

Effect of different methods of Zn application on rice growth, yield and nutrients dynamics in plant and soil

Adel Mohamed Ghoneim¹, Azza Ibrahim Ebid²

¹Agricultural Research Center, Field Crops Research Institute, Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt.

²Department of Biology, College of Science, Princess Nora Bint Abdul Rahman University, Saudi Arabia.

Authors' contributions

This study research was carried out in collaboration between the authors. Author AMG designed the study and carried out field experiments. Author AIE contributed in commenting and facilitating necessary materials for the research work and manuscript preparation. All authors read and approved the final manuscript.

ABSTRACT

Zinc (Zn) deficiency is widely spread in Egyptian paddy soils and has negative impact on national rice (*Oryza sativa* L.) production. Field experiments were conducted at the research farm of the Rice Research and Training Center, Sakha, Egypt, to evaluate the effects of different methods of Zn application on rice growth, yield of Sakha 104 and nutrients dynamics in soil and plant. The experiment included four treatments: no Zn, root soaking, foliar and soil application. The results indicated that Zn application by different methods, significantly increased number of tillers, panicles, plant height, 1000-grain weight, filled grains% and grains yield of Sakha 104. Among the different of Zn application, soil application of 15 kg ha⁻¹ as ZnSO₄.H₂O caused highest increase in total N percentage, total K percentage and available Zn content in both grain and straw, however, the percentage of total P decreased significantly. Zinc content in soil after harvesting was significantly affected by Zn application. Different methods of Zn tend to increase the total N and total K contents of soil but decreased P concentration significantly.

Key words: Method of Zn application, soil properties, Rice yield, Rice nutrient uptake.

1. INTRODUCTION

Zinc deficiency in rice has been widely reported in many rice-growing regions of the world [1, 2, 3]. Zinc deficiency in crop plants results in not only yield reduction but also Zn malnutrition in humans, where a high proportion of rice is consumed as a staple food [4, 5]. Low bioavailability of Zn in soil generally results in Zn deficiency in rice plants, and thus becomes one of the common constraints for Zn bio-fortification in rice grain. Zn deficiency in rice can be alleviated through Zn fertilization, which is considered to be a cost-effective method to alleviate Zn malnutrition [6].

Recently, water crisis in Egypt, has resulted in a move towards water-saving cultivation, from flooded to alternate wetting and drying to aerobic rice systems [7, 8]. Importantly, adoption of these water saving systems may decrease Zn availability [9]. High-yielding varieties and greater fertilizer inputs in Egypt were the strategies to raise the crop yield potential and feed increasing population. The increased application of fertilizers was not sufficient to compensate for over-use of cultivated land. Zinc deficiency is considered as a major threat to the global and regional food security [10]. Amongst several factors of low plant Zn availability in paddy fields is soil pH, CaCO_3 content and low redox potential [11]. Widespread occurrence of Zn deficiency in traditional lowland [12] and newly developed alternate wetting and drying or aerobic rice systems [13] necessitates harnessing of breeding efforts to increase Zn uptake [14, 15].

Widespread Zn deficiency has been found responsible for yield reduction in rice [16]. Globally, more than 30 % of soils are low in plant-available Zn [17]. Nonetheless, frequency of Zn deficiency is greater in rice than other crops, with more than 50 % of the crop worldwide prone to this nutritional disorder [18]. Zinc application methods and sources are aimed at improving Zn availability for plant uptake. Zn can be applied to soil, seed and leaves [18] and by dipping seedlings into a fertilizer solution. Zinc fertilization to cereal crops improves productivity and grain Zn concentration [19, 20] and thus improves grain nutritional value for human beings. However, the vast majority of Zn fertilizer trials and resulting fertilizer recommendations in rice have been in the context of managing the Zn deficiency, with very few studies related to Zn bio-fortification [21]. Selection of appropriate Zn sources for soil application is considered to be an alternative strategy to improve plant availability of Zn under lowland conditions. Generally, ZnSO_4 is the most widely applied Zn source for its high solubility and low cost. In

addition, Zn-EDTA (Ethylene Diamine Tetra acetic Acid) is also being recommended due to its efficiency of Zn availability for the plants [22].

Alternate wetting and drying (AWD) management is one of the most important water-saving strategies commonly practiced, which is being widely adopted in Egypt. It has been reported that AWD potentially results in decreased water inputs by 5%-35% when compared with continuous flooding, with the yield of rice grain either being maintained [23, 24, 25] or even increasing [26]. This study was aimed to investigate the effects of different methods of Zn applied through soil, root soaking and foliar on rice growth, yield and nutrients dynamics status in grain and straw of rice variety Sakha 104.

