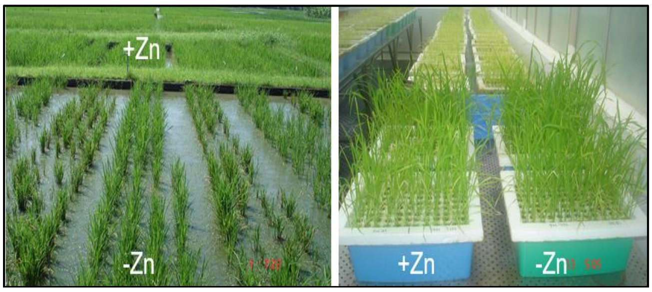


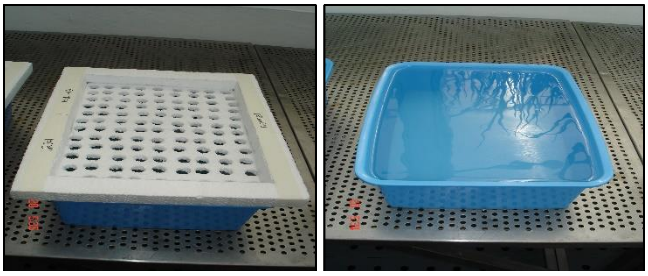
Fig 1: Plants grown in field and phytotron

The seeds of rice varieties IR74 and RIL46 were sown in shallow seedling trays filled with untreated soil and after three weeks, the seedlings were transplanted as single-plant hills in microplots measuring 2.4 m^2 with randomized complete block design with three replications. Similarly, pre-germinated seeds were sown in plastic trays maintained at phytotron (Fig. 2). Trays were filled with culture solution which contained 0.35g $ZnSO_4.7H_2O$ per 10 L of distilled water along with other required elements. For "-Zn", culture solution was prepared without $ZnSO_4.7H_2O$ (Singh & Mishra, 2004) [3], modified from Yoshida *et al*., 1976 [2].

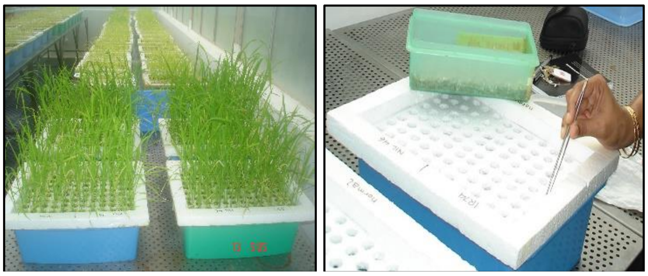
Thirty days after transplanting, observations on physiological parameters were recorded. Root and shoot length were measured (data not shown). Plants were pulled from the soil and the nutrient solution without damaging roots for Zn estimation, glutathione reductase activity and Hydrogen peroxide accumulation. After harvest Zn content in seeds were estimated in IR 74 and RIL 46 at "+Zn" and "-Zn" condition.

Samples were collected for Zn estimation, enzyme (glutathione reductase) analysis and root staining for hydrogen peroxide accumulation both in Zn efficient and sensitive lines and the data presented in the paper are from the plants grown in the field condition.

The samples were washed with Nanopure quality water, wrapped in aluminium foils and were snap-frozen in liquid nitrogen (Fig. 3). The collected samples were stored at -80 °C refrigerator until measurements were done.



Plants were grown in phytotron in plastic trays



Sowing of seeds 20 days after sowing

Fig 2: Sowing of seeds in plastic trays maintained in phytotron

Fig 3: Collection and soring of samples for biochemical analysis

Fig 4: Root growth of Zn efficient and sensitive lines with +Zn and –Zn in field

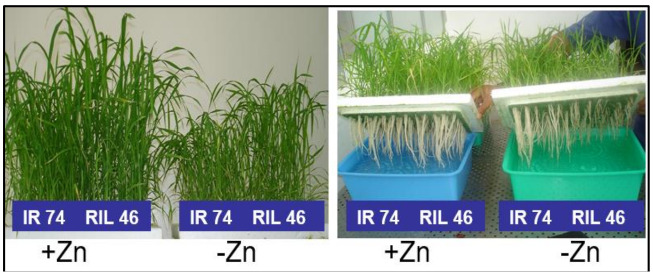


Fig 5: Difference in shoot and root growth of sensitive and efficient lines under Zn deficit and sufficient condition

Zinc estimation in root, shoot and grains

Finely powdered sample of root, leaves and grains (500 mg) were taken into polyethylene tubes, to which 25 ml 1N HCL solution was added and homogenized once the solution along with the powder. The sample was left over night for 24 hrs, without shaking by allowing it to digest in acid. The following day, once again the solution was mixed by shaking before filtering. The extract was collected in clean (acid washed) polyethylene tube. Observations of the sample extract were recorded using Atomic Absorption Spectrophotometer.

