***Pak. J. Bot.*, 58(4): 819-826, April 2026.**

ARTICLE **DOI: http://dx.doi.org/10.30848/PJB2026-4(14)**

LYSINE-CHELATED ZINC ALLEVIATES DROUGHT STRESS IN RICE CALLUS BY ENHANCING GROWTH AND NUTRIENT UPTAKE

NAILA MUKHTAR[1], RIFFAT NASIM FATIMA[2], RIFFAT BATOOL[2], GULNAZ PARVEEN[3]*, JOHAR JAMIL[4], SHAMAILA IRUM[5], SABA IQBAL[3], AND MUHAMMED MUMTAZ KHAN[6]

[1]Department of Botany, University of Okara, Pakistan (nailamukhtar@uo.edu.pk)
[2]Department of Botany, Government College Women University, Faisalabad, Pakistan (riffat@gcwuf.edu.pk; riffatbatool@gcwuf.edu.pk)
[3]Department of Botany, Women University Swabi (gulnaz.parveen@wus.edu.pk;
[4]Department of Microbiology, University of Swabi, Pakistan (Johar.jamil@uoswabi.edu.pk)
[5]Department of Zoology, University of Gujrat, Pakistan (shamaila.irum@uog.edu.pk)?
[6]Department of Microbiology, The University of Haripur, Pakistan (mumtaz.muhammadee@gmail.com)

*Corresponding author's email: gulnaz.parveen@wus.edu.pk (G.P)

Abstract

Drought stress significantly limits rice (Oryza sativa L.) growth and nutrient uptake. This study evaluated the effect of lysine-chelated zinc (LCZ) on mitigating drought-induced stress in callus tissues of two rice genotypes, Bas-370 and Kashmir Basmati. One-month-old calli were treated for 15 days with polyethylene glycol (PEG) to simulate drought stress (0%, 10%, and 20% PEG), both with and without 20 µM LCZ, under controlled conditions. PEG-induced stress led to a substantial reduction in callus fresh weight, relative growth rate (RGR), and the uptake of key nutrients, including phosphorus (P), manganese (Mn), magnesium (Mg), and iron (Fe). In contrast, LCZ treatment improved callus growth and partially restored nutrient uptake under drought conditions. The results suggest that LCZ can alleviate osmotic stress effects at the cellular level and support nutrient assimilation. These findings indicate the potential of LCZ as a supplement to enhance drought resilience in rice, though further validation in whole plants is recommended.

Key words: Correlation; Lysine chelated zinc; PEG induced drought; Relative growth rate; Rice callus

Introduction

Rice, one of the most important staples grains, provides nourishment and energy to millions. The number of rice consumers is rising as the global population grows. Water is a crucial abiotic factor essential for rice development and growth (Byrnes & Bum, 2017). However, drought stress can reduce the potassium (K^+) levels in rice callus, leading to a decrease in relative water content (RWC) and water use efficiency (WUE). It continues to impact global rice production significantly, with a reduction of up to 53% (Byrnes & Bum, 2017). Pakistan is renowned for its basmati rice, which is famous for its long, slender grains, gelatinization, strong aroma, and significant grain elongation during cooking (Mathure *et al.,* 2011). However, rice production is severely threatened by drought stress, which can lead to significant yield losses and poor grain quality (Hasanuzzaman *et al.,* 2013; Raza *et al.,* 2023).

Drought represents a major environmental challenge for agriculture, exerting extensive impacts (Eid *et al.,* 2020). Researchers are actively working to improve agricultural yield in water-scarce environments. The global population has increased from approximately 1.67 billion in the early 20th century to 5.99 billion currently, with projections of 8.10 billion by 2050 and 8.99 billion by 2150. This population increase is concentrated in developing regions, particularly in Asia and Africa, which account for 90% of the growth (Byrnes & Bum, 2017).

Drought stress directly affects several physiological processes in rice, including osmoregulation, the accumulation of soluble sugars and proline, and maintaining high water content (Choudhury *et al.*, 2024). Lysine, referred to as K or Lys, is an essential α-amino acid for human health (Lukasheva *et al.,* 2021). Adequate lysine content is defined as food containing at least 50 mg of lysine per gram of protein. Lysine is known to greatly influence the growth of prevalent cereal crops, specifically wheat, maize, and rice (Schmidt *et al.,* 2015; Verslues *et al.,* 2006). Lysine is mostly found in cereal grains but abundantly found in pulses (Schmidt *et al.,* 2015). Zinc is an essential micronutrient that plays a critical role in rice growth and development, particularly under stress conditions (Kaur & Garg, 2021; Seleiman *et al.,* 2023). It acts as a fertilizer for plants; however, excessive concentrations can be highly toxic (Kaur & Garg, 2021). It also serves as a cofactor for various enzymes, including oxidases, peroxidases, anhydrases, and dehydrogenases, and is a critical component of metallo enzymes (Hänsch & Mendel, 2009). Moreover it is often used in a chelated form to enhance absorption. Chelates are ligands that can bind to a single metal ion (like Zn^{2+}) to form a unique, relatively stable structure known as a chelation complex. When in chelated form, metal ions are less likely to react and become immobilized by soil components, increasing their availability to plant roots (Khan *et al.,* 2021). Additionally, rice callus, a type of undifferentiated plant tissue, has been reported to possess drought stress-tolerant properties (Maleki *et al.,* 2019). Callus culture is extremely important in plant biotechnology for the determination of plant tolerance to

Received: 26-05-2025 Revised: 26-08-2025 Accepted: 29-08-2025 Online: 09-01-2026

environmental stresses such as drought. Considerable development was obtained with drought tolerance in selected plants in *In vitro* studies, such as sorghum, winter wheat, maize, and rice (Bi *et al.,* 2007). In a short duration, *In vitro* mutants with beneficial agronomic traits such as salt or water stress tolerance and disease resistance can be found (Tahir *et al.,* 2018; Farooq *et al.,* 2009).

To mitigate the adverse effects of drought stress on rice, researchers have been exploring various strategies, including the use of zinc (Zn) fertilizers and plant growth regulators. Beneficial agronomic traits, including resistance to diseases and salt or water stress, can be quickly identified *In vitro* Sundararajan *et al.,* 2017; Hallajian, 2016; Ali *et al*., 2022). Polyethylene glycol (PEG)-induced drought stress is also a widely used experimental approach to simulate drought conditions in controlled environments. Lysine-chelated zinc (LCZ) improves callus growth and mineral nutrient uptake in basmati rice genotypes under drought stress. Hence, LCZ could be a great source for improving growth and other physiological parameters under abiotic stresses, especially under drought stress (Slaton, 2005). However, no considerable work has been done in rice to understand the mechanism of drought tolerance by applying LCZ at the callus/cellular level. Therefore, we aimed to investigate the role of LCZ in alleviating drought-induced stress in rice (Bas-370 and Kashmir Basmati) callus tissues. In this respect, rice calli were treated with different PEG concentrations to simulate drought stress (Control, 10%, and 20% PEG), both with and without LCZ (20 µM) for 15 days in controlled conditions.