2. Materials and Methods

2.1 Area Description and Soil Sampling

Field experiment was carried out at the research area of Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt (31°6'42" N, 30°56'45" E). Represented soil samples were taken in bulk from 0-25 cm depth before the growing season. The samples were air-dried, ground and passed through 2-mm sieve. Composite samples were taken and analyzed for physical and chemical characteristics namely, EC, pH, texture, cations and anions following standard methods [27]. The characterization of the soil used is presented in Table 1.

Table 1. Selected soil characteristics of the experimental site

pH	EC (dS m ⁻¹)	Cations (meq L ⁻¹)				Anions (meq L ⁻¹)				Particles size %			Zn (mg kg ⁻¹)*
		Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cl ⁻	HCO ₃ ⁻	CO ₃ ²⁻	SO ₄ ²⁻	Sand	Silt	Clay	
7.89	1.60	2.70	1.65	3.75	2.69	3.80	2.25	3.69	2.67	12.0	28.9	59.1	0.29

* Extracted by using Diethylene Triamine Pentaacetic Acid (DTPA).

2.2 Field Experiment

Field experiment was laid out in RCBD_[A1] with four treatments with plot size of 2x5m. The experiment included four treatments as given in Table 2.

Table 2. Treatments

Symbolic names	Method of Zn	Zn application rate
----------------	--------------	---------------------

	application	(kg ha ⁻¹)
T1 (no Zn applied)	-	-
T2 (Root soaking)	Root soaking in 1.50% solution	7.50
T3 (Foliar spray)	Spray at 20 DAT	2.50
T4 (Soil application)	Soil application just after transplanting	15.0

79 DAT, Days after transplanting

80 All plots except T1 received 150 kg ha⁻¹ N as urea, 81 kg ha⁻¹ of P as P_[A2] and 72 kg ha⁻¹
81 K as K_[A3]. Full dose of P and K was applied just before transplanting. Nitrogen fertilizers
82 were applied in two split application: the first split_[A4] was applied at the time of final land
83 preparation and second split_[A5] was added at panicle initiation stage. Zinc was applied
84 as ZnSO₄·H₂O. All other cultural practices were kept same and uniform for all
85 treatments. Plant height and number of tillers were recorded during the growth stage. At
86 crop maturity_[A6], rice plants were manually harvested from 1m² area in the center of
87 each plot. These plants were used to determine grain yield and its components [28] after
88 separating into straw and grains. Dry weight of straw was determined after oven-drying
89 at 70°C to constant weight. Yield components included panicle number; spikelets per
90 panicle, percentage of filled grain and 1000-grain weight were recorded.

91

92 | 2.3 Plant **a**Analysis

93 Samples (0.10 g) from each treatment were digested with 5.0 ml HNO₃-H₂O₂ (4:1, v/v)
94 using a hot block heater. After cooling, the digest was transferred to a 20-ml volumetric
95 flask and then filtered. Zn concentrations of these samples were determined using
96 inductively coupled plasma mass spectrometry (ICP-MS; Agilent Modell 7500a, Agilent
97 Technologies, USA).

98

99 | 2.4 Soil **s**amples

100 Samples of rhizosphere soil were collected by gently shaking off from the root system,
101 available Zn extracted by DTPA according to the method of [Lindsay and Norvell](#) [29] and
102 determined using ICP-MS (Agilent 7500a, USA).

103

2.5 Statistical Analysis

All the data including soil and plant samples were analyzed for critical differences using the ANOVA statistical package [30].

3. Results and Discussion

3.1 Growth Characters

The height of rice plant as affected by different methods of Zn application are shown in Figure 1. It is observed from the figure that methods of Zn application significantly affected the plant height. Maximum plant height of 100 cm was recorded from the treatment with 15 kg Zn ha⁻¹ as soil application which differs significantly from the other treatments. Minimum plant height was recorded with T1. The tillers (numbers/m²) of rice plant as affected by different methods of Zn application are shown in Figure 2. The application of different methods of Zn application, significantly increased the tillers number over control.

