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

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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^{-1}) compared to the control (3.20 ton ha^{-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.

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Zinc; acid sulphate soils; rice; lime; amino acids; biofortification

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.[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.[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.[4,26] Generally, the acid sulphate soils in tropical regions are characterised by low pH (<4), high Al^{3+} and Fe^{2+} and low available Zn concentration that can adversely affect rice growth.[4,5] Moreover, the continuous weathering and leaching processes,[6] and the low total Zn content of lowland tropical paddy soils accelerate Zn deficiency in most of the rice fields.[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.[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 $CaCO_3$.[27]

Extensive field research has addressed Zn application for better rice growth and development,[12–14] as well as the liming effect on the improvement of the chemical conditions of tropical soils,[5,10,11] separately. But, the results of the combined application of lime and Zn were less conclusive,[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.

Materials and methods

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

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Table 1. Physico-chemical properties of selected acid sulphate soil.

	EC × 10^{-6}	pH	OC	CEC	Ca	Mg	K	Ava. Zn
Oil series	ds m^{-1}		%			cmol$_c$ kg^{-1}		mg kg^{-1} soil
Tupus	245	3.96	2.48	7.15	0.66	0.03	0.001	0.56
	Mn	Fe	Clay	Silt	Sand	Texture	Soil order	Great group
Soil series	meq 100 g^{-1} soil			%				
Tupus	63	19.84	35.6	57.86	6.41	Si. C. L	Ultisols	Kandiaquult

growing states of Malaysia during the rice growing seasons of 2013 and 2015. Composite soil samples (three samples per plot) were collected from the untreated topsoil plough layer (0–30 cm), air-dried, ground and sieved through a 2-mm metal sieve. The physico-chemical properties of the soil samples were determined: soil pH, organic carbon (OC), cation exchange capacity (CEC), clay content, available Zn, Fe and Mn. A pipette method was used to determine soil texture.[17] Soil pH and electrical conductivity (EC) were determined in a 1:2.5, soil to water ratio suspension. The CEC was measured by the leaching and replacing method by NH_4 OAc.[18] The OC and available Zn were measured using the Walkley–Black [19] and the double acid method,[20] respectively (Table 1).

Crude protein and amino acids measurement

The quantitative crude protein (CP) content of the MR219 rice variety was determined by two different methods. First, the CP was calculated by multiplying a conversion factor of 6.25 with the total *N* of rice grain. Secondly, it was measured by the sum of amino acids by high performance liquid chromatography (HPLC) method. The total amino acid content and the amino acid profiles were measured using a reversed-phase HPLC method. The 0.02 g air-dried grains were hydrolysed by adding 15 mL 6 N HCl to the ground grain sample and mixing them well in a stoppered test tube for 24 h at 110 °C. Subsequently, 20 µL of the sample was injected into the HPLC system equipped with a HPLC photodiode array detector (model MD-2010; Jasco, Tokyo, Japan). The linear gradient system was used with buffer A (0.1 M ammonium acetate, pH 6.5) and buffer B (0.1 M ammonium acetate containing acetonitrile and methanol, 44:46:10, v/v, pH 6.5) at a flow-rate of 1 mL min^{-1}, by using a C18 reversed phase column (Thermal C18 5U, 250 × 4.6 mm) in an oven at 43 °C. The UV absorption detection at a wavelength of 254 nm was used to measure the total content of amino acids. The results were analysed using the Borwin chromatography software (Version 1.5, Jasco Co. Ltd., Japan).[21,22]

Experimental setup

Due to long-term fallow, weed control was done three times before ploughing and puddling. The common Malaysian and moderately zinc efficient rice variety, 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 KH_2PO_4 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.