Materials and Methods

An *In vitro* experiment was conducted to examine the role of lysine-chelated zinc in alleviating drought stress at the cellular level in Basmati rice. Two genotypes, Bas-370, and Kashmir Basmati, were used. Grains (caryopsis) were sterilized and cultured on MS basal medium (Murashige & Skoog, 1962). Supplemented with 4 mg L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid) for callus induction. The experiment was carried out in the Advanced Tissue Culture Laboratory, Department of Botany, Government College Women University, Faisalabad, Pakistan.

Seed sterilization: To initiate the callus culture, dehusked rice grains were surface sterilized with 70% ethanol for 10 sec. After the removal of ethanol, grains were dipped in 20% sodium hypochlorite for 20 minutes. Then, it was washed with 0.1% mercuric chloride ($HgCl_2$) for 2-3 minutes under a Laminar Air Flow Cabinet. The grains were rinsed with autoclave distilled water and dried before inoculation on MS media. After inoculation, the culture was kept in the growth room at 25 ± 2°C under a 16/8 h light and dark photoperiod.

Treatments: In Erlenmeyer flasks, 2 g of one-month-old callus was added to liquid MS medium, along with optimized concentrations of PEG and LCZ (control (0), 10% PEG, 20% PEG, 20 µM LCZ, 10% PEG + 20 µM LCZ and 20% PEG + 20 µM LCZ). The flasks were incubated on a gyratory shaker at 25 ± 2°C under 3000 lux light for 15 days. Each treatment was replicated three times, using 10 flasks for each replicate. A factorial layout with a completely randomized design was used for the experiment. After 15 days of treatment, callus was collected to assess the subsequent parameters (Fig. 1).

Growth determination: After harvesting, calli were first washed by gently agitating in deionized distilled water for a few minutes, continuously agitated, and then dried with tissue papers. Callus fresh weight, dry weight, RGR (Relative Growth rate), mineral nutrients (K, Ca, Mg, Mn, Fe, and P), and STI (stress tolerance index) were recorded.

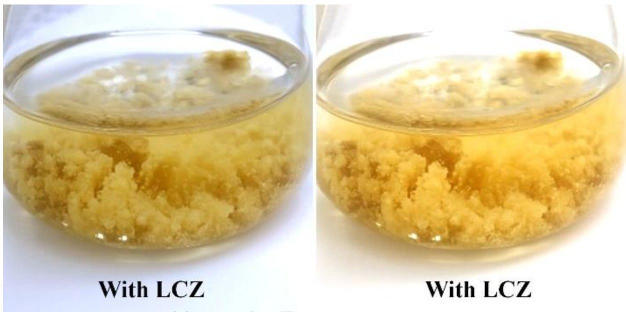

Fig. 1. Callus formation in control and LCZ-treated samples.

Relative Growth Rate was calculated as follows:

$$\text{RGR} = \ln(\text{Final fresh weight}) - \ln(\text{Initial fresh weight})$$

The callus STI was calculated as follows:

$$\text{STI} = \frac{\text{Average dry weight of callus treated x 100}}{\text{The average dry weight of callus control}}$$

Determination of mineral nutrients: A 0.5 g sample of dried callus was digested in 5 mL of concentrated HNO_3 at 100°C, with the temperature subsequently increased to 150°C in digestion flasks. After digestion, the volume was brought up to 50 mL in a volumetric flask. The extract was then filtered and analyzed for concentrations of mineral nutrients, including potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), and iron (Fe).

Determination of phosphorus (P): To prepare the Br-reagent, 25 grams of ammonium molybdate was completely dissolved in 400 mL of distilled water (Solution A). Separately, 1.25 grams of ammonium metavanadate was dissolved in 300 mL of distilled/deionized water, and then 250 mL of concentrated HNO_3 was added to this solution (Solution B). After allowing Solution B to cool, it was slowly mixed with Solution A in a 1-liter volumetric flask. The combined solution was then diluted with distilled water to a final volume of 1000 mL and thoroughly mixed to ensure homogenization. The reagent was allowed to stand for 30 minutes before use. The optical density (O.D.) of the test solution was measured at 460 nm using a spectrophotometer, Hitachi U-2001.

Statistical analysis: The collected data were subjected to analysis of variance (ANOVA) using the COSTAT software package (Cohort Software, Berkeley, California) under a factorial arrangement. Differences between treatment means were compared using the Least Significant Difference (LSD) test at a 5% probability level.