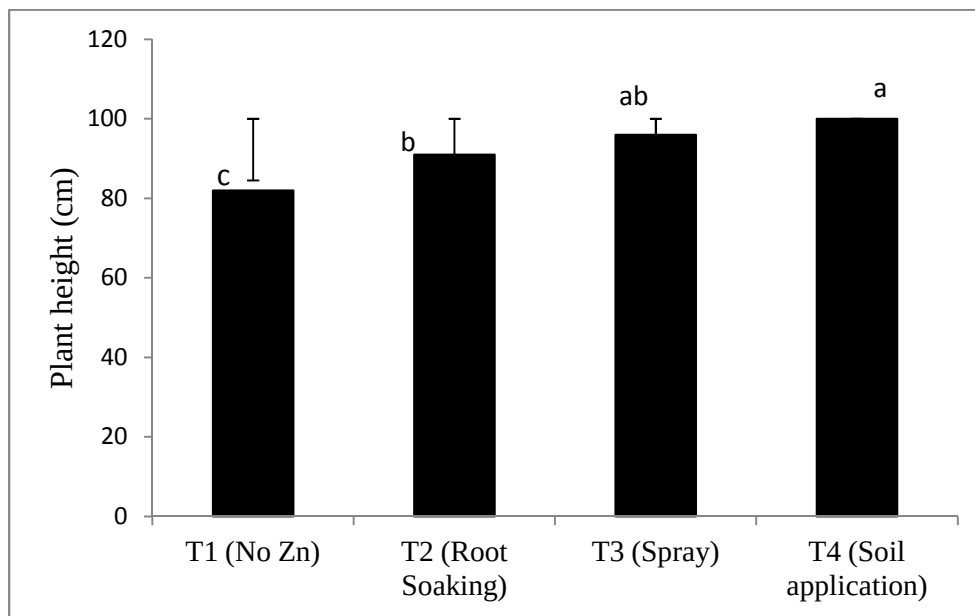


Fig. 1. Plant height of Sakha 104 as affected by different methods of Zn application. Mean followed by the same letters are not significantly different at 5% level of probability. Vertical bars represent $\pm$ standard error (SE, n=4)

Tillering capacity is one of the most important rice components which are responsible for yield production. Maximum tillers numbers/m² of 430 were recorded for T4 followed by

T3; while the minimum tillers numbers was recorded in T1. The increased of tillers number by soil application of Zn may be attributed due to increased of nutrients availability in soil compared with other treatments. Similar results were reported [by ?? authors names](#) [31, 32].

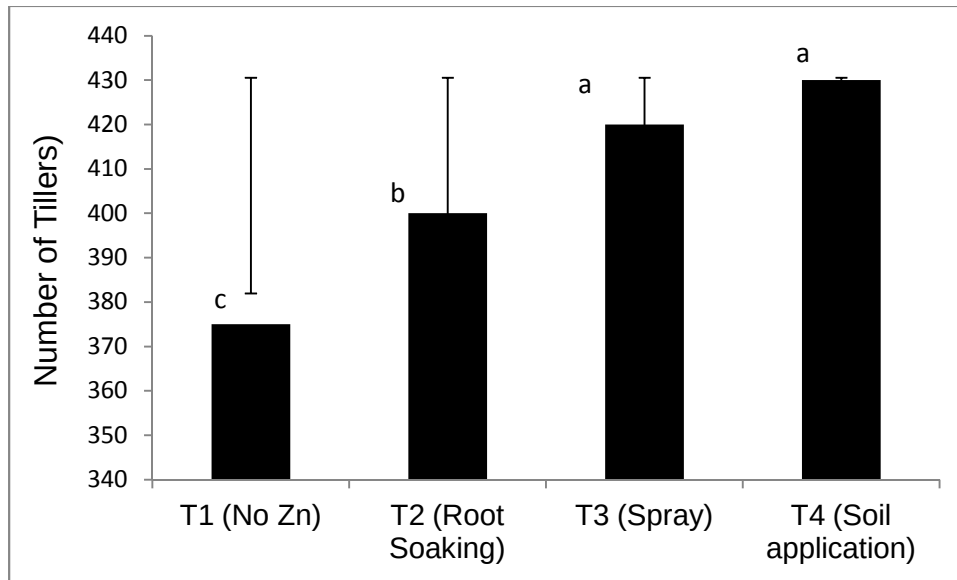


Fig. 2. Number of tillers of Sakha 104 as affected by different methods of Zn application. Mean followed by the same letters are not significantly different at 5% level of probability. Vertical bars represent $\pm$ standard error (SE, n=4)

3.2 Yield Components

~~Table 3 shows the number of panicles/m², number of spikelet/panicle, filled grain percentage and 1000-grain weight as affected by different methods of Zn application.~~

The results indicated that different methods Zn application (T2, T3 and T4) significantly increased the number of panicles/m² compared with T1 (Table 3). The maximum number of panicles/m² was recorded in T4 and followed by T2 and followed by T3, while the minimum number of panicles/m² rice plant was recorded in T1. The number of spikelets/panicle, percentage of filled grain and 1000-grain weight followed the same trend of response i.e. increased with different methods of Zn application compared to control but, no significant differences were found amongst the various methods. Similar results have been reported in previous research [33, 34, 35].

