Rice growth improvement and grains bio-fortification through lime and zinc application in zinc deficit tropical acid sulphate soils

Shahram Mahmoud Soltani\textsuperscript{a}, Mohamed Musa Hanafi\textsuperscript{b,c}, Abdol Wahid Samsuri\textsuperscript{c}, Sharifah Kharidah Seyed Muhammed\textsuperscript{d} and Mohammad Abdol Hakim\textsuperscript{b}

\textsuperscript{a}Laboratory of Food Crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Malaysia; \textsuperscript{b}Laboratory of Plantation Crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Malaysia; \textsuperscript{c}Faculty of Agriculture, Department of Land Management, Universiti Putra Malaysia, Serdang, Malaysia; \textsuperscript{d}Faculty of Food Science and Technology, Department of Food Science, Universiti Putra Malaysia, Serdang, Malaysia

\textbf{ABSTRACT}

A two years field study was conducted to explain the effect of Zn and lime application on morphological characteristics, rice yield and yield components, and more broadly, grains bio-fortification (Zn and protein content (CP), and amino acid profiles). The lime and Zn interaction increased grains and straw yield more than two times (6.64 ton ha\textsuperscript{-1}) compared to the control (3.20 ton ha\textsuperscript{-1}). The maximum increase in the Zn content of grain, white rice and bran was obtained about 30\% in whole grain, 42\% in bran and 56\% in white rice. Furthermore, CP increased by about 8\% in bran, 12.3\% in whole grain, and 27\% in white rice compared to control. Also, the Zn and lime application and their interaction were significantly increased the amino acids, especially essential parts.

\section*{Introduction}

Rice is the predominant staple food for a large section of the world population, especially in Asia and Africa, where 90\% of the rice is produced and consumed.\cite{1,47} Over the last five decades, rice yields have experienced two jumps, with rice production tripling as a result of genetic and agronomic improvements. However, the rapid growth in world population and economic developments have created a tremendous pressure for higher rice production.\cite{2,3} In order to further increase rice production to meet growing demand, two options are available, namely expanding the rice growing area and enhancing the rice yields per unit area.

In Asian countries, acid sulphate soils have been targeted for expanding rice production, with huge acreages of this soil existing under tropical climate conditions, exclusively in coastal plains.\cite{4,26} Generally, the acid sulphate soils in tropical regions are characterised by low pH (<4), high Al\textsuperscript{3+} and Fe\textsuperscript{3+} and low available Zn concentration that can adversely affect rice growth.\cite{4,5} Moreover, the continuous weathering and leaching processes,\cite{6} and the low total Zn content of lowland tropical paddy soils accelerate Zn deficiency in most of the rice fields.\cite{7–9}

Liming is a common practice to improve acid sulphate soil fertility and remediate toxicity problems for economic rice production. By increasing the soil pH, by precipitating toxic acidic cations, thereby reducing their phyto-availability, and by restoring adequate calcium and magnesium cation concentrations, lime application can improve rice growth.\cite{10,11} However, the uptake, translocation, metabolism, and plant use of essential micronutrients, such as Zn, may be inhibited by lime application, through increases in soil pH and the surface adsorption of Zn with crystalline CaCO\textsubscript{3}.\cite{27}

Extensive field research has addressed Zn application for better rice growth and development,\cite{12–14} as well as the liming effect on the improvement of the chemical conditions of tropical soils,\cite{5,10,11} separately. But, the results of the combined application of lime and Zn were less conclusive,\cite{15,16} and have shown complicated in field conditions. Therefore, the current study will explore the best Zn and lime requirements for proper rice yield and yield components (singly and in combination), and more broadly, for grain Zn bio-fortification, protein content and amino acid profiles in tropical acid sulphate soils of Malaysia.

\section*{Materials and methods}

\textbf{Soil selection and physico-chemical analysis}

A two years experiment was conducted in the acid sulphate soils research field, Kelantan, one of the major rice
MR219 [23] was transplanted in the experimental plots (5 m × 5 m) as a rice genotype in 30 cm × 30 cm configuration with three seedlings per hill. The two factors experiment was conducted in a split plot design with lime requirement as the main plot and Zn levels as sub-plots with three replications. Two levels of lime (0 and lime requirement to bring the soil pH to about 5.5 (10,000 kg ha⁻¹)) as calcium carbonate, three levels of Zn (0, 5 and 10 kg ha⁻¹) in the form of Zn sulphate were applied before ploughing and thoroughly mixed with the surface layer. Regionally recommended n, P and K fertilizers were applied in all treatments plots, according to the local application timing. nitrogen fertilizer as urea at the rate of 150 kg ha−¹ (50 basal, 50 top dressing at the start of tillering and 50 top dressing at the start of the flowering stage) was applied to each plot. Potassium as muriate of potash and Phosphorus as KH₂PO₄ at the rate of 70 kg ha−¹ were applied to each pot as a basal fertilizer to maintain a constant level of K and P. All the conventional managerial practices such as watering, fertilizer split application, weeding and pest control were conducted on time and when necessary. The soil and plant sampling and data recording started at the vegetative stage and continued with approximately 30 day intervals at the flowering and harvesting. According to rice growth, the whole plant was taken out intact and washed carefully with tap water followed by 0.01 n HCl and rinsed with double distiled water twice. Separation of leaves, stems, panicles, roots and grains then took place. The aerial parts and the roots were dried in the air and then in an oven at 65 °C, except for the grains that were dried at 45 °C. Once their dry matter weights were recorded, they were powdered and stored for chemical analysis. The collected morphological and physiological characters were: yield and yield components, including: plant height (PH), tiller number (TN), panicle length (PL), grains per panicle (GPP), total grain yield (ton ha⁻¹), stem and leaf dry weight, and rice quality factors (whole grain, bran and white rice Zn content, protein content and protein profiles).

