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Effect of Different Rates of Zinc on Root Morphological Traits among Different Upland Rice Landraces in Malaysia

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Abstract Billions of people globally are estimated to suffer from Zn deficiency due to their low dietary intake, especially those with rice. Global efforts are under way to improve the Zn concentrations in rice to increase Zn in diets. Zinc uptake in relation to morphological root parameters among 7 upland rice varieties were studied by conducting a solution culture experiment using modified Yoshida solution in Agriculture Faculty of University Putra Malaysia. Five Zinc levels were developed by the addition of 0, 5, 10, 20, 30 mg L^{-1} $ZnSO_4$. Seedlings were harvested in week 4. Zn uptake in roots and shoots of upland rice showed significant differences among all varieties. Root Zn uptake significantly increased at all rates. Other root parameters (length, average diameter, surface area, volume, and number of root tips) did not show any significant differences in 0 to 20 mg Zn L^{-1}, but they decreased significantly in 30 mg Zn L^{-1} in 4th week of observation. In addition shoot Zn uptake like other root parameters followed this trend and decreased significantly in 30 mg Zn L^{-1} in 4th week of observation.

Keywords Zinc, Rice, Root morphology, Zn Uptake, Upland Rice

1. Introduction

Zinc (Zn) is an essential micronutrient for normal growth and development of plants (Broadley*, et al.*, 2007). The normal concentration of this element is 25 to 150 mg kg^{-1} in plants. Deficiencies of Zn are usually associated with concentrations of less than 20 mg kg^{-1}, and toxicities will occur when the Zn leaf concentration exceeds 400 mg L^{-1} (Dobermann and Fairhurst, 2000). Cultivars differ in their ability to take up Zn, which may be caused by differences in Zinc translocation and utilization, differential accumulation of nutrients that interact with Zn and differences in plant roots to exploit for soil Zn (Tisdale*, et al.*, 1993). Earlier studies have shown that Zinc mass concentration (ZnMC) in all plant organs increased with an increase in Zn supply but to various degrees. At higher uptake levels, the ZnMC in stems increased most, while the ZnMC in hulled grains (brown rice) increased least (Jiang*, et al.*, 2008).

Rice, the main staple food of Asia, is inherently very low in Zn and its high consumption relative to other foods contributes to high incidence of Zn deficiency in human populations in Asia (Gibson*, et al.*, 2007; Stein*, et al.*, 2007). Upland rice is water saving rice production system depending on irrigation water management and anticipated yield (Wang*, et al.*, 2009). Upland rice needs to have a deeper rooting and a higher root length and density than lowland rice cultivars because of the limited water availability under aerobic as compared to flooded conditions (Matsuo*, et al.*, 2010). The primary source of Zn for rice plants is trough root uptake (Welch and Graham, 2002), To increase Zn uptake by roots, the Zn availability in the rhizosphere must be increased (Welch and Shuman, 1995). Some researchers reported that under nutrient-deficient conditions, plants tend to alter their root size and morphology for efficient nutrient acquisition. Enhanced root growth under Zn deficiency, both in length and number of roots, has been associated with Zn-deficiency tolerance of lowland rice genotypes. (Chen*, et al.*, 2009). In addition researchers showed Under moderate Zn deficiency, damage to root tip cells was observed in some susceptible genotypes (Widodo*, et al.*, 2010). Most recent studies in rice suggest that among numerous other mechanisms, Zn uptake is most important. However, the root traits in upland rice are not well understood. This study was undertaken to evaluate the effect of different rates of Zinc on root morphological traits

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among different upland rice landraces in Malaysia.

2. Materials and Methods

Seven upland varieties (Table 1) were collected from different parts of Malaysia. This study was conducted at Faculty of Agriculture, University Putra Malaysia from April to May 2012.