Table 3. Yield attributes of rice as affected by different methods of Zn application

Treatments	Number of panicles/m ²	Number of spikelet/panicle	Filled grain %	1000-grain weight (g)
T1 (No Zn)	325 d	110 b	80 b	20.1 b
T2 (Root soaking)	348 a	130 a	83 a	25.9 a
T3 (Foliar spray)	339 c	128 a	81 b	26.5 a
T4 (Soil application)	350 a	129 a	82 a	27.1 a

Mean values on each column followed by the same letters are not significantly different at 5% level of probability.

3.3 Grain and Straw Yield

The impact of different methods of Zn application on grains and straw yield of Sakha 104 are shown in Figure 3. The results indicated that the different methods of Zn application significantly increased the grain yield compared to no Zn application (T1). Maximum grain yield of 9.60 tones ha⁻¹ was recorded by soil application of Zn. No significant differences were observed in grain yield with T2 or T3. The increase in yield with Zn as soil application may be attributed to Zn has more residual effect compared with root soaking or foliar. The results indicated that straw yield of rice significantly increased with different methods of Zn application (T2, T3 and T4) compared with T1, but, no significant difference were observed between Zn application methods. Similar findings have been reported in previous studies [36, 37, 38].

3.4 Effect of Different Methods of Zn Application on Nutrients Content

Zinc content in grains and straw of rice as affected by different methods of Zn application are presented in Table 4. Highest Zn content of 31.1 mg kg⁻¹ was recorded in T3 followed by T4 and T2. The lowest Zn content in grain and straw was recorded in T1. The treatment differences were significant at 5% level. Although foliar application is effective in increasing grains Zn content [39, 40, 41], time of foliar Zn application is an important factor in this regard [42].

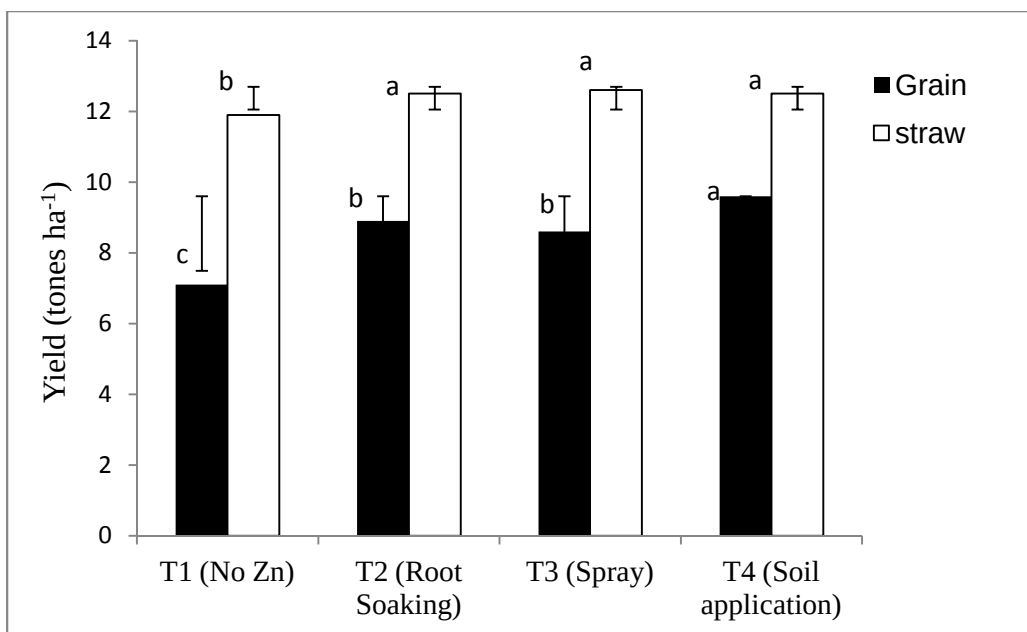


Fig. 3. Grain and straw yield of Sakha 104 as affected by different methods of Zn application. Means followed by the same letters are not significantly different at 5% level of probability. Vertical bars represent $\pm$ standard error (SE, n=4_[A15])

The increases in Zn content by grain occur when it is applied as foliar at later stages of plant growth. Zinc translocation towards rice grains in a nutrient solution using aerobic rice genotypes was studied. The results indicated that, when Zn was applied to roots or as foliar spray; under sufficient Zn supply, Zn partitioning to the grain was greater from root-supplied than foliar form [43]. Foliar application can avoid the problems of Zn binding in soil, but the time of Zn application should be around flowering for increasing grain-Zn concentration. The increase in grain-Zn concentration may be due to improved leaf remobilization of Zn during grain filling.