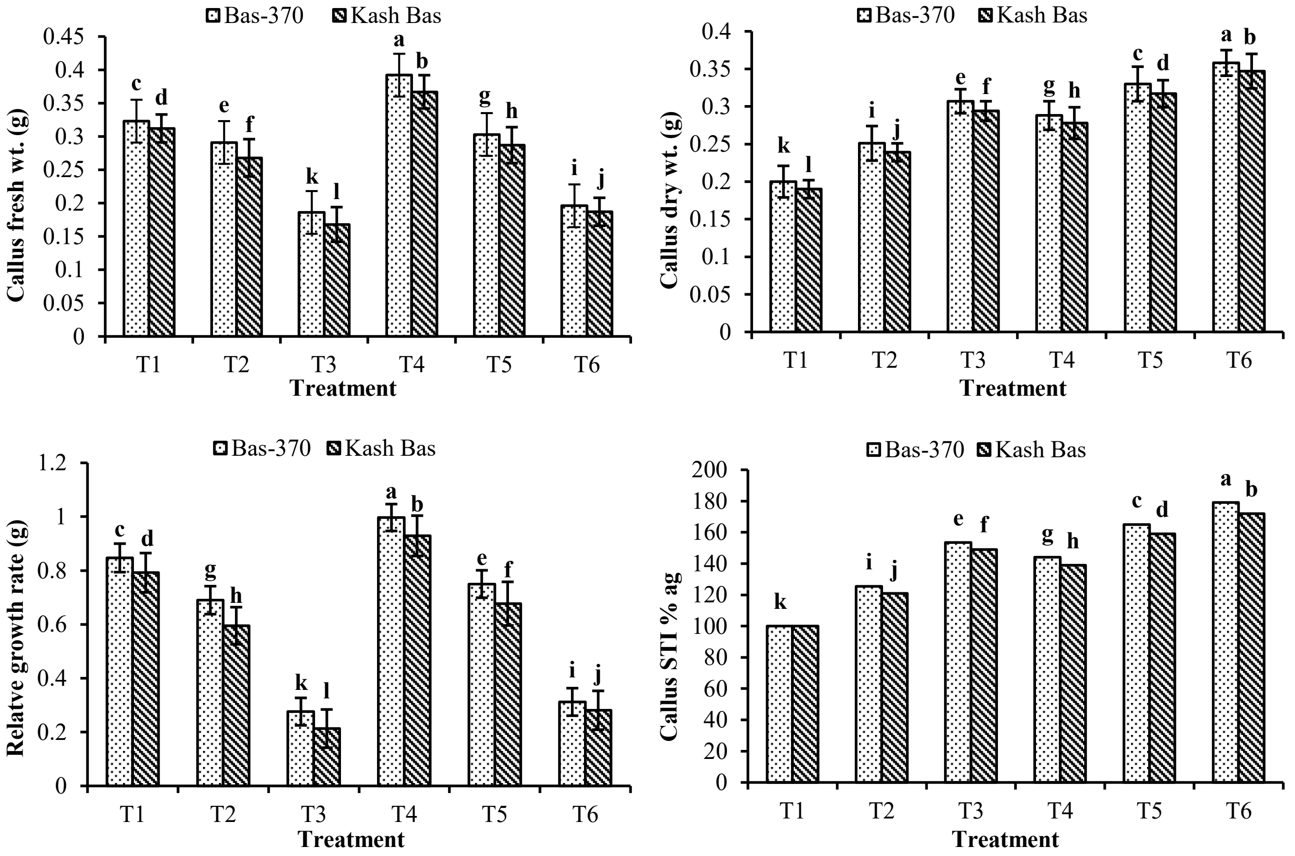

Fig. 2. Effect of LCZ on fresh weight., dry weight., RGR (Relative Growth Rate), and STI (Stress Tolerance Index) of basmati rice callus under drought stress

T1= control, T2= 10% PEG, T3= 20% PEG, T4= 20 *µM*, T5= 10% PEG+20 *µM*, T6= 20% PEG+20 *µM*

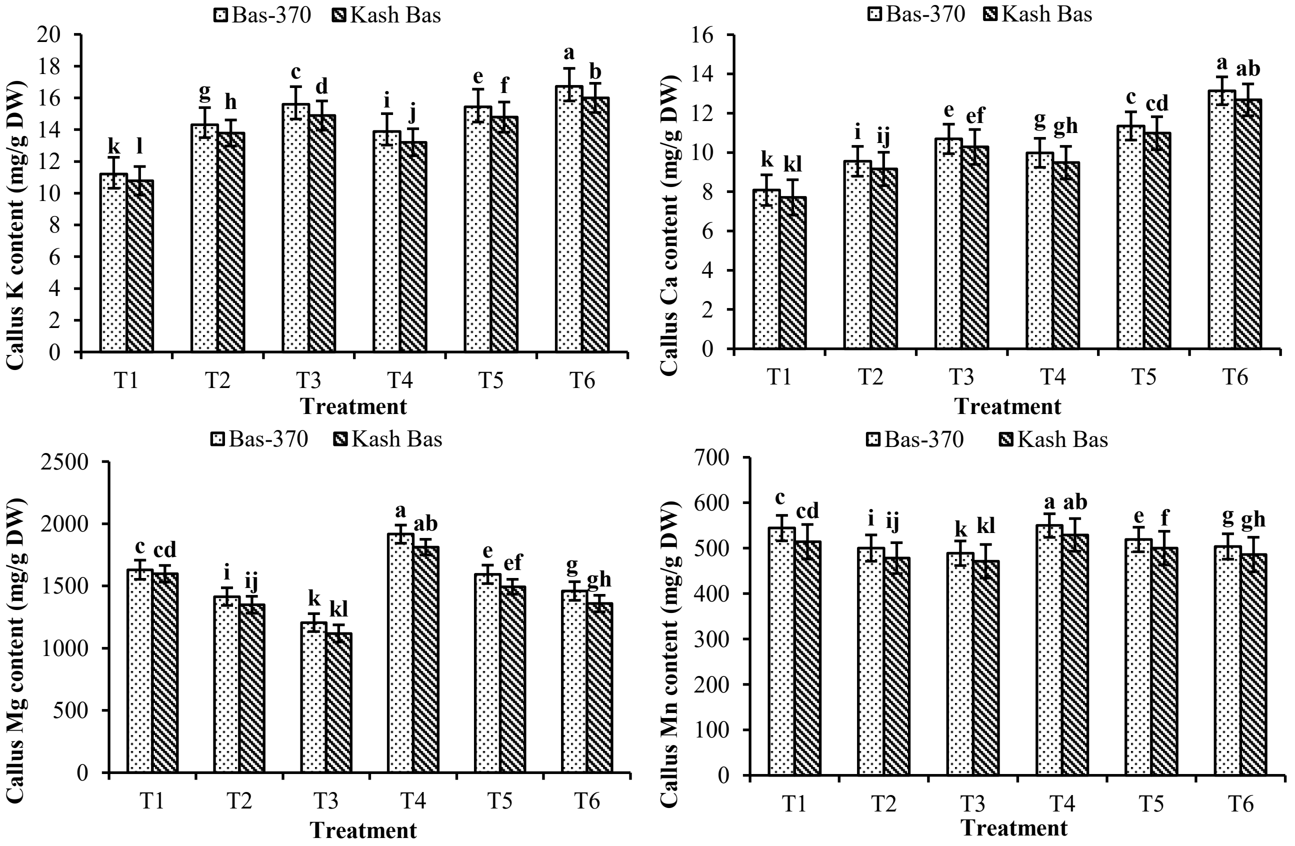

Fig. 3. Effect of LCZ on K, Ca, Mg, and Mn of basmati rice callus under drought stress

T1= control, T2= 10% PEG, T3= 20% PEG, T4= 20 *µM*, T5= 10% PEG+20 *µM*, T5= 20% PEG+20 *µM*

Results

Growth parameters

Callus fresh weight: Fresh weight was reduced in callus at different levels of drought stress in the culture medium compared to the control (Fig. 2; Supplementary Table 1). Both Bas-370 and Kashmir Basmati showed a reduction in callus fresh weight, but more was observed in Kashmir Basmati than in Bas-370. The fresh weight of the callus was increased with the addition of LCZ in the medium compared to the control. Application of LCZ with PEG improved the callus fresh weight in both genotypes, but more improvement was observed in Bas-370.

