Madras Agric. J., 104 (4-6): 157-160, June 2017

Genotypic Divergence in Per Cent Zinc Derived from Variable Sources and on Zinc Uptake in Rice [*Oryza sativa* (L.)] as Established by Radiotracer Techniques

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A pot culture experiment was conducted at Tamil Nadu Agricultural University, Coimbatore to establish the genotypic divergence in per cent zinc (Zn) derived from fertilizer (% Zndff) or from soil (% Zndfs) and on zinc uptake of two rice genotypes Zn -efficient Norungan and Zn –inefficient PMK 3. To assess the partitioning of Zn in the shoot and root of the genotypes, radiotracer technique was employed, in which graded levels of Zn (0.0, 12.5, 25.0, 37.5 and 50.0 kg ha^{-1} of $ZnSO_4$) as ^{65}Zn labeled $ZnSO_4$ were applied. Per cent Zndff showed an increase by 50 % for Zn-inefficient PMK 3 than that for Norungan while reverse was the trend for % Zndfs implying the fact that Zn inefficient genotypes have the ability to use only readily available sources of Zn. Zinc uptake from fertilizer increased with increasing dose of applied zinc for PMK 3 while in Norungan it showed a peak at 25 kg ha^{-1} of $ZnSO_4$ and thereafter it declined. Irrespective of the sources of zinc, root zinc accumulation was higher in PMK 3 than that in Norungan. The Zn-efficient genotype Norungan had better translocation of Zn from root to shoot than PMK 3.

Key words : Rice genotypes, Zn – efficient, Zn uptake

Zinc deficiency is a ubiquitous problem constraining rice production worldwide. Plethora of evidences have accumulated stating that the resilience of rice production is very often put to acid test due to the prevalence of two major limiting nutrients *viz*., nitrogen and zinc. Today, than ever before, the importance of zinc in crop production is being felt. It is estimated that about 50 percent of soils used for cereal production in the world have low levels of plant available Zn (Graham and Welch, 1996). Due to Zn deficiency about two million hectares of rice production are affected in Asia (Ferno *et al*., 1970), which is an economic and humanitarian problem.

Today, there is a shift in research trend from plant production point to food chain (Slingerland *et al*., 2006) or food systems (Graham *et al*., 2007). Though potential mechanisms including root Zn uptake and translocation, Zn sequestration in leaves and biochemical utilization of Zn have been studied, multitude of questions concerning zinc efficiency mechanisms between genotypes remain unanswered. Rhizosphere of plants always remains an exploratory venue for the scientists and radiotracer is a scientific tool for the determination of the nutrient translocation in plant system. It is possible to delineate beyond doubt, the crop uptake from fertilizer or from native source employing ^{65}Zn labeled fertilizers. Hence in the present study ^{65}Zn labeled compounds were exploited to establish the genotypic variation in % Zndff or % Zndfs and Zn uptake of the rice genotypes, Norungan (Zn efficient) and PMK 3 (Zn inefficient) (Sudhalakshmi, 2007).

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Material and Methods

A pot culture experiment was performed at the Radioisotope (Tracer) Laboratory, Tamil Nadu Agricultural University, Coimbatore to study the variation in %Zndff and %Zndfs in the rice genotypes Norungan and PMK 3.

About 10 kg of the soil (passed through 2 mm sieve) representing clay loam texture of Noyyal series (*Vertic Ustropept*) was transferred to the ceramic pots. The soil was neutral in reaction, low in $KMnO_4$ nitrogen, high in Olsen phosphorus and $1NNH_4OAc$ potassium and low in DTPA extractable Zn. The seeds of the rice genotypes *viz*., Norungan and PMK 3 were sown on the well - puddled soil. The seedlings after establishment were thinned to three plants per pot. The fertilization programme was adopted as per the crop production guide. The experiment was laid out in a factorial completely randomized block design with the following treatments each replicated thrice.

T_1 : Control

T_2 : $ZnSO_4$ @ 12.5 kg ha^{-1}

T_3 : $ZnSO_4$ @ 25 kg ha^{-1}

T_4 : $ZnSO_4$ @ 37.5 kg ha^{-1}

T_5 : $ZnSO_4$ @ 50 kg ha^{-1}

Zinc sulphate was labeled with ^{65}Zn @ 1 mCi / g of Zn.

The ^{65}Zn isotope was obtained from Board of Research and Isotope Technology, Mumbai as $ZnCl_2$ in dilute HCl. The labeled $ZnSO_4$ was applied on 24th day after sowing as per the treatment schedule. The pots were watered as judged by the soil moisture. Plant protection measures were adopted as and when required.

At the physiological growth stages (active tillering, fifty per cent flowering and harvest), five grams of the plant material from each treatment was ashed in a silica crucible and transferred to a scintillation vial. The vial was then placed in a T1- activated NaI crystal, well type gamma ray spectrophotometer (Type GRS, 23 B, Electronics Corporation of India Ltd., Hyderabad) and the radioactivity of the samples was determined by integral counting, keeping the single channel analyser at optimal window and lower level settings. The radioactivity of the fertilizer standards was also determined. Of the two varieties tested, Norungan turned photosensitive and did not attain the reproductive phase. Hence, zinc partitioning in root and shoot alone was accounted for both the genotypes. The data were subjected to statistical analysis as per the methods outlined for factorial completely randomized block design by Panse and Sukhatme (1978). Critical differences (CD) were determined at the P = 0.05 level of significance.