Estimation of glutathione reductase activity

The assessment of glutathione reductase (GR) enzyme activity, as well as protein and GR content, was conducted following the protocol outlined by Halliwell and Foyer in 1978.

Hydrogen peroxide accumulation in roots of sensitive and efficient genotypes

The roots of both Zn-sensitive and Zn-efficient lines were subjected to staining with CM-H2DCFDA (Chao *et al*., 2008) [11] and subsequently observed for the accumulation of hydrogen peroxide (H_2O_2) using a fluorescence microscope with excitation at 488nm and emission in the range of 510-560nm, with a bandpass filter set at 510-530nm.

Results and Discussion

Figure 4 & 5 illustrate the root growth patterns, revealing that under normal zinc conditions, both RIL 46 and IR 74 demonstrated superior root systems. Conversely, in the absence of zinc, root growth was hindered in both genotypes. Nevertheless, IR 74 exhibited more constrained root growth compared to RIL 46. This distinction suggests that, under the stress of zinc deficiency, IR 74 was more adversely affected in terms of root development compared to RIL 46.

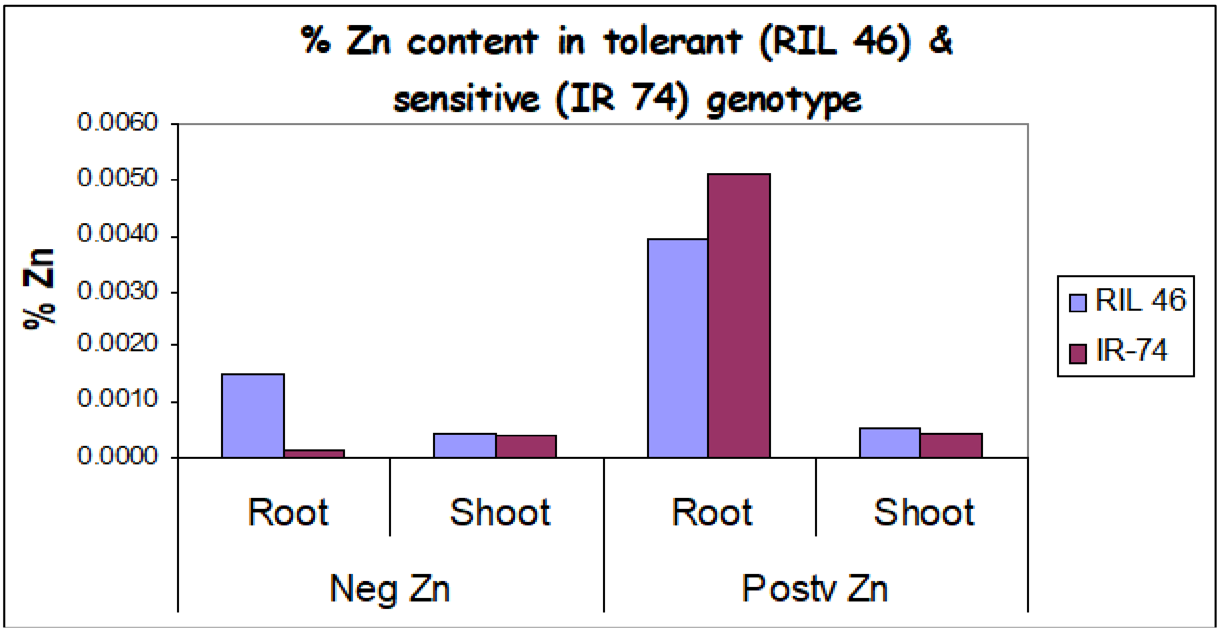


Fig 6: Root and shoot Zn concentration (%) in plants grown in presence and absence of Zn in the soil