Statistical analysis

The SAS programme was used for the analysis of variance (ANOVA) and the mean comparison through LSD (0.05) of all data. The correlation coefficient analysis was used to determine the relationship between variables.
Results and discussion

Physico-chemical soil properties

The two years average of physico-chemical properties of the acid sulphate soil used for the current experiment summarised in Table 1. The available Zn content of the soil (0.56 mg kg\(^{-1}\)) was less than the critical limit in paddy soils (2 mg kg\(^{-1}\)).\[24\] Furthermore, the soil suffered from a low pH (3.96), a lack of basic cations and acidic cations toxicity due to the high leaching in Ultisols under tropical conditions. The soil EC value did not reveal any salinity problem. Soil Zn deficiency disorder would restrict the soil Zn content and capacity to supply proper Zn amounts for normal growth of rice in agricultural soils.\[25\] Nearly 72% of the Malaysian soil series are classified as Oxisols and Ultisols,\[26\] which are extremely leached, and show a low pH (4–5), a high AL activity and low basic cations (Ca and/or Mg) capacity. These soil characters could inversely affect the rice production.\[27\]

Table 2. Analysis of variances of morphological characteristics of rice MR219 at maximum tillering and flowering stages.

| Sources            | Maximum tillering stage | Flowering stage |
|--------------------|--------------------------|-----------------|
|                    | TN | PH  | DW   | TN  | PH  | DW   |
| Lime               | 531.55**  | 0.053** | 2429057.02** | 651.1**  | 648.00** | 958245.80** |
| Zinc               | 4.955*  | 22.46**  | 383024.28* | 14.85**  | 15.72**  | 670422.90** |
| Lime × Zinc        | 5.055**  | 5.61**  | 44380.03* | 19.32**  | 21.16**  | 1681305.58** |

Notes: **, * = significant at 0.01, 0.05 and no significant; SDW = straw dry weight, PH = plant height, TN = tiller number.

Morphological parameters

At the maximum tillering stage, the TN and the SDW were significantly affected by lime (\(p \leq 0.01\)), the SDW by Zn and their interactions (\(p \leq 0.05\)), whereas the PH did not show significant effect with any of treatments separately. The interactions of lime and Zn just affected the SDW (Table 2). Although, at the flowering stage, the PH and SDW were significantly affected by all Zn and lime levels and their interactions (\(p \leq 0.01\)), the TN was only influenced by lime at the 1% confidence level (Table 2 and Figure 1). The response pattern of morphological characters at maximum tillering and flowering stages with Zn application and across the lime levels was different. The highest increases in PH, TN and SDW were obtained with 10 kg Zn ha\(^{-1}\) in limed plots, whereas in zero lime plots the maximum increases in TN and SDW were recorded at the 5 kg Zn ha\(^{-1}\) application. The maximum percentage increase in PH, TN and SDW was 63, 7, and 34% at maximum tillering (Table 3 and Figure 2), and 31, 15, and

Figure 1. Effect of lime and zinc on some selected morphological characteristics of rice at different growth stages. Note: Max. till = maximum tillering.
23% at flowering stage in the applied lime and Zn plots, respectively (Table 4 and Figure 1). These data suggest that the applied Zn sulphate in zero lime plots (acidic soil pH) is more mobile than in the limed plots. Further, in zero lime plots only 5 kg Zn ha\(^{-1}\) was equally effective as 10 kg Zn in limed plots. Interestingly, although increasing the Zn levels in limed plots increased the morphological character values, in zero lime plots the rice response trend varied between the two Zn levels applied (5 and 10 kg ha\(^{-1}\)). This would mean that the 5 kg Zn ha\(^{-1}\) increased the Tn, PH and SDW, while with 10 kg Zn ha\(^{-1}\) applied, almost all of them decreased numerically but not statistically (Tables 2 and 3). This might be due to the high solubility of Zn sulphate under high acidic pH conditions that could increase the available Zn concentration to near toxic levels. Therefore, it adversely influenced
rice growth and development. The results were in line with the findings of Khan et al. [31], who reported that the PH (14%) and TN (8%) significantly increased with the application of Zn (9 kg Zn ha$^{-1}$) at slightly acidic to near neutral soil reaction condition [31] and lime application (2–8 t ha$^{-1}$).[11,28,29]

At harvesting stage SDW, 1000GW, SPP and GY were significantly affected and increased by all Zn and lime levels with different interaction response patterns for lime and zero lime plots (p ≤ 0.01) (Tables 5 and 6). The highest morphological factors increased with 5 and 10 kg Zn ha$^{-1}$ in zero lime and limed plots, respectively. The maximum percentage increase of PH (31%) and TN (17.6%) was recorded at 10 kg Zn ha$^{-1}$ in limed plots compared to the control (Table 6 and Figure 1).