Callus relative growth rate (RGR): The relative growth rate (RGR) was reduced with the increase of PEG concentration in the media, but more reduction was observed at 20% PEG. RGR in both genotypes was reduced under drought stress, but more reduction was seen in Kashmir Basmati than Bas-370 (Fig. 2; Supplementary Table 1). Application of LCZ improved RGR in both genotypes under drought stress, but more improvement was observed in both Bas-370 alone and combination with PEG.

Callus dry weight: Callus dry weight was increased with elevated levels of PEG in Basmati-370 compared to Kashmir Basmati. LCZ application alone, as well as in combination with PEG, improved the callus dry weight compared to the control (Fig. 2, (Supplementary Table 1). The application of LCZ alone and in combination with different PEG levels exhibited a positive response in both genotypes, and more effective results were seen in Basmati-370 than in Kashmir Basmati.

Stress tolerance index (STI): Both genotypes showed a significant increase in STI with the increase in PEG concentration in the media. STI in Kashmir Basmati was reduced more than in Bas-370 (Fig. 2, Supplementary Table 1). The application of LCZ improved the STI of callus in both genotypes compared to the control alone and in combination with the PEG concentration.

Mineral nutrients

Callus potassium (K^+) content: Potassium (K) increased in callus tissues of Basmati-370 compared to Kashmir Basmati with PEG treatment. With the addition of LCZ in the culture medium, more increase in uptake of K content was observed than control in callus tissue of both genotypes (Fig. 3; Supplementary Table 2). More increase in K content was observed with the application of LCZ in combination with PEG than alone PEG treatment.

Callus calcium (Ca^{2+}) content: Elevated levels of PEG in the culture medium showed an increase in the uptake of callus Ca content in Basmati-370 compared to Kashmir Basmati. More reduction was observed at 20% PEG than at 10% PEG (Fig. 3, Supplementary Table 2). The addition of LCZ alone and in combination with PEG also improved the uptake of Ca content in the callus tissue of both genotypes. Basmati-370 showed an increase in the uptake of callus Ca content more than Kashmir Basmati.

Callus magnesium (Mg^{2+}) content: Mg content was reduced in Basmati-370 compared to Kashmir Basmati with elevating PEG treatments. With the addition of LCZ in the culture medium, the uptake of callus Mg content was improved compared to that of the control (Fig. 3, (Supplementary Table 2). Application of LCZ in combination with different PEG levels exhibited a positive response in ameliorating the negative effects of drought stress in both genotypes and improved the callus Mg content but not to the extent of control.

Callus manganese (Mn^{2+}) content: The uptake of callus Mn content was reduced in Basmati-370 compared to Kashmir Basmati, with elevated levels of PEG. The addition of LCZ alone and in combination with high PEG levels increased the uptake of callus Mn in both genotypes but increased in Basmati-370 compared to Kashmir Basmati (Fig. 3; Supplementary Table 2). The application of LCZ exhibited a positive response by reducing the negative effects of drought stress in both genotypes and improving the callus Mn content, but not to the extent of control.

Callus iron (Fe) content: More reduction in callus Fe content was found in Kashmir Basmati than in Basmati-370 as the PEG levels increased in the culture medium. Application of LCZ alone in culture medium increased the callus Fe content compared to control. When LCZ was applied in combination with elevated levels of PEG, less negative effects of drought in both genotypes were observed (Fig. 4; Supplementary Table 2). Basmati-370 showed more improvement in callus Fe content than Kashmir Basmati.

Callus phosphorus (P) content: Less reduction in callus P content was observed in Basmati-370 compared to Kashmir Basmati with elevating PEG treatments. Callus P content decreased more at 20 PEG than at 10 % PEG (Fig. 4). LCZ alone and in combination with elevated levels of PEG exhibited positive responses in both genotypes. More improvement in Basamati-370 was observed than in Kashmir Basmati.

Correlation: Stress Tolerance Index (STI). The lines represent the linear relationships for RGR and DW with STI, and their equations and R^2 values are provided. RGR and STI have a weak, inverse relationship ($p>0.05$), suggesting that RGR decreases with higher stress tolerance but not in a strongly predictable manner. DW and STI have a strong, direct relationship ($p>0.05$), indicating an increase of DW with higher stress tolerance (Fig. 5).

Both K and Ca contents decrease as RGR increases. This suggests that higher growth rates might result in a depletion of these nutrients or reduced accumulation in the callus tissue. Calcium appears to have a slightly stronger inverse relationship with RGR compared to potassium, based on the steeper slope and higher R^2 Potassium (K) is essential for osmotic regulation, enzyme activation, and stress responses. Its decline with higher RGR might suggest that rapidly growing tissues prioritize biomass expansion over maintaining optimal nutrient reserves (Fig. 5) (Marschner, 2011; White & Broadley, 2003). Both K and Ca contents increase with the increase of callus DW, suggesting that heavier callus tissues (indicating greater biomass accumulation) are associated with higher nutrient accumulation. While the relationship between DW and K is strong ($R^2=0.8793$), it is slightly weaker compared to Ca. This implies that K content is highly dependent on biomass but with a small degree of variability influenced by other factors. The relationship between DW and Ca content is exceptionally strong (R2=0.9378). This indicates that Ca accumulation is almost directly proportional to DW, suggesting a highly predictable dependency (Fig. 5).

In Fig. 6, the relationship between Relative Growth Rate (RGR) (x-axis) and the contents of iron (Fe) and phosphorus (P) in callus tissue (y-axis) is presented. Fe shows a steeper increase in content with increasing RGR than P, as indicated by its higher regression slope (slope for Fe = 580.62, slope for P = 8.59). However, the coefficient of determination (R^2) for P ($R^2 = 0.78$) is higher than that for Fe ($R^2 = 0.62$), suggesting that RGR better explains the variation in P content than in Fe content. Across all growth rates, P levels remain consistently lower than Fe levels.

The second part of the figure illustrates the relationship between Callus Dry Weight (DW) and the Fe and P contents. Both Fe and P contents show a slight decline as DW increases, but the relationships are weak, with low R^2 values ($R^2 = 0.22$ for Fe, $R^2 = 0.18$ for P), indicating that DW accounts for only a small portion of the variation in these nutrient levels. This suggests that callus dry weight is a poor predictor of Fe and P content.