Table 4 showed that, total N percentage in rice grain and straw of Sakha 104 affected significantly by different methods of Zn application compared with no Zn (T1). The highest total N percentage in grains (0.97%) was recorded in T2, and followed by T4 and followed by T3, respectively. The increases in total N percentage in grains by applying different methods of Zn application may be attributed due to the enhancing the micrograms for N recycling and organic N mineralization. The results are in agreement with findings of [44].

Phosphorus (P) content of plant as affected by different methods of Zn application is shown in Table 4. The results indicated that, application of different methods of Zn significantly decreased the total P percentage in grain and straw of Sakha 104 compared with T1. The highest total P percentage 0.26% and 0.041% were observed in T1, however, there was no significant [A16] difference was observed between different methods of Zn application.

Table 4. Effect of different methods of Zn application on total N, P, K, and Zn content of rice plant

Treatments	Zn (mg kg ⁻¹)		N		P		K	
			%					
	Grains	Straw	Grains	Straw	Grains	Straw	Grains	Straw
T1 (No Zn)	22.1 c	23.2 b	0.73 b[A17]	0.33 b	0.26 a	0.041a	0.160 c	1.40 d[A18]
T2 (Root soaking)	29.1 b	27.3 a	0.97 a	0.50 a	0.23 b	0.036 b	0.210 a	0.17 c[A19]
T3 (Foliar spray)	32.1 a	28.3 a	0.94 c[A20]	0.48 a	0.24 b	0.031 b	0.207 a	0.18 b[A21]
T4 (Soil application)	30.1 ab	29.1 a	0.96 a	0.47 a	0.24 b	0.026 c	0.180 b	1.80 a[A22]

Mean values on each column followed by the same letters are not significantly different at 5% level of probability.

All the Zn applied by different methods decreased the total P percentage. This may be attributed due to the antagonistic effect of Zn on P uptake. Application of P, not only decreased water-soluble and exchangeable-Zn, but also increased bound forms of adsorption of Zn with soil particles [45]. These effects were more pronounced under flooded than non-flooded water regime.

The data of total K percentage in grain and straw are presented in Table 4. The results showed that with application of different methods of Zn, total K percentage increased significantly compared with T1. These results are in agreement findings of [46], that

reported increase of K concentration of both rice grain and straw with Zn fertilizer application.

3.5 Nutrients Dynamics in Soil

Zinc content of soil after harvesting as influenced by different methods of Zn application is presented in Table 5. The results indicated that the application of different methods of Zn application significantly increased Zn of soil compared with control (T1). Maximum Zn content were recorded in the order T4>T3>T2. However, the Zn content of soil after harvesting time was increased compared with the initial Zn content of soil as presented in Table 1.

Table 5. Effect of different of methods of Zn application on available Zn, total N, P and K concentrations of soil after harvesting

Treatments	Zn content (mg kg ⁻¹)	N	P	K
		%		
T1 (No Zn)	0.35 c	0.73 d	0.26 a	0.15 b
T2 (Root soaking)	0.69 b	0.96 b	0.21 b	0.16 b
T3 (Foliar spray)	0.75 ab	0.91 c	0.22 b	0.18 a
T4 (Soil application)	0.81 a	0.99 a	0.23 b	0.19 a

Mean values by on each column followed by the same letters are not significantly different at 5% level of probability.

Zinc content in soil is present in a number of chemical forms with varying solubility. These forms include soluble Zn present in soil solution (water soluble), adsorbed on exchange sites (exchangeable), associated with OM, co-precipitated as secondary minerals or associated with sesquioxides [47]. These different forms mainly control solubility and availability of Zn to plants [48]. In addition, soil chemical properties such as pH, Rh, soil organic matter (OM), have strong influence on Zn adsorption-desorption reactions and play a critical role in regulating Zn solubility and its fractionation in soils

[49]. Varied responses of rice to the Zn fertilizers have been reported, depending on the source, application time, methods of application and soil chemical properties [49].

Nitrogen (N) content of soil at harvesting time as affected by applying different methods of Zn are presented in Table 5. The data showed that, there is significant difference in total N percentage among the methods of Zn application. The maximum total N percentage of 0.99 was recorded by T4, followed by T2 and T3, while the T1 has the lowest total N percentage value. Maximum total N percentage of soil content was observed with combined application of N and Zn. Among Zn application methods, soil application was considered superior treatment [50].