The tillering capacity and consequently, the active tiller numbers are the morphological parameters that can most affect the rice production potential. Therefore, their increases indirectly caused an increase in the grain yield.[30,31,42] Higher morphological characters, due to Zn application and better soil conditions by liming, attributed to the enhanced synthesis of carbohydrates and the storage of essential micronutrients with their increases indirectly caused an increase in the grain yield.[30,31,42] The growth and development of rice are also strongly influenced by the availability of Zn. At low Zn levels, the growth and development of rice are reduced due to the deficiency of Zn, which affects the synthesis of chlorophyll and other pigments. This results in poor photosynthesis and reduced yield.[31,42,59] Therefore, the application of Zn is crucial for the production of high-yielding rice varieties.

Despite significantly positive effects of lime and Zn application on rice growth, the morphological characters response pattern to Zn levels differed in limed and zero lime plots. In contrast to the limed plots, the values of the morphological factors increased with the application of 5 kg Zn ha$^{-1}$ and numerically, but not statistically, increased at the 10 kg Zn ha$^{-1}$ level (Figure 1). Although, under anoxic soil conditions (paddy soils), the pH tends to rise to neutral values and the Zn availability decreases sharply. The results suggested that in acid sulphate soils, the pH was already close to neutral before the application of Zn, and the increase in Zn availability was not significant.[31,42]

The results of the study indicated that the application of Zn and lime had a significant effect on the morphological characteristics of rice. The results are in accordance with the findings of Khan et al. [31] and other researchers who reported that Zn and lime application had a positive effect on the growth and development of rice.[31,42,59] The results also suggested that the combined effect of Zn and lime application is more effective than the application of either nutrient alone.[31,42,59] Therefore, the application of Zn and lime is recommended for the production of high-yielding rice varieties in acid sulphate soils.

### Table 5. Analysis of variances of morphological characteristics of rice MR219 at harvesting stage.

| Sources | Mean square | GPP | PL | 1000GW | IMM | SPP | SDW | PH | TN | AT | GY |
|---------|-------------|-----|----|--------|-----|-----|-----|----|----|----|----|
|         |             | cm  | g  | cm     | g   | cm  | g   | cm | g  | cm | g  |
| Lime    | 3463.86**   | 16.07** | 48.74** | 206.45** | 18522.99** | 27925.55.56** | 470.22** | 755.55** | 4.440** | 9.886** |
| Zinc    | 3507.05**   | 2.04*  | 6.34**  | 83.44**  | 3891.27**  | 112435.56**  | 52.16**  | 21.5*  | 46.61**  | 6.14** |
| Lime x zinc | 5650.16** | 0.68** | 7.12** | 11.71** | 6091.89** | 2760622.22** | 0.72** | 22.38* | 2.80* | 5.07** |

**Notes:** GPP = grain per panicle, PL = panicle length, 1000GW = 1000 grain weight, IMM = immature grain, SDW = straw dry weight, PH = plant height, TN = tiller number and GY = grain yield.

### Table 6. Effect of lime and zinc application interactions on morphological characteristics of rice MR219 at harvesting stage.

| TRT | L0 | LS | LSD | L0 | LS | LSD | L0 | LS | LSD | L0 | LS |
|-----|----|----|-----|----|----|-----|----|----|-----|----|----|
| N0  | 105.66Bb* | 115.67Ba  | 4.96 | 21.40Ba  | 22.56Ba  | 1.85 | 34.66ab  | 41.66Ba  | 6.57 | 27.40Ba  | 24.83Bb |
| N5  | 111.00Ab  | 117.67Ba  | 5.74 | 22.10ab  | 23.60aa  | 1.38 | 35.33ab  | 47.00aa  | 3.79 | 30.21aa  | 30.13aa |
| N10 | 108.00Ab  | 122.00Aa  | 4.30 | 21.13ab  | 24.26aa  | 0.28 | 34.33ab  | 49.00aa  | 3.79 | 30.00Bb  | 24.66Ba |
| LSD (5%) | 3.02 | 2.56  | 1.38 | 0.97   | 2.80  | 1.12 |

**Notes:** Captial and small letter = mean comparisons of lime across Zn levels and Zn across lime levels, respectively.
with the findings of Fageria et al. [35], Khan et al. [31], Wijebandara [30] and Rahman et al. [29]. Furthermore, the SDW numerically but not significantly decreased about 10% in zero lime plots by application of 10 kg Zn ha\(^{-1}\) compared to 5 kg Zn ha\(^{-1}\) (Table 7). This result suggests that in acid sulphate soils, the highest amount of added Zn governs the available Zn pool in the soil and may have a toxic effect on rice growth. The results are in accordance with Khan et al. [31], who in a field experiment found that the application of 15 kg ha\(^{-1}\) Zn compared to 10 kg Zn ha\(^{-1}\) numerically decreased the SDW.