These weak correlations imply that factors other than biomass accumulation—such as nutrient transport efficiency, metabolic activity, or stress-related ion uptake mechanisms—may play a more significant role in governing Fe and P accumulation in the callus tissue under the experimental conditions.

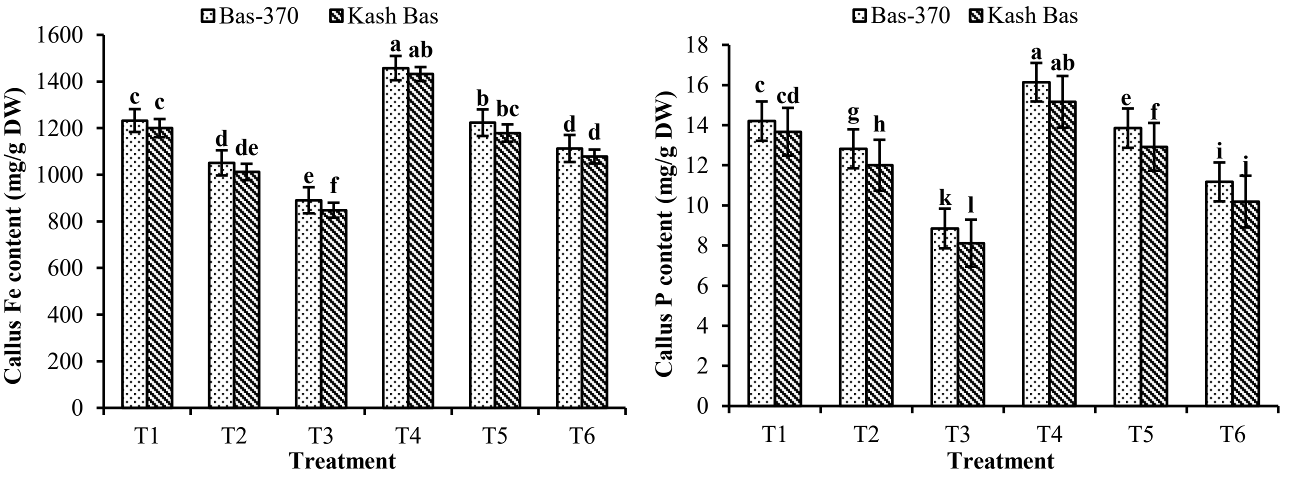

Fig. 4. Effect of LCZ on Fe and P of basmati rice callus under drought stress
T1= control, T2= 10% PEG, T3= 20% PEG, T4= 20 μM, T5= 10% PEG+20 μM, T5= 20% PEG+20 μM

Discussion

Drought stress induces a wide range of physiological changes in plants, including osmoregulation, proline accumulation, and alterations in water content and soluble sugars (Hasanuzzaman *et al.*, 2013). These responses are often accompanied by reductions in photosynthetically active radiation (PAR), transpiration rate, photosynthetic efficiency, pigment concentration, stomatal conductance, and relative water content (RWC), ultimately resulting in decreased water use efficiency (WUE) and growth inhibition (Seleiman *et al.*, 2021). Such physiological traits can serve as effective indicators for evaluating drought tolerance in various crop species (Hussain *et al.*, 2018).

In the present study, PEG-induced drought stress significantly affected the growth parameters of rice callus. A notable reduction in fresh weight was observed under increasing PEG concentrations, while dry weight paradoxically increased. This inverse trend may be explained by the accumulation of osmo protectants such as proline, soluble sugars, and proteins, which contribute to osmotic adjustment and increased cellular density under stress (Boutchouang *et al.*, 2024). These compounds help maintain cell turgor and metabolic activity, potentially explaining the higher dry weight despite reduced water content. Similar observations have been reported in indica rice (Sagar *et al.*, 2020), date palm (Al-Khayri & Ibraheem, 2014), and other species including tomato and wheat under PEG-induced stress (Sharma & Srivastava, 2014; Bouiamrine & Diouri, 2012).

Relative Growth Rate (RGR) was also decreased with increasing PEG, consistent with osmotic stress limiting cell division and elongation (Mohammad *et al.*, 2019). However, the application of lysine-chelated zinc (LCZ) mitigated the negative effects of drought stress, improving both fresh and dry weights across genotypes. LCZ application, alone or in combination with PEG, consistently enhanced callus growth, with Bas-370 showing a greater positive response than Kashmir Basmati. This genotypic difference may reflect variations in the efficiency of nutrient uptake, osmotic adjustment capacity, or zinc metabolism pathways—though further molecular studies are needed to confirm these mechanisms.

Zinc plays a central role in plant physiology, regulating processes such as enzyme activation, protein synthesis, antioxidant defense, and hormone regulation (Cakmak & Kutman, 2018). When applied in chelated form, as LCZ, zinc is more bioavailable and effective in promoting growth under stress conditions. Previous studies have shown that amino acid-chelated zinc enhances antioxidant activity, protects against oxidative damage, and improves nutrient transporter gene expression (Weisany *et al.*, 2012; Kumar *et al.*, 2023). These mechanisms may underlie the observed improvements in Stress Tolerance Index (STI) in our study following LCZ treatment under PEG stress.

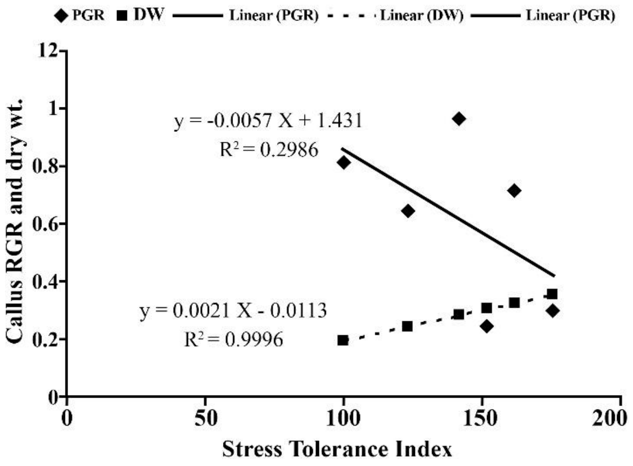

Fig. 5. Correlation between callus stress tolerance index, and, callus relative growth rate (RGR) and callus dry weight of calli of two Basmati-rice genotypes under drought stress with or without lysine chelated zinc (LCZ).