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

	Mean square					
	Maximum tillering stage			Flowering stage		
	TN	PH	DW	TN	PH	DW
Sources		cm	g		cm	g
Lime	531.55**	0.053^{NS}	2429057.02**	651**	648.00**	9582455.80**
Zinc	4.955^{NS}	22.46^{NS}	383024.28*	14.85^{NS}	15.72**	670422.90**
Lime × Zinc	5.055^{NS}	5.61^{NS}	44380.03*	19.32^{NS}	21.16**	1681305.58**

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

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]

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

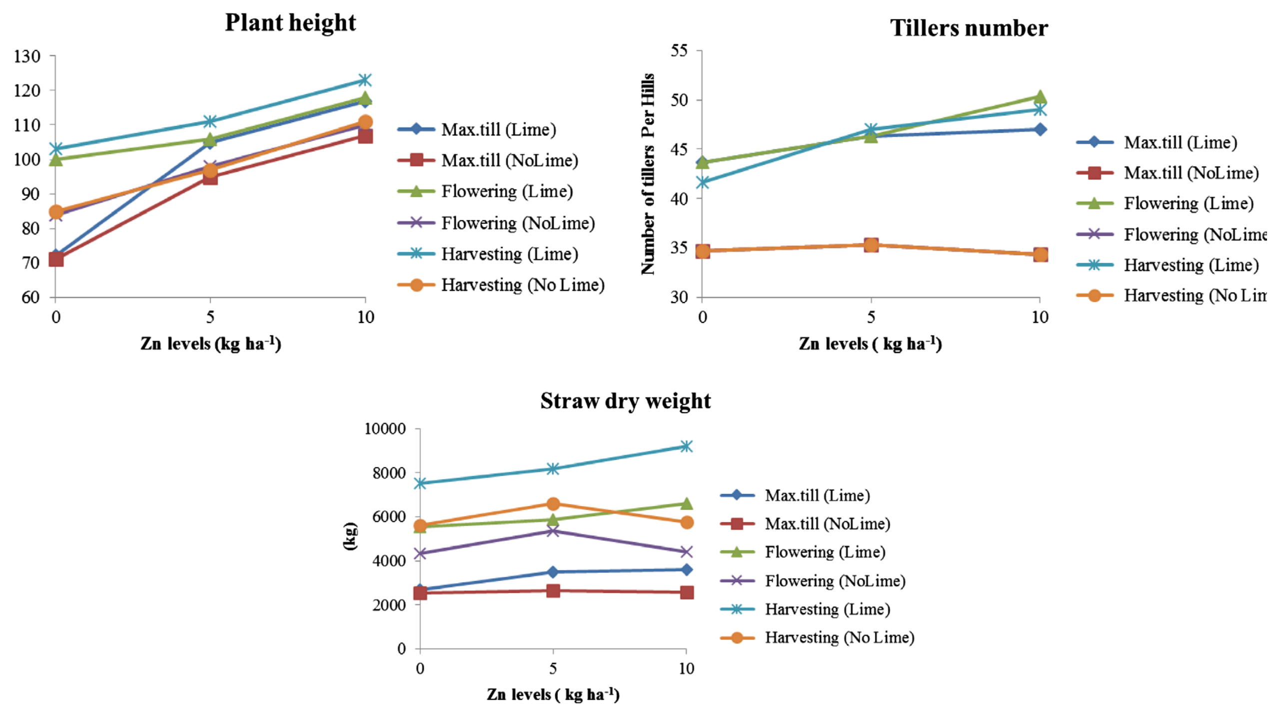

Figure 1. Effect of lime and zinc on some selected morphological characteristics of rice at different growth stages.
Note: Max. till = maximum tillering.

Table 3. Effect of lime and zinc application on morphological characteristics of rice MR219 at maximum tillering and flowering stages.

	Maximum tillering stage								
	PH			TN			DW		
	cm						g		
TRT	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD
Zn0	69.63Ba	69.67Aa	3.79	34.45Aa	42.97Aa	19.40	2.52Aa	2.73Ba	0.13
Zn5	70.21ABa	71.12Aa	5.17	35.42Ab	46.36Aa	4.30	2.71Ab	3.52Aa	0.20
Zn10	75.00Aa	72.53Aa	2.69	34.28Ab	47.10Aa	7.58	2.39Ab	3.61Aa	0.82
LSD (5%)	2.69	3.99		4.40	11.26		0.67	0.34	

Note: Capital and small letter = mean comparisons of lime across Zn levels and Zn across lime levels, respectively; SDW = straw dry weight, PH = plant height, TN = tiller number.