Total of 420 seedlings including, five treatments of different Zinc concentrations (0, 5, 10, 20 and 30 mg L^{-1}) were applied on the seven upland rice varieties ($ZnSO_4.7H_2O$ as source of Zn was applied). Experimental units were grown in Yoshida solution culture (Yoshida, 1981) in growth chamber and pH was daily adjusted on 5.5. Plants were irrigated twice a day and 105 seedlings harvested weekly until week four. The roots that developed after 28 days were scanned using WinRHIZO root scanning software. Root parameters, such as volume, surface area, average diameter, length and number of root tips were recorded. Dry-matter weights, root and shoot Zn uptake were determined. The experiment was laid out in a three factorial using randomized complete block design (RCBD) in three repetitions and three replication.. The data obtained were subjected to ANOVA using the SAS 9.2 version. Pairs of treatment means were compared (or declared significantly or not significantly different at 5% level) by applying Duncan's New Multiple Range Test.

Table 1. Name of varieties

Variety	Bertih (V1)	Tenom (V2)	Kesum (V3)	Sintuk (V4)	Polut wangi (V5)	Hita (V6)	Nabawan (V7)
State of origin	Pahang	Sabah	Pahang	Pahang	Pahang	Pahang	Sabah

Table 2. Means comparison of root and shoot Zn uptake of seven rice varieties in five rates at four weeks(Means in a column with the same letters are not significantly different at 5% level)

Variety	(Root)				(Shoot)			
	7 day	14day	21day	28day	7 day	14day	21day	28day
	Control				Control			
V1	0.005 a	0.008 a	0.008 b	0.011 b	0.0009 e	0.0016 e	0.0020 d	0.0026 c
V2	0.004 ab	0.004 c	0.008 b	0.008 d	0.0019 c	0.0022 d	0.0022 cd	0.0026 c
V3	0.004 ab	0.004 c	0.008 b	0.010 c	0.0038 a	0.0048 a	0.0058 a	0.0070 a
V4	0.004 b	0.004 c	0.007 b	0.013 a	0.0023 b	0.0041 b	0.0050 b	0.0059 b
V5	0.002 c	0.005 bc	0.011 a	0.012 b	0.0015 cd	0.0032 c	0.0048 b	0.0065 ab
V6	0.004 ab	0.004 bc	0.006 b	0.008 d	0.0017 c	0.0020 d	0.0023 cd	0.0025 c
V7	0.002 c	0.005 b	0.006 b	0.008 d	0.0012 de	0.0023 d	0.0028 c	0.0032 c
	5 mgl^{-1}				5 mgl^{-1}			
V1	0.007 a	0.014 b	0.018 bc	0.023 c	0.0043 a	0.0089 b	0.0103 c	0.0148 c
V2	0.003 d	0.021 a	0.034 a	0.043 a	0.0017 d	0.0098 a	0.0165 a	0.0262 a
V3	0.004 c	0.013 b	0.014 cd	0.016 d	0.0023 cd	0.0041 c	0.0058 de	0.0087 d
V4	0.004 c	0.008 d	0.012 cd	0.013 e	0.0024 cd	0.0038 c	0.0059 de	0.0084 d
V5	0.005 b	0.011 c	0.022 b	0.027 b	0.0032 b	0.0086 b	0.0124 b	0.0162 b
V6	0.004 c	0.007 de	0.008 d	0.010 f	0.0030 cb	0.0046 c	0.0063 d	0.0072 e
V7	0.003 d	0.006 e	0.010 d	0.013 e	0.0024 cb	0.0042 c	0.0054 e	0.0079 de
	10 mgl^{-1}				10 mgl^{-1}			
V1	0.022 a	0.024 a	0.036 a	0.040 c	0.0152 a	0.0193 a	0.0231 b	0.0293 b
V2	0.014 b	0.021 b	0.028 d	0.033 e	0.0091 b	0.0121 b	0.0182 c	0.0223 d
V3	0.007 cd	0.019 c	0.034 b	0.044 b	0.0038 cd	0.0113 c	0.0167 d	0.0256 c
V4	0.007 cd	0.015 d	0.023 e	0.047 a	0.0043 d	0.0122 b	0.0264 a	0.0315 a
V5	0.005 d	0.014 d	0.030 c	0.036 d	0.0040 d	0.0064 e	0.0136 e	0.0269 c
V6	0.009 c	0.010 e	0.018 f	0.021 f	0.0058 c	0.0102 d	0.0136 e	0.0150 e
V7	0.005 d	0.008 f	0.011 g	0.011 g	0.0030 e	0.0043 f	0.0051 f	0.0060 f
	20 mgl^{-1}				20 mgl^{-1}			
V1	0.023 a	0.041 a	0.053 b	0.071 a	0.0150 a	0.0234 b	0.0386 a	0.0475 a
V2	0.015 b	0.045 a	0.060 a	0.060 ab	0.0088 d	0.0132 f	0.0278 b	0.0388 b
V3	0.014 b	0.024 b	0.044 c	0.056 ab	0.0080 e	0.0168 d	0.0264 c	0.0315 e
V4	0.011 c	0.020 b	0.026 f	0.059 ab	0.0067 f	0.0164 d	0.0276 b	0.0366 c
V5	0.008 d	0.024 b	0.033 e	0.049 b	0.0058 g	0.0249 a	0.0214 d	0.0349 cd
V6	0.020 e	0.020 b	0.039 d	0.045 b	0.0139 b	0.0189 c	0.0278 b	0.0333 d
V7	0.020 e	0.016 c	0.024 g	0.031 c	0.0127 c	0.0154 e	0.0163 e	0.0167 f
	30 mgl^{-1}				30 mgl^{-1}			
V1	0.027 a	0.062 a	0.076 a	0.102 a	0.0218 a	0.0356 a	0.0518 a	0.0644 a
V2	0.015 c	0.049 b	0.063 b	0.102 a	0.0098 b	0.0184 b	0.0288 b	0.0441 b
V3	0.010 f	0.042 c	0.052 c	0.071 b	0.0054 d	0.0166 c	0.0255 c	0.0311 c
V4	0.012 e	0.029 e	0.035 d	0.059 c	0.0047 d	0.0097 f	0.0100 f	0.0194 f
V5	0.019 b	0.032 d	0.037 d	0.056 d	0.0095 b	0.0142 e	0.0203 d	0.0282 d
V6	0.014 d	0.020 f	0.050 c	0.056 d	0.0071 c	0.0154 d	0.0192 e	0.0230 e
V7	0.010 f	0.019 f	0.028 e	0.040 e	0.0047 d	0.0077 g	0.0099 f	0.0134 g