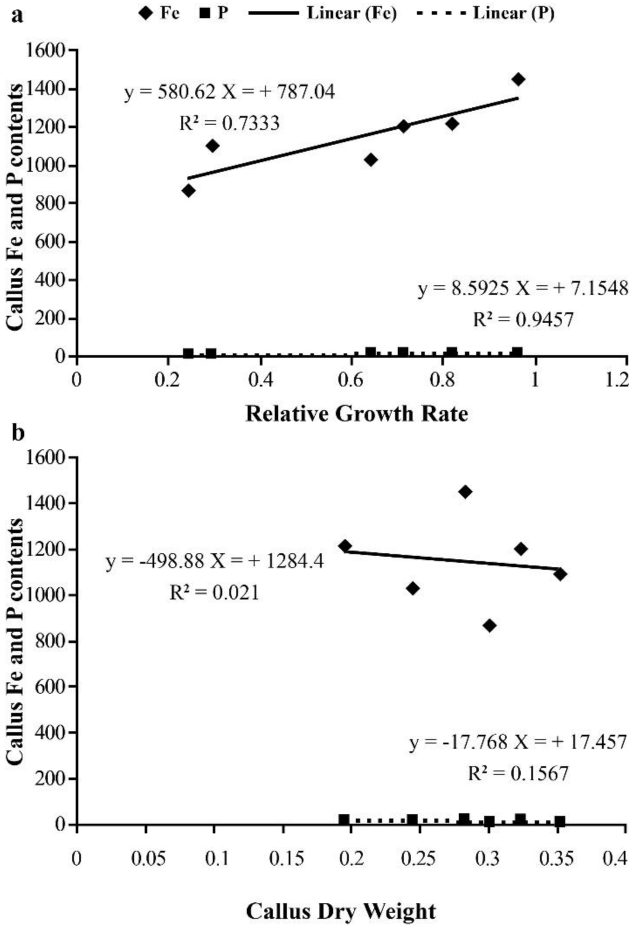

Fig. 6. Correlation between callus relative growth rate (a) and callus dry weight (b), and, callus Mg and Mn contents of calli of two Basmati-rice genotypes under drought stress with or without lysine chelated zinc (LCZ).

Drought stress also had a profound effect on mineral nutrient accumulation. We observed a general reduction in the contents of Mg, Mn, Fe, and P, while K and Ca levels were increased under elevated PEG concentrations. The increase in K^+ and Ca^{2+} may contribute to osmotic adjustment, as these cations are key in maintaining cell turgor and membrane stability under dehydration (Ashraf *et al*., 2013). These findings align with previous reports indicating that plants enhance the accumulation of compatible solutes and specific ions to cope with water limitation (Tavakol *et al*., 2018; Niazkhani *et al*., 2021). Conversely, the reduced uptake of other nutrients could be due to decreased membrane permeability, reduced transpiration, and impaired active transport under stress conditions (Rouphael *et al*., 2012; Qi *et al*., 2019).

Interestingly, the accumulation of Fe and P showed a weak or inconsistent correlation with callus dry weight and RGR. This suggests that factors beyond biomass, such as transporter regulation, cellular redox status, or root-shoot communication (in whole plants), may play a stronger role in controlling Fe and P uptake and storage in callus cultures.

Zinc deficiency is well-documented to impair growth and mineral nutrition, while excessive levels (>50 mg/L) may be toxic (Imtiaz *et al*., 2010; Noulas *et al*., 2018). In this research, the application of LCZ offered a balanced zinc source that not only prevented deficiency but also improved the uptake of other nutrients even under drought stress. Our data confirm that LCZ application improved the all tested mineral nutrients and significantly reduced the negative impact of PEG induced osmotic stress.

Conclusion

In conclusion, lysine-chelated zinc (LCZ) played a significant role in enhancing drought tolerance in rice callus exposed to polyethylene glycol (PEG)-induced stress. Its application, both alone and in combination with PEG, improved growth parameters and mineral nutrient uptake in both rice genotypes, with Bas-370 exhibiting greater resilience than Kashmir Basmati. LCZ proved more effective than lysine or zinc alone, highlighting the advantage of chelation in improving nutrient availability and stress mitigation. These findings support the potential of LCZ not only in *In vitro* culture systems but also as a foliar or soil application in whole plants to enhance drought resistance and nutrient efficiency. Further research at the whole-plant and molecular level is recommended to validate its broader applicability in crop improvement strategies.

Statements and Declarations

Funding: "The authors declare that no funds, grants, or other support were received during the preparation of this manuscript."

Conflict of Interests: "The authors have no conflict of interest including financial, academic or any other."

Author Contributions: Study conception and design: Naila Mukhtar, Riffat Nasim Fatima and Riffat Batool; data collection: Gulnaz Parveen, Shamaila Irum and Muhammad Mumtaz Khan; analysis and interpretation of results; Johar Jamil, Saba Iqbal and Naila Mukhtar draft manuscript preparation: Naila Mukhtar, Rifat Naseem supervision: Naila Mukhtar, Gulnaz Parveen and Naila Batool project administration: Naila Mukhtar, Riffat Nasim Fatima and Riffat Batool. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials.

Supplementary Table 1. Analyses of variance (ANOVA) for relative growth rate (RGR), dry weight, and stress tolerance index (STI) in the callus of the two Basmati rice genotypes under drought stress with or without lysine chelated zinc (LCZ).

Source of variance	DF	Relative growth rate (RGR)	Dry weight of callus	Stress tolerance index (STI)
Drought (PEG)	2	1.194***	0.023***	5409.798***
Lycine Chelated Zinc (LCZ)	1	0.068***	0.047***	11095.111***
Genotype (G)	1	0.039***	0.0013***	186.778***
PEG x LCZ	2	0.00715***	0.00107***	261.715***
PEG x G	2	0.00133***	0.00000619ns	8.0486**
LCZ x G	1	0.00059**	0.000001ns	28.444***
PEG x LCZ x G	2	0.000502***	0.00000208ns	1.798ns
Error	24	0.000053	0.0000505	1.1944

, * = Significance at 0.01 and 0.001, respectively, ns = Non-significant

Supplementary Table 2. Analyses of variance (ANOVA) for uptake of mineral nutrients in the callus of the two Basmati rice genotypes under drought stress with or without lysine chelated zinc (LCZ).