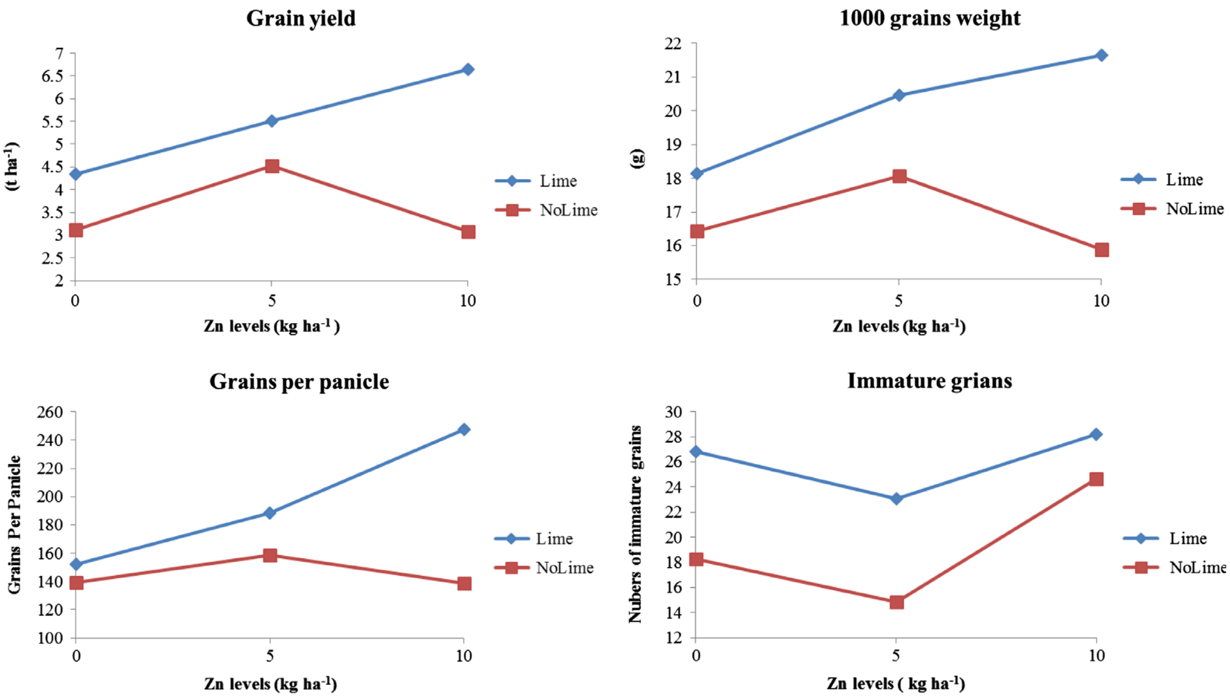

Figure 2. Effect of lime and zinc application on yield and some selected yield components.
Note: Max. till = maximum tillering.

Table 4. Effect of lime and zinc application interactions on morphological characteristics of rice MR219 at maximum tillering and flowering stages.

	Flowering stage								
	PH			TN			DW		
	cm						g		
TRT	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD
Zn0	93.56Ab	102.97Ba	4.96	33.55Aa	42.97Aa	19.40	4.34Bb	5.63Ca	0.023
Zn5	96.12Ab	105.68Ba	5.73	35.33Ab	46.33Aa	4.30	5.34Ab	5.77Ba	0.063
Zn10	93.63Ab	109.30Aa	5.17	34.91Ab	51.33Aa	7.45	3.93Cb	6.58Aa	0.66
LSD (5%)	2.87	2.56		4.4	10.98		0.33	0.08	

Note: Capital and small letter = mean comparisons of lime across Zn levels and Zn across lime levels, respectively; SDW = straw dry weight, PH = plant height, TN = tiller number.