3. Result and Discussion

Effects of different rates of Zinc on Zinc uptake in roots showed V1 in control treatment was shown the highest Zn uptake in roots (0.005 mg plant^{-1}) Then, V5 and V7 were shown the lowest Zn uptake in root (0.002 mg plant^{-1}) (Table 2). The highest Zn uptake in root was recorded in V1and V2 at 30 (mg L^{-1}) at week 4, (0.102 mg plant^{-1}) and the lowest was recorded in V7 (0.04 mg plant^{-1}) as indicated in table 2.

Concentration levels of Zn in solution were impressed the uptake of Zn in all varieties (Table 2). Also, among all seven varieties it was observed that, by one step increase of Zn source supply in this experiment, Zn uptake (mg plant^{-1}) in roots of upland rice was enhanced significantly (Figure 1B). Accordingly, Zn was accumulated in roots. The maximum uptake (0.0424 mg plant^{-1}) was noticed at 30 mgZn L^{-1} and the minimum value (0.0061 mg plant^{-1}) was found at control (Figure 1B). A gradual increase of Zinc concentration was observed in higher Zn levels (Figure 1B). (Figure 1C) showed the maximum uptake (0.0371 mg plant^{-1}) was noticed at week 4 and the minimum value (0.0096 mg plant^{-1}) was found at week 1. Increase of Zinc uptake was observed week by week. The average of Zn uptake in all rates and weeks showed that Bertih variety had the most Zn uptake (0.0329 mg plant^{-1}) and Nabawan had the lowest Zn uptake (0.0138 mg plant^{-1}) (Figure 1A).

Zn uptake in shoots of rice seedling was showed significantly improve in all treatment subjected to different Zn levels weekly (Table 2). Shoot Zn uptake of V1 in 30 (mgZn L^{-1}) at week 4 was 0.0644 (mg plant^{-1}). It has been shown the highest Zn uptake rate. On the contrary, V7 was showed the lowest Zn uptake at week 4 in 30 (mgZn L^{-1}). In control treatment Zn uptake in shoots was illustrated the lowest. Rice shoots accumulated less Zn than roots. The average Zn uptake ranged from 0.0031 to 0.0223 (mg plant^{-1}) in shoots in different rates (Figure 2B). The maximum uptake (0.0223 mg plant^{-1}) was noted at 20 (mgZn L^{-1}) and the minimum value (0.0031 mg plant^{-1}) was recorded at control (Fig 4.B). By the increasing Zn levels from 0 to 20 (mgZn L^{-1}) augment of uptake was observed. It worth to say, in 30 (mgZn L^{-1}), Zn uptake in shoot decreased significantly in most varieties (Table 2, Figure 2B).