The PL was significantly affected by Zn (\(p \leq 0.05\)) and lime (\(p \leq 0.01\)) application, whereas the interactions between lime and Zn did not significantly influence the PL. Although the PL increased with both lime and Zn application, the liming effect was found to be two times greater than the Zn effect (Tables 5 and 6). It suggests that the lime application compared to the Zn application, by reducing the Al availability and increasing the soil pH to more suitable conditions, can enhance rice growth and its morphological characters. Also, the increased PL might be due to higher Zn efficiency in the treatment rather than to higher Zn levels. [36] The maximum percentage increase was observed by adding 10 kg Zn ha\(^{-1}\) in limed plots (7%). A similar result was found by Wijebandara [30], who reported that the PL increased by 15% when Zn levels were increased from 10 to 25 kg ha\(^{-1}\). Further, the addition of lime, which increased the pH of the soils to 5.8, significantly influenced the PL. [29]

### Yield and yield components

The rice grain yield (t ha\(^{-1}\)) and yield components (GPP, and 1000 GW) were significantly affected by Zn lime application, and their interactions (\(p \leq 0.01\)), whereas the IMM only was influenced by lime (\(p \leq 0.01\)) (Table 5). All Zn levels averaged across the lime levels increased the grains yield. However, Zn levels separately showed different effects on grain yield across the lime levels. The highest yield (5.52 t ha\(^{-1}\)) at 5 kg Zn ha\(^{-1}\) was obtained in zero lime plots, whereas, the maximum yield at 10 kg Zn ha\(^{-1}\) was recorded in limed plots (6.64 t ha\(^{-1}\)) and was 2.13 times higher than the control plots (Table 7 and Figure 2). The GPP and 1000 GW responded to Zn similarly as yield. In limed plots the highest values of 247.33 and 21.66 g were obtained by adding 10 kg Zn ha\(^{-1}\), but in zero lime plots, the values of the yield components were recorded at about 158.4 and 18.06 g, respectively at 5 kg Zn ha\(^{-1}\) (Table 7 and Figure 2). The increases in rice grain yield and yield components due to Zn application are attributed to the Zn function in several metallic enzyme activities, regulatory functions and auxin production, thereby enhancing carbohydrates synthesis and their upward movement to

| TRT  | Grain yield | Strain dry weight | Spikelet per panicle | 1000 grain weight | Grain per panicle | 1000 grain weight | Spikelet per panicle |
|------|-------------|-------------------|----------------------|------------------|-----------------|-------------------|----------------------|
| Zn0  | 139.33Bb    | 152.27Ca          | 13.49                | 16.44aa          | 18.13Ba         | 3.54Cb            | 5623.30Bb            |
| Zn5  | 158.40ab    | 188.27Ba          | 7.86                 | 18.06aa          | 20.47aa         | 5.52Ba            | 6616.70Ba            |
| Zn10 | 130.65Cb    | 257.33aa          | 26.88                | 15.89ab          | 21.66aa         | 1.79Cb            | 5156.70C             |

LSD (5%) 3.95 19.76 2.21 1.17 0.20 0.5 449.10 704.64 10.81 15.22

Notes: Capital and small letter = mean comparisons of lime across Zn levels and Zn across lime levels, respectively.

Table 7. Effect of lime and zinc application interactions on straw and grain yield, and selected yield components at harvesting stage.
of 30, 42 and 56% in whole grain, bran and white rice, respectively. However, the CP content was 8, 12.3 and 27% in bran, whole grain, and white rice, respectively (Table 9 and Figure 3). Furthermore, the values of CP following two different measurement methods (conversion of total N and sum of amino acids) showed a variation of 10%. The reason for these increases may be related to the enhancement of rice growth in general and, hence increased Zn uptake. A possible contribution of S could be considered by increasing Zn sulphate in cereal crops. [39] The Zn bio-fortification with four different Zn compounds and three rice varieties indicated that the Zn content of white rice was significantly increased (64%) by application of ZnSO4 [40] and CP (10%) by soil application of Zn in India. [41] Although, all levels of applied Zn (0, 2.5, 5 and 7.5) significantly increased the Zn content of rice grain, the highest content (45 mg kg−1) was observed at 7.5 kg ha−1. Cakmak [12] findings have also indicated that the Zn concentration in brown rice increased by soil application of Zn by about 17% over the control. On average, Zn-amino acid and ZnSO4 increased Zn bioavailability in polished rice up to 68.37 and 64.43%, respectively. Also, protein content increased by 1.88–4.79% depending on cultivar (Table 10). [42]