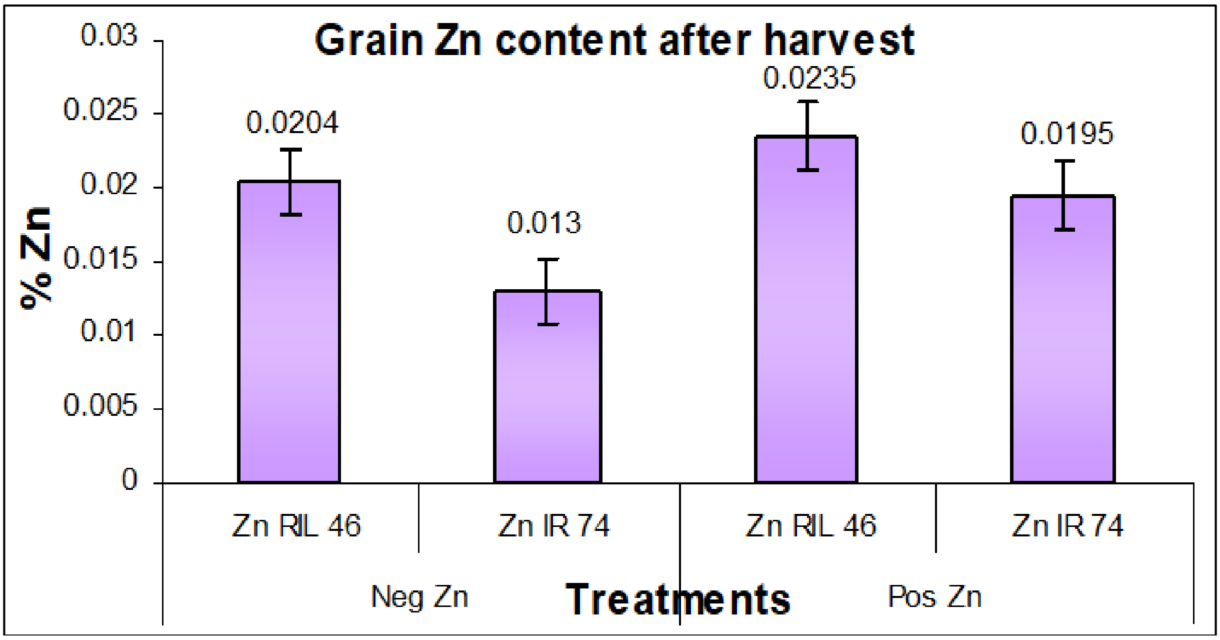


Fig 7: Grain Zn content after harvest in +Zn and –Zn condition

The zinc content analysis in leaves conducted 30 days after transplanting revealed that while IR 74 exhibited a notable capacity to absorb and translocate more zinc under normal zinc conditions ("+Zn"), its ability to uptake and translocate zinc was compromised in the absence of zinc (Fig 6). In contrast, RIL 46 demonstrated a higher zinc uptake even under zinc-deficient conditions, surpassing the susceptible variety IR 74. This highlights RIL 46's enhanced resilience to zinc deficit conditions compared to IR 74.

The Zn content analysis in grains post-harvest revealed distinct patterns (Fig 7). Under normal Zn conditions ("+Zn"), RIL accumulated 0.0235% zinc, outperforming IR 74, which accumulated 0.0195% Zn. In the absence of Zn, IR 74 showed a significant decrease in zinc accumulation, reaching only 0.013% zinc. In contrast, the efficient line, RIL 46, maintained a higher Zn content even under zinc stress conditions, recording 0.0204% zinc. This underscores the resilience of the efficient line to zinc stress, as evidenced by its sustained zinc accumulation compared to the susceptible variety IR 74.