Song *et al.,* (2011) stated that Zn concentration in both shoots and roots of the two cultivars increased in response to an altered Zn supply in the nutrient solution (from 0.15 μM to 2 mM). The Zn concentration in roots of the two cultivars was higher than this amount in shoots.

The minimum value (0.006 mg plant^{-1}) was found at week one. A gradual increase of Zinc uptake in shoot was observed week by week (Figure 2C). Based on the average of Zn uptake in all rates and weeks it could conclude that, the Bertih variety had the most Zn uptake (0.0215 mg plant^{-1}) and Nabawan had the lowest Zn uptake (0.0072 mg plant^{-1}) (Figure 2A).

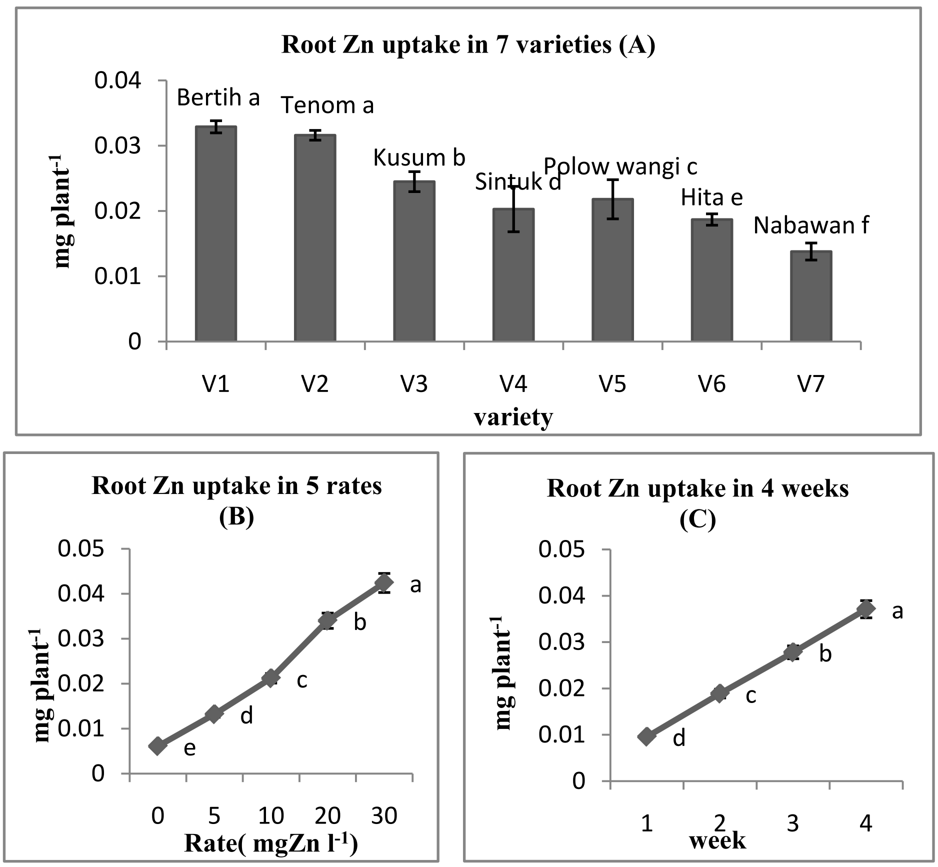

Figure 1. Means comparison of root Zn uptake of (A) seven rice varieties (B) five rates (C) four weeks