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

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

	Mean square									
	GPP	PL	1000GW	IMM	SPP	SDW	PH	TN	AT	GY
Sources		cm	g			g	cm			Ton ha^{-1}
Lime	4362.86**	16.07**	48.74**	206.45*	18522.99**	27925355.56**	470.22*	555.55**	4.440*	9.886**
Zinc	3507.05**	2.04*	6.34**	83.44^{NS}	3891.27**	1124355.56**	52.16**	21.5*	46.61**	6.14**
Lime × zinc	5650.16**	0.68^{NS}	7.12**	11.71^{NS}	6091.89**	2760622.22**	0.72^{NS}	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.
**, * and NS = significant at 0.01, 0.05 and no significant, respectively.

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

	Plant height			Panicle length			Tiller number			Active tillers		
	cm											
TRT	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD
Zn0	105.66Bb*	115.67Ba	4.96	21.40Ba	22.56Ba	1.85	34.66Ab	41.66Ba	6.57	27.40Ba	24.83Bb	2.72
Zn5	111.00Ab	117.67Ba	5.74	22.10Ab	23.60Aa	1.38	35.33Ab	47.00Aa	3.79	30.21Aa	30.13Aa	0.6
Zn10	108.0ABb	122.00Aa	4.30	21.13Bb	24.26aa	0.28	34.33Ab	49.00Aa	3.79	25.00Ba	24.66Ba	1.88
LSD (5%)	3.02	2.56		1.38	0.97		4.40	5.20		2.41	1.12	

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

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 ($p \le 0.01$) (Tables 5 and 6). The highest morphological values were observed 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 upward transportation to the site of grain production.[37] Field study findings in Entisols of Pakistan indicated that the PH significantly increased by about 8% (108 cm) when 12 kg Zn ha^{-1} was applied compared to the control (100 cm).[31]

The TN and AT were significantly affected lime levels ($p \le 0.01$ and $p \le 0.05$, respectively), Zn application ($p \le 0.05$ and $p \le 0.01$, respectively), and their interaction ($p \le 0.05$) (Table 5). Khan et al. [31] also found that the PH, TN and AT were increased by increasing the Zn levels until 12 kg ha^{-1}. The results are in accordance with the findings of Shamshuddin and Ismail [32]; Rahman et al. [29] Shamshuddin and Anda [27] and Shamshuddin et al. [5]. 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, decreased 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, this process was more time consuming and, therefore, the highest amount of added Zn was more available and might cause the toxic effect on growth.

The response of SDW to applied Zn and lime levels and their interactions was positively significant (Tables 5 and 6 and Figure 1). The maximum percentage increase in SDW was observed with 5 and 10 kg Zn ha^{-1} application in zero lime (30%) and limed plots (34%) compared to the control (Figure 1). According to Elisa et al. [33], the most suitable soil pH for the MR219 rice variety growing in paddy fields is about 6. The pH of acid sulphate soil is almost always less than 4. The rice plant is not only subjected to Al^{3+} toxicity and H^+ stress but also to low available Zn conditions. The combined effect can cause stunted rice growth and eventually reduce the straw and grain yields. Lime and Zn application can overcome these problems through an increase of the soil pH, a reduction of the Al concentration and a higher Zn content of the soil. It means that the soil conditions for rice would reach suitable levels and the yield increased to be close to yields in soils with good conditions.[5] Similar results were observed by Muthukumararaja and Sriramachandrasekharan [34]. They found that the highest SDW (44–60% more than the control) was achieved by adding 5 mg Zn kg^{-1} compared to the control. The results are in accordance 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 he 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

Table 7. Effect of lime and zinc application interactions on straw and grain yield, and selected yield components at harvesting stage.

	Grain per panicle			1000 grain weight			Grain yield			Straw dry weight			Spikelet per panicle		
				g			tonha^{-1}			kgha^{-1}					
TRT	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD
Zn0	139.33Bb	152.27Ca	13.49	16.44Aa	18.13Ba	1.85	3.11Ba	3.54Cb	0.28	5623.30Bb	7496.70Ba	487	155.93Ba	176.10Ca	25.87
Zn5	158.40Ab	188.27Ba	7.86	18.06Aa	20.47Aa	3.19	5.11Aa	5.52Ba	0.70	6616.7Ab	8170.00Ba	883.65	175.60Ab	210.67Ba	7.24
Zn10	130.65Cb	257.33Aa	26.88	15.89Ab	21.66Aa	1.79	2.85Cb	6.43Aa	0.80	5156.7C	9203.30A	1212	148.29Bb	285.53Aa	21.19
LSD (5%)	3.95	19.76		2.21	1.17		0.20	0.5		449.10	704.64		10.81	15.22	