Phosphorus (P) content of soil after harvesting as affected by applying different methods of Zn are presented in Table 5. The application of different methods Zn application, content in soil decreased P significantly compared with no Zn. The highest P% content observed in T1, however no significant differences were recorded among the methods of Zn application. Phosphorus interacts with Zn in soil which reduces Zn translocation from roots to the shoots [51] and also imbalanced P: Zn ratio in plant (dilution effect) has a negative effect on rice yield. Other studies also indicated that P application influenced Zn uptake by rice and translocation into shoots [52].

The content of total K percentage of soil at harvesting time as affected by different of methods Zn application are presented in Table 5. The results indicated that the application of different of methods Zn application has a little effect of increase the total N percentage compared with no Zn added (control). The maximum total N percentage of 0.19% was recorded with T4, followed by T3 and T2. Similar results have been reported [53].

4. CONCLUSION

The study aimed to provide most suitable technology to tackle Zn deficiency in rice, by comparing the effects of different methods of Zn application on rice growth, yield and nutrients concentration. Zinc application by any of the methods increased significantly the rice growth parameters, straw and grain yield over control. Maximum soil Zn concentration was found in plots with soil Zn application as compared to foliar or root soaking application. Zinc content in soil after harvest was also significantly affected by methods of Zn application. Maximum soil Zn concentration was found in soil Zn application treatment as compared with foliar and root soaking. The study suggested

that, soil application of Zn resulted in better results and easier as compared to foliar and root soaking treatment. The obtained results of this study will help to mitigate Zn deficiency in rice and therefore, improve zinc use efficiency.

ACKNOWLEDGEMENTS

Agricultural Research Center, Field Crops Research Institute, Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt is acknowledged for financially supporting of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fitzgerald MA, Mccouch SR, Hall RD. Not just a grain of rice: the quest for quality. Trends Plant Sci., 2009; 14(3): 133–139.
2. Mandal B, Hazra GC, Mandal LN. Soil management influences on zinc desorption for rice and maize nutrition. Soil Sci. Soc. Am. J., 2000; 64: 1699–1705.
3. Tiong J, McDonald GK, Genc Y, et al. HvZIP7 mediates zinc accumulation in barley (*Hordeum vulgare*) at moderately high zinc supply. New Phytol., 2014; 201(1): 131–143.
4. Yao F, Huang J, Cui K, et al. Agronomic performance of high-yielding rice variety grown under alternate wetting and drying irrigation. Field Crops Res., 2012; 126: 16–22.
5. Zhang H, Xue Y, Wang Z, et al. An alternate wetting and moderate soil drying regime improves root and shoot growth in rice. Crop Sci., 2009; 49(6): 2246–2260.
6. Zhao FJ, Mcgrath SP. Biofortification and phytoremediation. Curr. Opin. Plant Biol., 2009; 12(3): 373–380.
7. Farooq M, Kobayashi NK, Wahid A, Ito O, Basra SMA. Strategies for producing more rice with less water. Adv Agron., 2009; 101: 351–388.

- 296 8. Farooq M, Rehman A, Aziz T, Habib M. Boron nutrimpriming improves the germination
297 and early seedling growth of rice (*Oryza sativa* L.). *J Plant Nutr.*, 2011a; 34:
298 1507–1515.
- 299 9. Gao XP, Zou CQ, Fan XY, Zhang FS, Hoffland E. From flooded to aerobic conditions
300 in rice cultivation: consequences for zinc uptake. *Plant Soil*, 2006; 280: 41–47.
- 301 10. Rana WH, Kashif SR. Effect of different Zinc sources and methods of application on
302 rice yield and nutrients concentration in rice grain and straw. *Journal of*
303 *Environmental and Agricultural Sciences*, 2014; 1–9.
- 304 11. Alloway BJ. Soils factors associated with zinc deficiency in crops and humans.
305 *Environ Geochem Health*, 2009; 31(5): 537–548.
- 306 12. Singh B, Natesan SKA, Singh BK, Usha K. Improving zinc efficiency of cereals
307 under zinc deficiency. *Curr Sci.*, 2003; 88: 36–44.
- 308 13. Dobermann A, Fairhurst TH. Nutrient disorders and nutrient management. Potash
309 and Phosphate Institute, PPI of Canada and IRRT, Singapore, 192 pp, 2000.
- 310 14. Sharma PK, Lav B, Ladha JK, Naresh KK, Gupta RK, Balsubramanian BV, Bouman,
311 BAM. Crop water relation in rice-wheat cropping under different tillage systems
312 and water management practices in a marginally sodic, medium textured soil. In:
313 Bouman, B.A.M., Tuong, T.P., Ladha, J.K. (Eds) *Water-wise production*. IRRI,
314 Los Baños, Philippines, 2002.
- 315 15. Rehman HU, Aziz T, Farooq M, et al. Zinc nutrition in rice production systems: a
316 review. *Plant Soil*, 2012; 361(1-2):203–226.
- 317 16. Fageria NK, Baligar VC, Clark RB. Micronutrients in crop production. *Adv Agron.*,
318 2002; 77: 185–268.
- 319 17. Hajiboland R, Yang ZE, Romheld V. Effects of bicarbonate and high pH on growth
320 of Zn efficient and Zn inefficient genotypes of rice, wheat and rye. *Plant Soil*,
321 2003; 250: 349–357.
- 322 18. Johnson SE, Lauren JG, Welch RM, Duxbury JM. A comparison of the effects of
323 micronutrient seed priming and soil fertilization on the mineral nutrition of