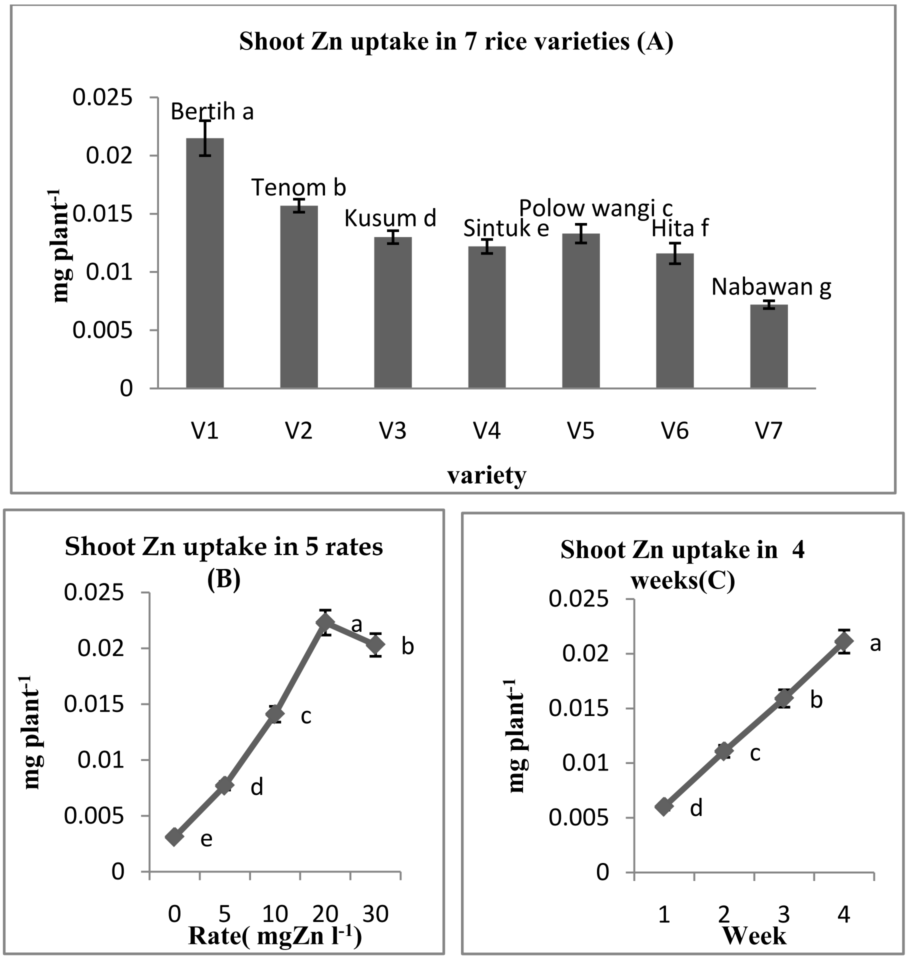

Figure 2. Means comparison of shoot Zn uptake of (A) seven rice varieties (B) five rates (C) four weeks

Table 3. Root lengths of varieties in different rate of Zinc (Means in a line with the same letters are not significantly different at 5% level)

	Length (mm)				
Variety	0	5 (mg L^{-1})	10 (mg L^{-1})	20 (mg L^{-1})	30 (mg L^{-1})
V1	136.293 d	181.100 c	200.390 b	222.723 a	140.057 d
V2	172.610 d	293.317 a	235.353 b	235.390 b	216.353 c
V3	164.627 d	226.317 b	253.027 a	253.060 a	214.990 c
V4	190.647 b	197.760 b	207.650 b	235.557 a	196.573 b
V5	224.317 d	289.010 a	270.990 b	264.300 b	238.277 c
V6	226.967 c	233.250 c	271.333 a	275.250 a	247.787 b
V7	225.987 d	244.390 c	282.637 a	264.957 b	195.007 e

Owing to analysing the results it could be concluded that, Zn uptake in the shoots of rice seedling was also influenced by Zn supply and genotype. Similar results by Genc, *et al.*, (2007) reported under researched genotypes had significantly higher shoot Zn uptake at adequate Zn than at deficient Zn supply.

The root length of Rice (mm) at week 4, as affected by different levels of Zinc is presented in table 3. It showed that, although root length increased significantly with increase in Zn rate, from 0 to 20 mgZn L^{-1} in some varieties, but some other varieties didn't show any significant differences between 0, 5, 10 and 20 mgZn L^{-1}, In contrast, root length decreased significantly in most varieties in 30 mgZn L^{-1} (Table 3).