A correlation analysis showed that the CP was positively and significantly correlated to grain Zn content (0.46**) (Table 11). Many researchers were found out that the grain Zn concentration in cereal crops was significantly correlated with grain protein content. [43–45] The correlation analysis between Zn and crude protein content in different parts of the grain indicated that the grain Zn was positively and significantly correlated to bran Zn (0.93**) and white rice Zn (0.91**) (Table 11). Also, the grain Zn content was significantly correlated with the CP content of bran (0.42*) and white rice (0.514*) (Table 11). The close relationship between whole grain Zn, bran and white rice Zn content would indicate that by increasing grain production and filling sites in the rice plant.[37] Furthermore, lime addition can lead to a more suitable pH for MR 219 rice growth (pH close to 6), to the reduction of Al and Fe toxicity, and to the increase in Ca and Mg supply.[5,27] Therefore, adequate Zn application in combination with liming can provide proper conditions for rice growth and development. Surprisingly, the grain and straw yield significantly decreased in zero lime plots by increasing the applied Zn level from 5 to 10 kg ha−1. This might be due to the soil available Zn which increased about 12.5 times compared to the control and 10 kg Zn ha−1 application. Results from a field experiment showed that by increasing the Zn level to 400 ppm, the grain and straw significantly decreased. [38] Also, Fageria, Santos [35] observed that maximum grain yield (43.23 g pot−1) was obtained at 5 kg ha−1 Zn in acidic soil, but the grain yield decreased with more than 10 kg Zn ha−1. These results are in accordance with Peda Babu et al. [37], Shamshuddin and Kapok [11], Shamshuddin and Anda [27] and Shamshuddin et al. [5], who they reported that by application of lime and Zn, the yield and yield components increased significantly similarly to soils without any problems of the low pH and toxicities of Al and Fe.

**Rice bio-fortification parameters**

The Zn biofortification and quality parameters including crude protein (CP), amino acid and Zn in whole grain, bran and white rice showed were positively significant affected and increased with the application of lime, Zn and their interactions (p ≤ 0.01). Whereas the significant effect of their interactions on bran was at 5% confidence level (Table 8). All parameters significantly increased by increasing 10 kg Zn ha−1 compared to 5 kg Zn ha−1. The maximum increase in Zn content was obtained with 10 kg Zn ha−1 compared to the control, whit increases of 30, 42 and 56% in whole grain, bran and white rice, respectively. However, the CP content was 8, 12.3 and 27% in bran, whole grain, and white rice, respectively (Table 9 and Figure 3). Furthermore, the values of CP following two different measurement methods (conversion of total N and sum of amino acids) showed a variation of 10%. The reason for these increases may be related to the enhancement of rice growth in general and, hence increased Zn uptake. A possible contribution of S could be considered by increasing Zn sulphate in cereal crops.

### Table 8. Analysis of variances of rice grain zinc content.

| Sources          | Mean square | Crude protein content (%) |
|------------------|-------------|---------------------------|
|                  | Whole grain | White rice | Bran | Whole grain | Bran | White rice |
| Lime             | 432.18**    | 468.18** | 4.87** | 16.45** | 3.058** | 4.87** |
| Zinc             | 1006.30**   | 1088.97** | 5.92** | 1.506** | 3.390** | 5.92** |
| Lime × zinc      | 35.84**     | 59.30** | 0.71*  | 0.142** | 0.429*  | 0.71*  |

Notes: **, * = significant at 0.01 and 0.05, respectively.

### Table 9. Effect of lime and zinc application interactions on crude protein content of rice MR219 grain.

| Treatments | Whole grain | Bran | White rice |
|------------|-------------|------|------------|
| Zn0        | 8.19Cb      | 8.81Ba | 0.25      |
| Zn5        | 8.63Ba      | 9.56Ba | 1.11      |
| Zn10       | 9.11Ab      | 10.66Ab | 0.71      |
| LSD (5%)   | 0.12        | 0.8   | 0.13      |

Notes: Capital and small letter = mean comparison of lime across Zn levels and Zn across lime levels, respectively; LSD = 10 ton ha−1 lime application.
Amino acid profiles

The ANOVA showed significant effects of Zn and lime, and their interaction on almost all amino acids, except for asparagine, cysteine and histamine (Tables 12 and 13 and Figure 3). The results of the amino acid profile analyses indicated that 38% of the total CP was essential amino acid (EAAs) (threonine, cysteine, tyrosine, valine, Zn content of the outer layer, Zn penetrated to the inner layers of rice grain.\cite{39} These findings are in accordance with the findings of Zhao and Selim \cite{43}, Cakmak et al. \cite{44}, Gomez-Becerra et al. \cite{45} and Phattarakul et al. \cite{39}, which reported that the distribution patterns of protein bodies in the different fractions of the grain, such as embryo, endosperm and bran were closely related to their N, Fe and Zn content.\cite{46}
lysine, isoleucine, leucine and phenylalanine) and 62% were non-essential amino acids (NEAAs) (asparagine, serine, glutamine, glycine, histamine, arginine, alanine, proline, cysteine, lysine, isoleucine, leucine and phenylalanine, respectively).

The results also showed that there was considerable variation in the concentration of measured amino acids. The highest concentration was found for glutamine with 18.55%, whereas the lowest concentration (<0.001%) was for methionine. Almost all amino acids increased by Zn and lime application, except for methionine and histidine. The mechanism by which Zn affects the protein synthesis is by increasing in RNA. In the rice, the level of RNA was dramatically increased by increasing Zn. Zinc is necessary for the activity of the enzyme in RNA. As a consequence of this, the earliest causal effect of Zn deficiency is a sharp decrease in the level of RNA. The importance of Zn in protein synthesis suggested that relatively high Zn concentrations are required by meristematic tissue where cell division as well as the synthesis of nucleic acids and protein is actively taking place. Therefore, by increasing the soluble Zn concentration, the amino acid or protein metabolism is enhanced significantly.[47] A field experiment with four Zn levels (Zn0 = 0, Zn1 = 5, Zn2 = 10, and Zn3 = 15 kg ha\(^{-1}\)) showed that applied Zn increased the protein concentration of rice grain at all levels of Zn over the control, but the highest protein content in rice grain was recorded at the 15 kg Zn ha\(^{-1}\) level.[48] Also, zinc application enhanced the Zn concentration in the plant about 5–19% over control which was associated with RNA and ribosome induction, the result of which accelerated protein synthesis.[49,50]