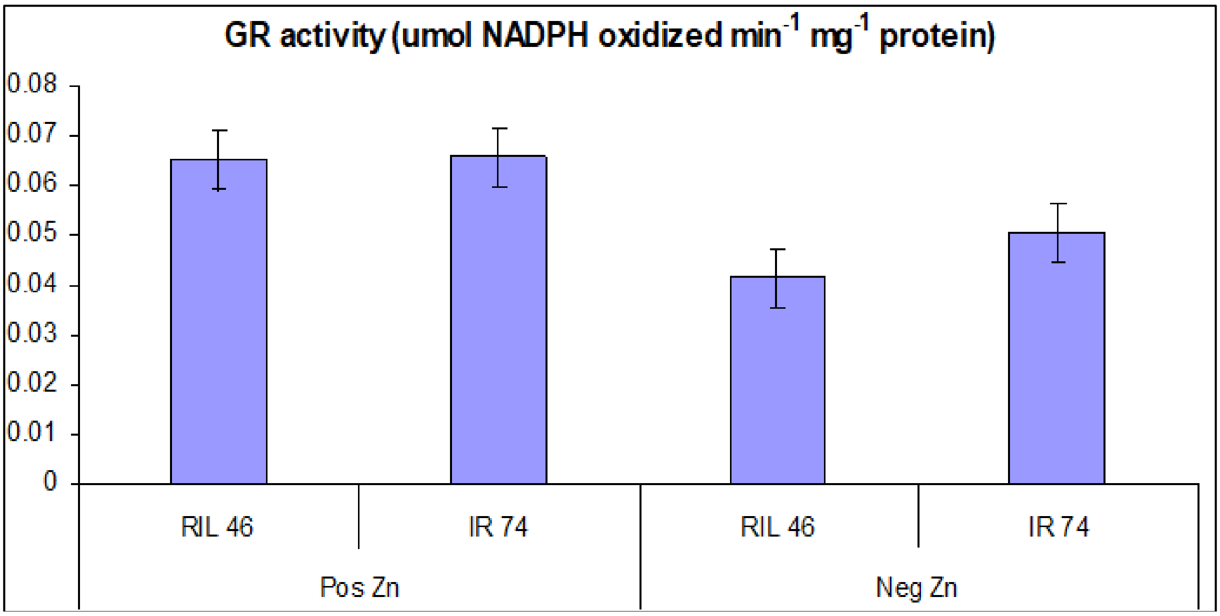


Fig 8: GR activity in efficient and sensitive lines under Zn sufficient and Zn deficit condition

In the context of this study, the investigation into Glutathione Reductase (GR) enzyme activity in leaves revealed that under normal conditions, both the sensitive and efficient lines exhibited comparable GR enzyme activity levels (Fig 8). However, when subjected to zinc deficit conditions, a noteworthy divergence in GR activity became apparent. The efficient line displayed significantly lower GR activity compared to the sensitive line.

This observed reduction in GR activity in the efficient line under zinc deficit conditions can be attributed to a strategic response to mitigate oxidative damage. Despite the zinc deficiency stress, the efficient line demonstrated less oxidative damage, as evidenced by the lower GR activity. This suggests that the efficient line has developed mechanisms to cope with zinc deficit-induced oxidative stress more efficiently.

Conversely, the sensitive line exhibited higher GR activity under zinc deficit conditions. This heightened enzymatic activity can be interpreted as a reactive response to counteract increased oxidative damage experienced by the sensitive line in the absence of sufficient zinc. The elevated GR activity in the sensitive line signifies an attempt to mitigate the oxidative stress and underscores the susceptibility of this line to the adverse effects of zinc deficiency.

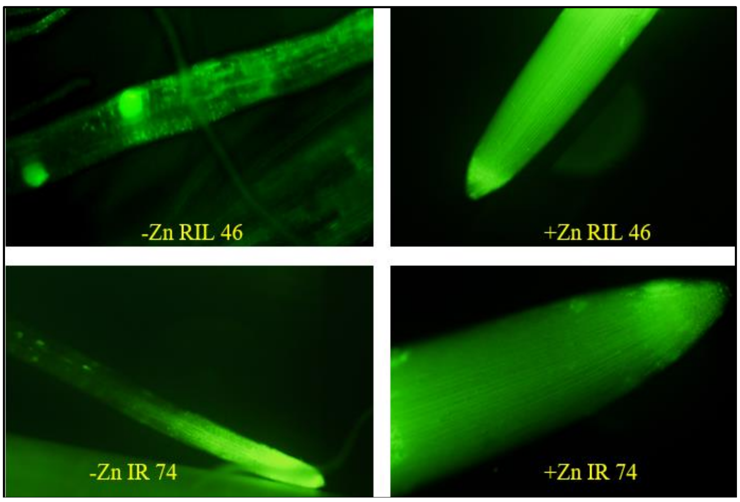


Fig 9: Hydrogen peroxide accumulation in efficient and sensitive lines

The assessment of hydrogen peroxide (H_2O_2) accumulation in the roots of both sensitive and efficient rice genotypes provided insights into the oxidative stress response under zinc-deficient conditions (Fig 9). The findings indicated that under zinc deficiency (-Zn), the sensitive line accumulated more hydrogen peroxide in its roots compared to the efficient line. Conversely, under normal zinc levels, both genotypes demonstrated minimized H_2O_2 production in their roots.