High level of Zinc showed severe phytotoxic effects rice and significantly inhibited its growth by interfering with certain important metabolic process were also observed by (Alam*, et al.*, 2002) and (Ebbs and Kochian, 1998). As clearly seen in the Table 4, the effects of application of different rates of Zinc on root surface area, number of root tips, and volume, followed the same trend as root length. Gao et al. (2011) reported that root surface area correlated significantly with Zn uptake, but only explained 32% of variation in Zn uptake the effects of application of different rates of Zinc on average root diameter showed that this root parameter didn't have any significant differences between all rates in most of the varieties.

Table 4. Root surface area, number of root tips, root volume and average root diameter of varieties at different rate of Zinc (Means in a line with the same letters are not significantly different at 5% level)

		Surface areas (cm^2)			
Variety	0	5 (mg L^{-1})	10 (mg L^{-1})	20 (mg L^{-1})	30 (mg L^{-1})
V1	25.127 c	31.117 ab	32.000 ab	34.900 a	28.347 bc
V2	30.643 b	47.640 a	31.133 b	34.570 b	34.183 b
V3	26.970 c	34.950 b	33.453 b	40.380 a	31.327 bc
V4	31.333 b	32.473 b	37.910 a	38.577 a	31.737 b
V5	36.243 c	46.783 a	41.963 b	40.327 bc	35.153 c
V6	34.960 b	32.073 b	44.450 a	41.900 a	40.193 a
V7	30.717 c	34.433 b	41.560 a	44.733 a	35.133 b
		N Tips			
V1	328.67 d	487.33 b	580.67 a	580.00 a	354.00 c
V2	505.67 c	867.00 a	596.67 b	589.00 b	490.00 c
V3	472.67 c	627.00 b	674.67 b	751.33 a	627.67 b
V4	560.67 b	607.67 ab	629.33 ab	672.00 a	574.67 b
V5	570.00 b	680.00 ab	740.67 ab	813.67 a	648.00 ab
V6	674.67 c	548.67 d	738.67 b	825.00 a	730.67 b
V7	547.33 c	816.33 a	670.67 b	640.67 b	624.33 b
		Volume (cm^3)			
V1	0.363 b	0.377 b	0.427 b	0.483 a	0.363 b
V2	0.363 b	0.420 b	0.427 b	0.593 a	0.420 b
V3	0.337 b	0.457 a	0.500 a	0.517 a	0.497 a
V4	0.273 c	0.383 b	0.450 ab	0.510 a	0.383 a
V5	0.467 a	0.577 a	0.523 a	0.517 a	0.467 a
V6	0.417 c	0.450 bc	0.597 a	0.523 ab	0.517 ab
V7	0.417 b	0.563 a	0.523 a	0.590 a	0.413 b
		Average diameter (mm)			
V1	0.55 b	0.56 a	0.57 a	0.58 a	0.56 a
V2	0.55 a	0.55 a	0.55 a	0.57 a	0.55 a
V3	0.53 b	0.55 b	0.55 b	0.57 a	0.54 b
V4	0.54 a	0.54 a	0.54 a	0.55 a	0.55 a
V5	0.53 c	0.55 ab	0.55 ab	0.57 a	0.54 bc
V6	0.53 a	0.54 a	0.55 a	0.55 a	0.54 a
V7	0.53 a	0.55 a	0.56 a	0.57 a	0.55 a

4. Conclusions

Increase of Zinc rate has a positive effect on morphological root parameters up to 20 mg L^{-1}. Soluble forms of Zn are readily available to plants and the uptake of Zn has been reported to be linear with concentration in the nutrient solution and soils. In addition, plants grown in Zn-contaminated soils accumulate a great proportion of the metal in the roots (Kabata-Pendias, 2000). This shows that Zn in high concentration causes root growth disorder. Results of this study showed at 30 mgZn L^{-1} young plants died, possibly due to toxic effect of Zn. Sensitive plant species are reported to be retarded in growth when their tissues contain 20 to 200 mgZn kg^{-1}. However, the upper toxic levels range in various plants are from 100 to 500 mgZn kg^{-1} (DW) (Kabata-Pendias, 2000).

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