**Conclusion**

The current study showed that in spite of lime induced Zn deficiency in paddy soils, their application not only promoted the rice growth but also increased the Zn and amino acids accumulation in grain. The result obtained in the present field experiments indicated that rice variety MR219 grew productively due to lime and Zn application, even if the low soil pH (<5), Al and Fe toxicity and Zn deficiency. Despite low effect of lime and Zn application on rice morphological characters in maximum tillering, they positive significantly increased in flowering and harvesting stages. Although the treatments application separately increased the yield, yield component, but the highest grain yield, about 6.5 ton ha\(^{-1}\) was obtained at the highest level of applied treatments (limed plus 10 kg Zn ha\(^{-1}\)). Furthermore, rice quality; grain Zn, amino acid contents also enhanced by the lime and Zn application.

**Acknowledgement**

The authors would like to acknowledge the Universiti Putra Malaysia and also acknowledge to Long Term Research Grant Scheme (LRGS) in Food Security – Enhance Sustainable Rice Production under the Ministry of High Education, Malaysia for Technical and financial support of this project. Also, the authors would like to appreciate the editor of this paper, Miss Shiva Dialami.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was financially supported by the Ministry of Higher Education, Malaysia.

**ORCID**

Shahram Mahmoud Soltani http://orcid.org/0000-0002-1300-9536
Mohamed Musa Hanafi http://orcid.org/0000-0003-1898-6819

---

**Table 13. Effect of lime and zinc application interactions on amino acid profiles of rice MR219 grain.**

| Treatments | Asp | Ser | Glu | Gly | His | Arg | Thr | Ala | Pro | Cys | Lys | Ile | Leu | Phe | Total |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| No limed  |
| Zn0       | 0.62a | 0.35C | 1.16C | 0.30B | 0.17B | 0.60B | 0.26C | 0.39C | 0.48B | 0.08a | 0.25B | 0.27C | 0.56B | 0.38b | 6.3C |
| Zn5       | 0.62a | 0.41b | 1.30B | 0.34AB | 0.19A | 0.70AB | 0.29B | 0.43B | 0.38A | 0.07A | 0.28AB | 0.30B | 0.64A | 0.43AB | 7.15 |
| Zn10      | 0.67a | 0.46A | 1.43A | 0.37A | 0.20A | 0.77A | 0.32A | 0.48A | 0.42A | 0.06A | 0.31A | 0.33A | 0.70A | 0.47A | 7.85A |
| LSD       | 0.15  | 0.03  | 0.11  | 0.04  | 0.02  | 0.10  | 0.02  | 0.04  | 0.04  | 0.02  | 0.03  | 0.02  | 0.06  | 0.05  | 0.64  |
| Limed     |
| Zn0       | 0.71a | 0.46B | 1.42B | 0.34B | 0.20B | 0.71B | 0.31A | 0.47B | 0.40A | 0.06A | 0.31A | 0.32A | 0.67B | 0.46A | 7.70A |
| Zn5       | 0.73a | 0.49AB | 1.46B | 0.36B | 0.34B | 0.74B | 0.35A | 0.49B | 0.45A | 0.16A | 0.36A | 0.38A | 0.72B | 0.53A | 8.67A |
| Zn10      | 0.78a | 0.56A | 1.72A | 0.45A | 0.34A | 0.90A | 0.37A | 0.57A | 0.50A | 0.10A | 0.37A | 0.38A | 0.82A | 0.57A | 9.30A |
| LSD       | 0.16  | 0.08  | 0.08  | 0.07  | 0.06  | 0.12  | 0.09  | 0.09  | 0.09  | 0.12  | 0.11  | 0.10  | 0.14  | 1.7   |
| Zn0 No limed | 0.62a | 0.35b | 1.16B | 0.30B | 0.17B | 0.60B | 0.26B | 0.39B | 0.34B | 0.06A | 0.29B | 0.27B | 0.56B | 0.38B | 6.3B  |
| Zn5 No limed | 0.62a | 0.41a | 1.30B | 0.34A | 0.19A | 0.70A | 0.29A | 0.43B | 0.38A | 0.07A | 0.28A | 0.30A | 0.64A | 0.43A | 7.15a |
| Zn10 No limed | 0.71a | 0.46a | 1.42a | 0.34a | 0.20a | 0.73a | 0.31a | 0.47a | 0.40a | 0.08a | 0.31a | 0.32a | 0.67a | 0.46a | 7.70a |
| LSD No limed | 0.10  | 0.04  | 0.10  | 0.05  | 0.02  | 0.14  | 0.03  | 0.05  | 0.03  | 0.05  | 0.02  | 0.07  | 0.06  | 0.61  |
| Zn0 Limed | 0.62a | 0.41a | 1.30B | 0.34A | 0.19A | 0.70A | 0.29A | 0.43B | 0.38A | 0.07A | 0.28A | 0.30A | 0.64A | 0.43A | 7.15a |
| Zn5 Limed | 0.73a | 0.49a | 1.46a | 0.36a | 0.34a | 0.74a | 0.35a | 0.49a | 0.45a | 0.16a | 0.36a | 0.38a | 0.72a | 0.53a | 8.67a |
| Zn10 Limed | 0.78a | 0.56a | 1.72a | 0.45a | 0.24a | 0.90a | 0.37a | 0.57a | 0.50a | 0.10a | 0.37a | 0.38a | 0.82a | 0.57a | 9.30a |