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

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

Sources	Mean square					
	Zn content (mgkg^{-1})			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	Crude protein (%)								
	Whole grain			Bran			White rice		
	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD
Zn0	8.19Cb	8.81Ba	0.25	12.31Cb	14.07Ba	0.44	7.16Bc	7.50Ac	0.11
Zn5	8.63Ba	9.56Ba	1.11	12.80Bb	14.52Ba	0.25	7.59Bb	8.33Ab	0.22
Zn10	9.11Ab	10.6Aa	0.71	13.00Ab	15.32Aa	0.43	8.13Ba	9.53Aa	0.34
LSD (5%)	0.12	0.8		0.13	0.20		0.13	0.23	

Notes: Capital and small letter = mean comparison of lime across Zn levels and Zn across lime levels, respectively; L5.5 = 10 ton ha^{-1} lime application.

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 effected and increased with the application of lime, Zn and their interactions ($p \leq 0.01$). 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 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 considers 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 $ZnSO_4$ [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' has 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 $ZnSO_4$ 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

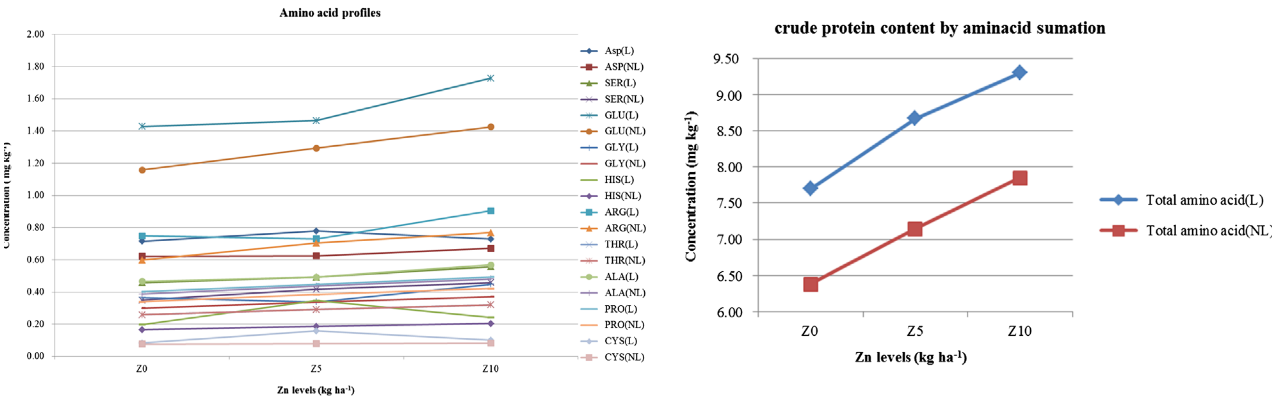

Figure 3. Effect of lime, zinc and their interactions on crude protein and amino acid profiles of rice grains.
Note: L and NL = lime and no lime.

Table 10. Effect of lime and zinc application interactions on zinc content of rice MR219 grain.

	Zn content ($mgkg^{-1}$)								
	Whole grain			Bran			White rice		
TRT	L0	L5.5	LSD	L0	L5.5	LSD	L0	L5.5	LSD
Zn0	54.06Cb	71.20Ca	4.97	71.20Ca	76.73Ca	6.6	26.73Ca	27.86Ca	12.42
Zn5	70.00Bb	78.60Ba	3.94	78.60Bb	87.33Ba	5.54	28.48Ba	34.46Ba	8.89
Zn10	87.06Ab	91.93Aa	2.29	91.93Ab	107.06Aa	0.28	34.2Ab	42.13Aaa	3.73
LSD (5%)	5.76	5.93		3.9	1.69		6.7	4.41	

Notes: Capital and small letter = mean comparison of lime across Zn levels and Zn across lime levels, respectively; L5.5 = 10 ton ha^{-1} lime application.