- 324 chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), rice (*Oryza sativa*) and wheat
325 (*Triticum aestivum*) in Nepal. *Exp. Agric.*, 2005; 41: 427–448.
- 326 19. Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic
327 biofortification. *Plant Soil*, 2008; 302, 1–17.
- 328 20. Phattarakul N, Rerkasem B, Li LJ, et al., Biofortification of rice grain with zinc
329 through zinc fertilization in different countries. *Plant Soil*, 2012; 361(1-2): 131-
330 141.
- 331 21. Impa SM, Schulin R, Ismail A, Beebout JS. Unravelling the mechanisms influencing
332 grain-zn content in rice genotypes. In: Abstracts, International Rice Congress,
333 Hanoi Veitnam, Nov 8-12, 2010.
- 334 22. Quijano-Guerta C, Kirk GJD, Portugal AM, Bartolome VI, McLaren GC. Tolerance of
335 rice germplasm to zinc deficiency. *Field Crops Res.*, 2002; 76: 123–130.
- 336 23. Bouman BAM, Hengsdijk H, Hardy B, et al. Water-Wise Rice Production.
337 International Rice Research Institute, Los Baños, Philippines, p.89–102, 2002.
- 338 24. Rehmani MIA, Wei G, Hussain N, Ding C, Li G, Liu Z, Wang S, Ding Y. Yield and
339 quality responses of two Indica rice hybrids to post-anthesis asymmetric day and
340 night open-field warming in lower reaches of Yangtze River delta. *Field Crops*
341 *Res.*, 2014; 156: 231–241.
- 342 25. Chapagain T, Yamaji E. The effects of irrigation method, age of seedling and
343 spacing on crop performance, productivity and water-wise rice production in
344 Japan. *Paddy Water Environ.*, 2010; 8(1): 81–90.
- 345 26. Yang XE, Chen WR, Feng Y. Improving human micronutrient nutrition through
346 biofortification in the soil plant system: China as a case study. *Environ Geochem*
347 *Health*, 2007; 29 (5): 413–428.
- 348 27. Page AI, Miller RH, Keeney DR. Methods of soil analysis. Madison, Wisconsin,
349 USA, 1982.
- 350 28. Ramesh SA, Shin R, Eide D, Schachtman DP. Differential metal selectivity and
351 gene expression of two zinc transporters from rice. *Plant Physiol.*, 2003; 133:
352 126–134.