Notes: Capital and small letter = mean comparison of lime across Zn levels and Zn across lime levels, respectively; LSD = 10 ton ha\(^{-1}\) lime application; Asp, Ser, Glu, Gly, His, Arg, Thr, Ala, Pro, Cys, Lys, Ile, Leu, and Phe = Asparagine, Serine, Glutamine, Glycine, Histamine, Arginine, Threonine, Alanine, Proline, Cysteine, Lysine, Isoleucine, Leucine and Phenylalanine, respectively.
References

[1] FAO. Rice market monitor. Vol. XVI, Trade and Markets Division. Rome: FAO; 2013.

[2] Khoshgoftarmanesh AH, Schulin R, Chaney RL, et al. Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agron. Sustain. Dev. 2009;30:83–107.

[3] Tonini A, Cabrera E. Opportunities for global rice research in a changing world. Metro Manila, Philippines: International Rice Research Institute; 2013.

[4] Enio MSK, Shamshuddin J, Fauziah CI, et al. Pyritization of the coastal sediments in the Kelantan Plains in the Malay Peninsula during the Holocene. Am. J. Agric. Biol. Sci. 2011;6:393–402.

[5] Shamshuddin J, Elisa AA, Shazana MARS, et al. Rice defense mechanisms against the presence of excess amount of Al3+ and Fe2+ in the water. Aust J. Crop Sci. 2013;7:314–320.

[6] Somani LL. Micronutrients for soil and plant health. Udaipur, Rajasthan: Agrotech Publishing Academy; 2008.

[7] Hafeez B, Khanif YM, Samsuri AW, et al. Zinc status of Kelantan state. Soil health: preserving resources for sustainable agriculture; Kuala Lampour: Malaysian Society of Soil Science; 2009. p. 290–293.

[8] Khairiah J, Ding-Woei Y, Habibah J, et al. Concentration of heavy metals in Guava plant parts and soil in the Sungai Wangi plantation, Perak, Malaysia. Int. J. Agric. Res. 2009;4:310–316.

[9] Habibah J, Lee PT, Khairiah J, et al. Speciation of heavy metals in paddy soils from selected areas in Kedah and Penang, Malaysia. Afr. J. Biotechnol. 2011;10:13505–13513.

[10] Anda M, Shamshuddin J, Fauziah CI, et al. Dissolution of ground basalt and its effect on Oxisol chemical properties and cocoa growth. Soil Sci. Soc. Am. J. 2009;174:264–271.

[11] Shamshuddin J, Kapok JR. Effect of ground basalt application on the chemical properties of an Ultisol and Oxisols in Malaysia. Pertanika J. Trop. Agric. Sci. 2010;33:7–14.

[12] Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 2008;302:1–17.

[13] Fageria NK. The use of nutrients in crop plants. Boca Raton: CRC Press; 2010.

[14] Kabeya MJ, Shankar A. Effect of different levels of zinc on growth and uptake ability in rice zinc contrast lines (Oryza sativa L.). Asian J. Plant Sci. Res. 2013;3:112–116.

[15] Fageria NK, Stone LF. Micronutrient deficiency problems in South America. In: Alloway BJ, editor. Micronutrient deficiencies in global crop production. New York (NY): Springer; 2008. p. 247–268.

[16] Impa SM, Johnson-Beebout SE. Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. Plant Soil 2012;361:3–41.

[17] Gee GW, Bauder JW. Particle size analysis. In: Klute AE, editor. Methods of soil analysis: part 1: physical and mineralogical methods. 2nd ed. Madison (WI): American Society of Agronomy and Soil Science Society of America; 1986. p. 383–411.

[18] Bower CA, Reitemeier RF, Fireman M. Exchangeable cation analysis of saline and alkali soils. Soil Sci. 1952;73:251–262.

[19] Jackson ML. Análisis Químico de Suelos [Soil chemical analysis]. Barcelona; Omega Editorial; 1964.

[20] Amacher MC. Nickel, cadmium and lead. In: Sparks DL, editor. Methods of soil analysis. Part 3: chemical methods. Madison (WI): SSSA; 1996. p. 212–216.

[21] Rozan P, Kuo YH, Lambein F. Free amino acids present in commercially available seedlings sold for human consumption: a potential hazard for consumers. J. Agric. Food Chem. 2000;48:716–723.

[22] Ohtsubo KI, Suzuki K, Yasui Y, et al. Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. J. Food Compos. Anal. 2005;18:303–316.

[23] Hafeezullah B. Evaluation of Malaysian rice genotype foe adaptability in zinc deficit soil [PhD thesis]. Serdang, Selangor: Universiti Putra Malaysia; 2010.