The observed increase in hydrogen peroxide accumulation in the roots of the sensitive line under zinc-deficient conditions implies a heightened oxidative stress response in these plants. Hydrogen peroxide is a reactive oxygen species (ROS) that is produced in plants as a response to various stressors, including nutrient deficiencies. In this case, the sensitive line appears to activate mechanisms leading to increased H_2O_2 production, possibly as part of a stress signaling pathway.

The restricted growth of plants under zinc stress conditions in the sensitive line is mentioned as a possible reason for the higher hydrogen peroxide accumulation. It suggests that the sensitive genotype may be experiencing more severe nutrient stress, leading to the activation of oxidative stress responses. The buildup of hydrogen peroxide can be both a consequence of oxidative stress and a signaling molecule that triggers adaptive responses in plants.

On the other hand, the observation of minimized H_2O_2 production in both genotypes under normal zinc conditions suggests that, in presence of adequate zinc, the plants can maintain a more balanced and controlled redox state, reducing the need for elevated oxidative stress responses.

RIL 46 exhibits better root growth and sustained zinc accumulation under zinc-deficient conditions, indicating genetic resilience to nutrient stress compared to IR 74. This resilience is further supported by reduced Glutathione Reductase (GR) activity in RIL 46, suggesting a more effective defense against oxidative damage induced by zinc deficiency. Additionally, the efficient line's lower hydrogen peroxide accumulation suggests superior mechanisms to mitigate oxidative stress, highlighting its overall robustness in coping with zinc deficiency.

Conclusion

The study suggests that the accumulation of zinc in leaves and seeds, along with Glutathione Reductase activity and hydrogen peroxide accumulation, can serve as valuable indicators for selecting rice lines that exhibit resilience and better adaptation to zinc deficit soil conditions. These parameters provide insights into the plant's ability to cope with nutrient stress, aiding in the identification and cultivation of varieties that can thrive under zinc-deficient environments.

References

1. Halliwell CH, Foyer C. Properties and physiological function of a glutathione reductase purified from spinach leaves by affinity chromatography. Planta. 1978;139:9-17.
2. Yoshida S, Douglas AF, James HC, Kwanchai AG. Laboratory manual for physiological studies of rice, IRRI, 3rd edition; c1976. p. 27.
3. Singh RK, Mishra B. Role of central soil salinity research Institute in Genetic Improvement of Rice in India. In: "Genetic Improvement of Rice Varieties of India" (eds. Sharma S.D. and Rao U.P.). Today and Tomorrow Printers and Publishers, New Delhi; c2004. p. 189-242.
4. Costa MI, Sarmento-Ribeiro AB, Goncalves AC. Zinc: From Biological Functions to Therapeutic Potential. Int J Mol Sci. 2023;24:4822.
5. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. J Res Med Sci. 2013;18(2):144-157.
6. Hussain A, Jiang W, Wang X, Shahid S, Saba N, Ahmad M, *et al*. Mechanistic Impact of Zinc Deficiency in Human Development. Front Nutr. 2022;9. https://doi.org/10.3389/fnut.2022.717064.
7. Saleem MH, Usman K, Rizwan M, Al Jabri H, Alsafran M. Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Front Plant Sci. 2022;13:1033092.
8. Khan ST, Malik A, Alwarthan A, Shaik MR. The enormity of the zinc deficiency problem and available solutions: An overview. Arabian J Chem. 2022;15(3):103668.
9. Gindri RG, Navarro BB, Dias PVC, Tarouco CP, Nicoloso FT, Brunetto G, *et al*. Physiological responses of rice (*Oryza sativa* L.) oszip7 loss-of function plants exposed to varying Zn concentrations. Physiol Mol Biol Plants. 2020;26(7):1349-1359.
10. Hasanuzzaman M, Nahar K, Anee TI, Fujita M. Glutathione in plants: Biosynthesis and physiological role in environmental stress tolerance. Physiol Mol Biol Plants. 2017;23(2):249-268.
11. Chao YE, Zhang M, Tian SK, Lu LL, Yang XE. Differential generation of hydrogen peroxide upon exposure to zinc and cadmium in the hyperaccumulating plant species (*Sedum alfredii* Hance). 2008. J Zhejiang Univ Sci B. 9(3):243-249.