Source of variance	DF	Calcium (Ca)	Potassium (K)	Magnesium (Mg)	Manganese (Mn)	Iron (Fe)	Phosphorus (P)
Drought (PEG)	2	24.955***	38.693***	626673.3***	7125.8***	370260.8***	83.4***
Lycine Chelated Zinc (LCZ)	1	36.764***	22.373***	433569.6***	2086.5***	389554.9***	23.8***
Genotype (G)	1	1.563***	3.447***	60a57.4***	4044.3***	11950.1***	6.2***
PEG x LCZ	2	0.377***	2.135***	7512.6***	77.4***	3318.5***	1.1***
PEG x G	2	0.00275**	0.0234ns	477.8**	44.3***	129.5***	0.001ns
LCZ x G	1	0.0064***	0.0374ns	3591.6***	38.8***	23.6ns	0.163ns
PEG x LCZ x G	2	0.00405***	0.0073ns	650.8***	17.1***	41.6*	0.024ns
Error	24	0.000353	0.0836	57.9	0.084	11.9	0.089

*, **, *** = Significance at 0.05, 0.01 and 0.001 respectively, ns = Non-significant

References

Ali, S., M.F.B. Mfarrej, A. Hussain, N.A.M. Akram, Rizwan, X. Wang, A. Maqbool, M. Nafees and B. Ali. 2022. Zinc fortification and alleviation of cadmium stress by application of lysine chelated zinc on different varieties of wheat and rice in cadmium stressed soil. *Chemosphere*, 295: p.133829. https://doi.org/10.1016/j.chemosphere.2022.133829.

Al-Khayri, J.M. and Y. Ibraheem. 2014. *In vitro* selection of abiotic stress tolerant date palm (*Phoenix dactylifera* L.): a review. *Emir. J. Food Agric*., 26(11): 921-933. https://doi.org/10.9755/ejfa.v26i11.18975.

Ashraf, M., M. Shahbaz and A. Qasim. 2013. Drought-induced modulation in growth and mineral nutrients in canola (*Brassica napus* L.). *Pak J Bot*., 45:93-98. https://doi.org /10.5555/20133114258.

Bi, R.M., M. Kou, L.G. Chen, S.R. Mao and H.G. Wang. 2007. Plant regeneration through callus initiation from mature embryo of *Triticum*. *Plant Breed*., 126(1): 9-12. https://doi.org/10.1111/j.1439-0523.2007.01327.x.

Bouiamrine, E.H and M. Diouri. 2012. Response of durum wheat (*Triticum durum* Desf) callus culture to osmosis induced drought stress caused by polyethylene glycol (PEG). *Ann. Biol. Res*., 3: 4555-4563. https://doi.org/ full/10.5555/ 20123330478.

Boutchouang, RP., O. Fliniaux, J.V.E. Eyamo, A.S.M. Djabou, J.X. Fontaine, R. Molinié, F. Mesnard and N. Niemenak. 2024. Metabolome profiling of cacao (*Theobroma cacao* L.) callus under drought stress conditions induced by polyethylene glycol (PEG) as osmoticant. *Phytochem. Anal.*, 35(4): 708-722. https://doi.org/ /abs/10.1002/pca.3323.

Byrnes, B.H. and B.L. Bumb. 2017. Population growth, food production and nutrient requirements. In: Nutrient use in crop production. *CRC Press*. 1-27. https://doi.org /10.1201/ 9780203745281-1

Cakmak, I. and U.A. Kutman. 2018. Agronomic biofortification of cereals with zinc: A review. *Europ. J. Soil Sci*., 69(1): 172-180. https://doi.org /10.1111/ejss.12437.

Choudhury, D., C. Mukherjee, S. Dey and S. Dutta. 2024. Drought stress tolerance in rice: A critical insight. *Plant Sci. Today*, 11: 241-257.

Eid, M.A., A.A. Abdel-Salam, H.M. Salem, S.E. Mahrous, M.F. Seleiman, A.A. Alsadon, T.H. Solieman and A.A. Ibrahim. 2020. Interaction effects of nitrogen source and irrigation regime on tuber quality, yield, and water use efficiency of *Solanum tuberosum* L. *Plants,* 9: 110. https://doi.org/ 10.3390/plants9010110

Farooq, M., A. Wahid, D.J. Lee, O, Ito and K.H. Siddique. 2009. Advances in drought resistance of rice. *Crit. Rev. Plant Sci*., 8(4): 199-217. https://doi.org /10.1080/07352680902952173.

Hallajian, M.T. 2016. Mutation breeding and drought stress tolerance in plants. drought stress tolerance in plants. *Mol. Gen. Persp.,* 359-383. https://doi.org/10.1007/978-3-319-32423-4-13.

Hänsch, R. and R.R. Mendel. 2009. Physiological functions of mineral micronutrients (cu, Zn, Mn, Fe, Ni, Mo, B, cl). *Curr. Opin. Plant Biol*., 12(3): 259-266. https://doi.org/10.1016/ j.pbi.2009.05.006.

Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury and M. Fujita. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*., 14(5): 9643-84. https://doi.org/10.3390/ijms14059643.

Hussain, M., S. Farooq, W. Hasan, S. Ul-Allah, M. Tanveer, M. Farooq and A. Nawaz. 2018. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agri. Water Manag.,* 201: 52-166. https://doi.org/10.1016/j.agwat.2018.01.028.

Imtiaz, M., A. Rashid, P. Khan, M.Y. Memon and M. Aslam. 2010. The role of micronutrients in crop production and human health. *Pak. J. Bot*., 42(4): 2565-2578.

Kaur, H. and N. Garg. 2021. Zinc toxicity in plants: A review. *Planta*, 253: 6-129.

Khan, I., S.A. Awan, M. Rizwan, S. Ali, M.J. Hassan, M. Brestic, X. Zhang and L. Huang. 2021. Effects of silicon on heavy metal uptake at the soil-plant interphase: A review. *Ecotoxicol. Environ. Safety*, 222: p. 112-510. https://doi.org/10.1016/j.ecoenv.2021.112510

Kumar, H., R. Dhalaria, S. Guleria, R. Cimler, R. Choudhary, D.S. Dhanjal, R. Singh, N. Kimta, K. Dulta, A.K. Pathera and A. Khan. 2023. To exploring the role of probiotics, plant-based fermented products, and paraprobiotics as anti-inflammatory agents in promoting human health. *J. Agri. Food Res*., 100896. https://doi.org/10.1016/j.jafr.2023.100896.

Lukasheva, E.V., G. Babayeva, S.S. Karshieva, D.D. Zhdanov and V.S. Pokrovsky. 2021. L-lysine α-oxidase: enzyme with anticancer properties. *Pharmaceut*., 14(11): 1070. https://doi.org /10.3390/ph14111070.