[24] Dobermann A, Fairhurst TH. Nutrient disorders and nutrient management. Singapore: Potash and Phosphate Institute of Canada and International Rice Research Institute; 2000.

[25] White JG, Zasoski RJ. Mapping soil micronutrients. Field Crops Res. 1999;60:11–26.

[26] Paramananthan S. Soils of Malaysia: their characteristics and identification. Kuala Lumpur: Academy of Sciences Malaysia; 2000.

[27] Shamshuddin J, Anda M. Enhancing the productivity of Ultisols and Oxisols in Malaysia using basalt and/or compost. Pedologist 2012;55:382–391.

[28] Rosmani H, Sarwani M. Responce of some rice cultivars to lime application on acid sulphate soils. Int. Rice Res. News. 1991;16:6–13.

[29] Rahman M, Meisner C, Duxbury J, et al., editors. Yield response and change in soil nutrient availability by application of lime, fertilizer and micronutrients in an acidic soil in a rice-wheat cropping system. 17th World Congress on Soil Science (WCSS); 2002; Citeseer, Bangkok, Thailand.

[30] Wijebandara DMDI. Studies on distribution and transformation of soil zinc and response of rice to nutrients in traditional and system of rice intensification (Sri) methods of cultivation. Dharwad: Dharwad University of agricultural sciences; 2007.

[31] Khan P, Memon MY, Imtiaz M. Determining the zinc requirements of rice genotype Sarshar evolved at nia, tandojam. Sarhad J. Agric. 2012;28:1–7.

[32] Shamshuddin J, Ismail H. Reactions of ground magnesium limestone and gypsum in soils with variable-charge minerals. Soil Sci. Soc. Am. J. 1995;59:106–112.

[33] Elisa AA, Shamshuddin J, Fauziah CI. Root elongation, root surface area and organic acid exudation by rice seedling under Al3+ and/or H+ stress. Am. J. Agric. Biol. Sci. 2011;6:324–331.

[34] Muthukumararaja TM, Siramachandrasekharan MV. Effect of zinc on yield, zinc nutrition and zinc use efficiency of lowland rice. J. Agric. Technol. 2012;8:551–561.

[35] Fageria NK, Santos AB, Cobucci T. Zinc nutrition of lowland rice. Commun. Soil Sci. Plant Anal. 2011;42:1719–1727.

[36] Cheema NM, Ullah N, Khan NU. Effect of Zn on the panicle structure and yield of coarse rice, IR-6. Pak. J. Agric. Res. 2006;19:33–37.
[37] Peda Babu P, Shanti M, Rajendra Prasad B, et al. Effect of zinc on rice in rice black gram cropping system in saline soils. Andhra Agric. J. 2007;54:47–50.

[38] Malik N, Chamon A, Mondol M, et al. Effects of different levels of zinc on growth and yield of red amaranth (Amaranthus sp.) and rice (Oryza sativa, Variety-BR49). J. Bangladesh Assoc. Young Res. 2011;1:79–91.

[39] Phattarakul N, Rerkasem B, Li L, et al. Biofortification of rice grain with zinc through zinc fertilization in different countries. Plant Soil 2012;361:131–141.

[40] Wei Y, Shohag M, Yang X. Biofortification and bioavailability of rice grain zinc as affected by different forms of foliar zinc fertilization. PLoS ONE 2012;7:e45428.

[41] Yadav B, Khamparia RS, Kumar R. Effect of zinc and organic matter application on various zinc fractions under direct-seeded rice in vertisols. J. Indian Soc. Soil Sci. 2013;61:128–134.

[42] Wei Y, Shohag M, Yang X. Biofortification and bioavailability of rice grain zinc as affected by different forms of foliar zinc fertilization. PLoS one 2012;9:e45428.

[43] Zhao K, Selim H. Adsorption–desorption kinetics of Zn in soils. Soil Sci. 2010;175:145–153.

[44] Cakmak I, Pfeiffer WH, McClafferty B. Review: biofortification of durum wheat with zinc and iron. Cereal Chem. 2010;87:10–20.

[45] Gomez-Becerra HF, Abugalieva A, Morgounov A, et al. Phenotypic correlations, G×E interactions and broad sense heritability analysis of grain and flour quality characteristics in high latitude spring bread wheats from Kazakhstan and Siberia. Euphytica 2010;171:23–38.

[46] Promthai C, Huang L, Rerkasem B, et al. Distribution of protein bodies and phytate-rich inclusions in grain tissues of low and high iron rice genotypes. Cereal Chem. J. 2008;85:257–265.

[47] Alloway BJ. Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Berlin: Springer; 2012.

[48] Sarkar JR, Paul AK, Paul SS. Effect of N and Zn fertilization on the uptake pattern and protein content in T-aman rice. Int. J. Bioresour. Stress Manag. 2010;1:133–136.

[49] Keram K, Sharma B, Kewat M, et al. Effect of zinc fertilization on growth, yield and quality of wheat grown under agro-climatic condition of Kymore Plateau of Madhya Pradesh, India. The Bioscan. 2014;9(4):1479–1483.

[50] Sudha S, Stain P. Effect of zinc on yield, quality and grain zinc content of rice genotypes. Int. J. Farm Sci. 2015;5:17–27.