- 353 29. Lindsay WL, Norvell WA. Development of a DTPA soil test for Zn, Fe, Mn, and Cu.
354 Soil Sci. Soc. Am. J. 1978; 42: 421–428.
- 355 30. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research, 2nd Edition.
356 A Wiley Interscience Pub., John Wiley and Sons, New York. USA. p. 628, 1984.
- 357 31. Ghoneim AM, Ueno H, Asagi N, Watanabe T. Indirect ¹⁵N Isotope Techniques for
358 Estimating N Dynamics and N Uptake by Rice from Poultry Manure and Sewage
359 Sludge. Asian Journal of Earth Sciences, 2012; 5 (2): 63–69.
- 360 32. Ghoneim AM. Nitrogen and carbon uptake by some Rice cultivars from ¹⁵NH₄Cl and
361 ¹³C-U-glucose labeling fertilizer. International Journal of Agronomy and
362 Agricultural Research, 2014; 4 (4): 20–27.
- 363 33. Cakmak I. Enrichment of fertilizers with zinc: an excellent investment for humanity
364 and crop production in India. J Trace Elem Med Biol., 2009; 23(4): 281–289.
- 365 34. Beebout SJ, Tuyogon D, Rubianes F, Castillo O, Larazo W, Bunquin M, Laureles, E.
366 Improved zinc management strategies for rice scientists and farmers. In:
367 Proceedings of 2010 International Annual Meetings of ASA-CSSA-SSSA,
368 October 31 to November 04, 2010, Long Beach, California, USA, 2010.
- 369 35. Farooq M, Wahid A, Siddique KHM. Micronutrients application through seed
370 treatments - a review. J. Soil. Sci. Plant Nutr., 2012; 12: 125–142.
- 371 36. Khan MU, Qasim M, Subhan M, Jamil M, Ahmad RD. Response of rice to different
372 methods of Zn application in calcaerous soils. Pak J Appl. Sci., 2003; 3:524–529.
- 373 37. Jiang W, Struik PC, Zhao M, van Keulen H, Fan TQ, Stomph TJ. Indices to screen
374 for grain yield and grain zinc mass concentration in aerobic rice at different soil
375 Zn levels. NJAS Wageningen J Life Sci., 2008b; 55: 181–197.
- 376 38. Bhaduri D, Purakayastha TJ. Soil available Zn: a potent soil quality indicator in a
377 rice–wheat system. 3rd International Zinc Symposium, 10–14 October 2011,
378 Hyderabad, India.
- 379 39. Welch RM. Breeding strategies for biofortified staple plant foods to reduce
380 micronutrient malnutrition globally. J Nutr., 2002; 132:495–499.

- 381 40. Hajiboland R, Salehi SY. Characterization of Zn efficiency in Iranian rice Genotypes
382 I. Uptake efficiency. *Gen Appl Plant Physiol*. 2006; 32: 191–206.
- 383 41. Chasapis CT, Loutsidou AC, Spiliopoulou CA, et al. Zinc and human health: an
384 update. *Arch. Toxicol.*, 2012; 86(4): 521–534.
- 385 42. Stomph TJ, Hoebe N, Spaans E, van der, Putten PEL. The relative contribution of
386 post-flowering uptake of zinc to rice grain zinc density. 3rd International Zinc
387 Symposium 10-14 October 2011, Hyderabad, India.
- 388 44. Gao X, Hoffland E, Stomph TJ, Grant CA, Zou C, Zhang F. Improving zinc
389 bioavailability in transition from flooded to aerobic rice. A review. *Agron Sustain*
390 *Dev.*, 2012; 32: 465–478.
- 391 45. Lal B, Majumdar B, Venkatesh MS. Individual and interactive effects of phosphorus
392 and zinc in lowland rice. *Indian J Hill Farming*, 2002; 13: 44–46.
- 393 46. Iqbal M, Aslam M, Ranja AM, Akhtar J. Salinity tolerance of rice as affected by Zn
394 application. *Pakistan J. Biol. Sci.*, 2000; 3: 2055–2057.
- 395 47. Arnold T, Kirk, GJD, Wissuwa M, Frei M, Zhao FJ, Mason TFD, Weiss DJ.
396 Evidence for the mechanisms of zinc uptake by rice using isotope fractionation.
397 *Plant Cell Environ.*, 2010; 33: 380–381.
- 398 48. Almendros P, Gonzalez D, Obrador A, Alvarez JM. Residual zinc forms in weakly
399 acidic and calcareous soils after an oilseed flax crop. *Geophysical Research*
400 *Abstracts*. EGU General Assembly, 2008; 10, EGU2008-A-12479.
- 401 49. Takrattanasaran N, Chanchareonsook J, Johnson PG, et al. Amelioration of zinc
402 deficiency of corn in calcareous soils of Thailand: zinc sources and application
403 methods. *J. Plant Nutr.*, 2013; 36(8): 1275–1286.
- 404 50. Depar N, Rajpar I, Memon MY, Imtiaz M, Zia-ul-hassan. (2011). Mineral nutrient
405 densities in some domestic and exotic rice genotypes. *Pak J Agric. Eng Vet Sci.*,
406 2011; 27: 134–142.
- 407 51. Haldar M, Mandal LN. Influence of soil moisture regimes and organic matter
408 application on the extractable Zn and Cu content in rice soils. *Plant Soil*, 1979;
409 53: 203–213.

- 410 52. Lonergan PF, Pallotta MA, Lorimer M, et al. Multiple genetic loci for zinc uptake and
411 distribution in barley (*Hordeum vulgare*). New Phytol., 2009; 184(1):168–179.
- 412 53. Wu CY, Feng Y, Shohag MJ, et al. Characterization of ⁶⁸Zn uptake, translocation,
413 and accumulation into developing grains and young leaves of high Zn-density
414 rice genotype. J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.), 2011; 12(5):
415 408–418.
- 416