Maleki, M., M. Ghorbanpour, S. Nikabadi and S.H. Wani. 2019. In vitro screening of crop plants for abiotic stress tolerance. In recent approaches in omics for plant resilience to climate change (pp. 75-91). *Cham: Springer International Publishing*. https://doi.org/10.1007/978-3-030-21687-0_4.

Marschner, P. 2011. *Marschner's Mineral Nutrition of Higher Plants* (3rd ed.). Academic Press.

Mathure, S., A. Shaikh, N. Renuka, K. Wakte, N. Jawali, R. Thengane and A. Nadaf. 2011. Characterisation of aromatic rice (*Oryza sativa* L.) germplasm and correlation between their agronomic and quality traits. *Euphytica*, 179: 237-46. https://doi.org/10.1007/s10681-010-0294-9.

Mohammad, S. M.A. Khan, A. Ali, L. Khan and M.S. Khan. 2019. Feasible production of biomass and natural antioxidants through callus cultures in response to varying light intensities in olive (*Olea europaea* L.) cult. Arbosana. *J. Photochem. Photobiol. B: Biol*., 193: 140-147. https://doi.org/10.1016/j.jphotobiol.2019.03.001.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Niazkhani M, M.B. Abdollahi, M. Jafari and M.H.S. Rasooli. 2021. Micronutrients concentrations in bread wheat cultivars with different Zn efficiency under Zn deficient and Zn sufficient conditions. *Water Soil*, 21: 35(1): 49-65. https://doi.org/10.22067/jsw.v35i1.84269

Noulas, C., M. Tziouvalekas and T. Karyotis. 2018. Zinc in soils, water and food crops. *J. Trace Elements Med. Biol*., 1; 49: 252-60. https://doi.org/10.1016/j.jtemb.2018.02.009

Qi, J., S. Sun, L. Yang, M. Li, F. Ma and Y. Zou. 2019. Potassium uptake and transport in apple roots under drought stress. *Hort. Plant J*., 5(1): 10-16. https://doi.org/ 10.1016/ j.hpj.2018.10.001

Raza, M.A.S., B. Zulfiqar, R. Iqbal, M.N. Muzamil, M.U. Aslam, F. Muhammad, J. Amin, H.M.U. Aslam, M.A. Ibrahim, M. Uzair and M. Habib-ur-Rahman. 2023. Morpho-physiological and biochemical response of wheat to various treatments of silicon nano-particles under drought stress conditions. *Sci. Rep*., 13(1): 2700. https://doi.org /10.1038/s41598-023-29784-6

Rouphael, Y., M. Cardarelli, D. Schwarz, P. Franken and G. Colla. 2012. Effects of drought on nutrient uptake and assimilation in vegetable crops. Plant responses to drought stress: From morphological to molecular features. *Springer*, 171-195. https://doi.org/10.1007/978-3-642-32653-0-7.

Sagar. A., F. Rauf, M.A. Mia, T.H. Shabi, T. Rahman and A.Z. Hossain. 2020. Polyethylene Glycol (PEG) induced drought stress on five rice genotypes at early seedling stage. *J. Bang. Agri. Uni*., 18(3): 606-614. https://doi.org/10.5455/ JBAU.102585.

Schmidt, D., V. Rizzi, S.A. Gaziola, L.O. Medici, E. Vincze, M. Kozak, P.J. Lea and R.A. Azevedo. 2015. Lysine metabolism in antisense C-hordein barley grains. *Plant Physiology and Biochemistry*, 87: pp. 73-83. https://doi.org/ 10.1016/j.plaphy.12.0

Seleiman, M.F., N. Al-Suhaibani, N. Ali, M. Akmal, M. Alotaibi, Y. Refay, T. Dindaroglu, H.H. Abdul-Wajid and M.L. Battaglia. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*, 10(2): p. 259. https://doi.org /10.3390/plants10020259

Seleiman, M.F., W.A. Al-Selwey, A.A. Ibrahim, M. Shady and A.A. Alsadon. 2023. Foliar applications of ZnO and SiO_2 nanoparticles mitigate water deficit and enhance potato yield and quality traits. *Agronomy*, 13: 466. https://doi.org /10.3390/agronomy13020466

Sharma, P. and D.K. Srivastava. 2014. *In vitro* plant regeneration from cotyledon and hypocotyls tissues of tomato (*Solanum lycopersicum* L. cv. Solan Vajr). *Vegetos*. 27(3): 151. https://doi.org/ 10.5958/2229-4473.2014.00083.4.

Slaton, N.A., R.J. Norman and Jr. C.E. Wilson. 2005. Effect of zinc source and application time on zinc uptake and grain yield of flood‐irrigated rice. *Agron. J*., 97(1): 272-278. https://doi.org/ abs/10.2134/agronj 2005.0272.

Sundararajan, S., B. Sivaraman, V. Rajendran and S. Ramalingam. 2017. Tissue culture and Agrobacterium-mediated genetic transformation studies in four commercially important indica rice cultivars. *J. Crop Sci. Biotech*., 20: 175-83. https://doi.org/10.1007/s12892-017-0045-0

Tahir, M., M.M. Zafar, A. Imran, M.A. Hafeez, M.S. Rasheed, H.S.B. Mustafa, A. Ullah, H.M. Saad and B. Mustafa. 2018. Response of tomato genotypes against salinity stress at germination and seedling stage. *Nature Sci*., 16(4): 10-17. https://doi.org/10.7537/marsnsj160418.03.

Tavakol, E., B. Jákli, I. Cakmak, K. Dittert, P. Karlovsky, K. Pfohl and Senbayram. 2018. Optimized potassium nutrition improves plant-water-relations of barley under PEG-induced osmotic stress. *Plant Soil*, 430: 23-35. https://doi.org /10.1007/s11104-018-3704-8.

Verslues, P.E., M. Agarwal, S. Katiyar-Agarwal, J. Zhu and J.K. Zhu. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant J*., 45(4): 523-539. https://doi.org /10.1111/j.1365-313X.2005.02593.x

Weisany, W., Y. Sohrabi, G. Heidari, A. Siosemardeh, K. Ghassemi-Golezani. 2012. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics*., 5(2): 60-67. https://doi.org/10.3316/informit.182984019960534.

White, P.J. and M.R. Broadley. 2003. Calcium in plants. *Ann. Bot*., 92(4): 487-511. https://doi.org/10.1093/aob/mcg164