Table 11. Correlation of zinc and crude protein content of rice MR 219 grain.

	Grain Zn	Bran Zn	White rice Zn	Crude Protein Grain	Crude Protein Bran	Crude Protein White Rice
Grain Zn	1	0.94**	0.90**	0.66**	0.42*	0.51*
Bran Zn		1	0.92**	0.31^{NS}	0.74**	0.41^{NS}
White rice Zn			1	0.24^{NS}	0.113^{NS}	0.41*
Crude Protein Grain				1	0. 875**	0.97**
Crude Protein Bran					1	0.78**
Crude Protein White Rice						1

Notes: **, * and NS = significant at 0.01, 0.05 and no significant, respectively.

Table 12. Analysis of variances of amino acid profiles in rice MR219 grain.

	Amino acid profiles								
Zn levels	Asp	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro
Zn	0.002^{NS}	0.01**	0.13**	0.011**	0.01^{NS}	0.04**	0.1**	0.01**	0.01**
Lime	0.04*	0.04**	0.26**	0.00**	0.02^{NS}	0.04^{NS}	0.00**	0.02**	0.02**
Zn × lime	0.003^{NS}	0.000^{NS}	0.007^{NS}	0.003^{NS}	0.007^{NS}	0.006^{NS}	0.000^{NS}	0.000^{NS}	0.000^{NS}

	Amino acid profiles								
Zn levels	Cys	Tyr	Val	Met	Lys	Ile	Leu	Phe	Total
Zn	0.002^{NS}	0.007*	0.01**	0.01^{NS}	0.005^{NS}	0.006**	0.03**	0.01*	3.5**
Lime	0.005^{NS}	0.02^{NS}	0.02**	0.009^{NS}	0.02*	0.01*	0.05**	0.03**	9.2**
Zn × lime	0.002^{NS}	0.001^{NS}	0.000^{NS}	0.01*	0.000^{NS}	0.000^{NS}	0.00^{NS}	0.000^{NS}	0.01**

Notes: 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.
**, * and NS = significant at 0.01, 0.05 and no significant, respectively.

Zn content of the outer layer, Zn penetrated to the inner layers of rice grain.[39] These findings are in accordance with the findings of Zhao and Selim [43], Cakmak et al. [44], Gomez-Becerra et al. [45] and Phattarakul et al. [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.[46]

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,

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

Treatments		Amino acid profiles														
		Asp	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Cys	Lys	Ile	Leu	Phe	Total
No limed	Zn0	0.62A	0.35C	1.16Cb	0.30B	0.17B	0.60B	0.26C	0.39C	0.34B	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	0.08A	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	0.20A	0.73B	0.31A	0.47B	0.40A	0.08A	0.31A	0.32A	0.67B	0.46A	7.70A
	Zn5	0.73A	0.49AB	1.46B	0.36B	0.34A	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	0.07	0.08	0.32	0.12	0.09	0.06	0.09	0.12	0.11	0.10	0.08	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	0.08a	0.25b	0.27b	0.56b	0.38b	6.3b
	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	0.10	0.04	0.10	0.05	0.02	0.14	0.3	0.05	0.05	0.3	0.05	0.02	0.07	0.06	0.61
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
	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
	LSD	0.16	0.11	0.06	0.16	0.58	0.14	0.15	0.05	0.13	0.21	0.2	0.16	0.09	0.22	2.52
Zn10	No limed	0.67a	0.46b	1.43b	0.37b	0.20b	0.77b	0.32b	0.48b	0.42b	0.08b	0.31b	0.33b	0.70b	0.47b	7.85b
	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
	LSD (5%)	0.16	0.03	0.13	0.04	0.03	0.10	0.04	0.07	0.04	0.01	0.04	0.04	0.07	0.05	0.97

Notes: Capital and small letter = mean comparison of lime across Zn levels and Zn across lime levels, respectively; L5.5 = 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.

lysine, isoleucine, leucine and phenylalanine) and 62% were non-essential amino acids (NEAAs) (asparagine, serine, glutamine, glycine, histamine, arginine, alanine, and proline).

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 histamine. 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.

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