The radioassay data were employed in the following formulae

$$\text{Specific activity of plant samples (Bq mg}^{-1}\text{of Zn)} = \frac{^{65}\text{Zn radioactivity (Bq)}}{\text{Content of Zn (mg)}}$$

$$\text{Specific activity of fertilizer standard (Bq mg}^{-1}\text{of Zn)} = \frac{^{65}\text{Zn radioactivity (Bq)}}{\text{Content of Zn (mg)}}$$

$$\text{Per cent Zn derived from fertilizer (\% Zndff)} = \frac{\text{Specific activity of plant sample}}{\text{Specific activity of fertilizer standard}} \times 100$$

$$\text{Per cent Zn derived from soil (\% Zndfs)} = 100\text{-\% Zndff}$$

$$\text{Uptake of Zn derived from the applied fertilizer (mg pot}^{-1}\text{)} = \frac{\text{\% Zndff x Total Zn uptake (mg pot}^{-1}\text{)}}{100}$$

$$\text{Uptake of Zn derived from soil (mg pot}^{-1}\text{)} = \frac{\text{\% Zndfs x Total Zn uptake (mg pot}^{-1}\text{)}}{100}$$

Results and Discussion

The per cent zinc derived from fertilizer showed an elevated trend for PMK 3 (zinc - inefficient genotype) with an overall average of 4.78 % than that observed (2.38 %) for Norungan (zinc efficient) (Table 1). This apparently implies the inability of PMK 3 to mobilize nutrient from the soil and that it can respond only to elevated doses of applied zinc. Correspondingly, the zinc uptake from fertilizer increased with increasing dose of applied zinc (till 37.5 kg ha^{-1}) for PMK 3 (Table 2) whereas in Norungan, the maximum uptake was realized at 25 kg $ZnSO_4$ ha^{-1}. Rajarajan (1991) attributed the increase in %Zndff with incremental dose of applied zinc to the enhanced supply of Zn ions in soil solution. In Norungan, the dose of $ZnSO_4$ beyond 25 kg ha^{-1} might have resulted in dilution effect or interference of other competing ions with zinc resulting in physiological malfunction causing reduced uptake. This was the trend for shoot. In root, with increasing dose of applied zinc, sequestration in the root exhibited an increase and this was more prominently visualized in PMK 3.

Table 1. Genotypic divergence on the per cent zinc derived from fertilizer (%Zndff) in rice

Treat ments	PMK 3	Noru ngan	Mean	PMK 3	Noru ngan	Mean	PMK 3	Noru ngan	Mean
	Tillering Stage			Flowering Stage			Harvest Stage		
Shoot									
T_1	-	-	-	-	-	-	-	-	-
T_2	2.01	2.18	2.10	7.69	2.75	5.22	3.05	4.65	3.85
T_3	6.63	2.18	4.41	8.55	4.24	6.40	7.10	6.50	6.80
T_4	6.96	1.73	4.35	9.23	3.54	6.39	8.45	5.94	7.20
T_5	3.07	1.64	2.36	7.99	3.19	5.59	8.14	5.62	6.88
Mean	3.73	1.55	2.64	6.69	2.74	4.72	5.35	4.54	4.95
Root									
T_1	-	-	-	-	-	-	-	-	-
T_2	2.14	2.36	2.25	7.83	1.76	4.80	2.69	2.65	2.67
T_3	4.22	1.08	2.65	8.26	1.61	4.94	4.97	3.08	4.03
T_4	4.38	0.98	2.68	9.16	1.74	5.45	5.19	4.60	4.90
T_5	3.69	0.62	2.16	6.10	1.54	3.82	5.53	5.14	5.34
Mean	2.89	1.01	1.95	6.27	1.33	3.80	3.68	3.09	3.39
	V : CD (P = 0.05) : 0.07			V : CD (P= 0.05) : 0.12			V : CD (P= 0.05) : 0.12		
	T : CD (P = 0.05): 0.17			T : CD (P= 0.05) : 0.27			T : CD (P= 0.05) : 0.28		
	V x T : CD (P = 0.05): 0.24			V x T : CD (P= 0.05) : 0.38			V x T : CD (P= 0.05) : 0.40		

The per cent Zndfs was higher for Norungan (Table 3) but it assumed lesser magnitude in PMK 3. This is a natural corollary that when two sources of nutrients are provided, the increase in uptake from one source will preclude the uptake from the other (Rajarajan, 1991). The zinc uptake from the soil (both shoot and root) was higher for Norungan (Table 4) than that observed for PMK 3. As is the case observed here, Erenoglu *et al*., 1999 attributed the higher efficiency of rye to the greater Zn uptake capacity from soils. PMK 3 set forth an increase in soil zinc uptake with increasing dose of applied zinc, which could probably be attributed to the increase in root activity with enhanced zinc fertilization, which would have facilitated the roots to explore greater volume of soil. In Norungan, the uptake increased till 25 kg $ZnSO_4$ ha^{-1} and thereafter it declined with increasing dose of applied zinc. Irrespective of the source of zinc, the root zinc accumulation was higher for PMK 3 than that for Norungan.

At flowering stage, the retention of zinc in the root was more conspicuous compared to the other two stages. Rengel *et al.*1998 postulated that the zinc -

efficient wheat plants transported larger amount of ^{65}Zn to the shoots than did the Zn - inefficient plants. Zinc deficient roots might have had the binding sites with high affinity for Zn^{2+} occupied by Ca or other cations, which quickly exchange with added Zn^{2+} (Hart *et al.*, 1998). Vacuolar sequestration of micronutrients in the root cells which consequently affect the transport across the plasma membrane was reported by Rengel and Graham (1995). Thus in the present study Zn efficient Norungan had better Zn translocation to the shoot than Zn inefficient PMK 3.

Table 2. Genotypic divergence on zinc uptake from fertilizer (mg plant^{-1}) in rice

Treatments	PMK 3	Norungan	Mean	PMK 3	Norungan	Mean	PMK 3	Norungan	Mean
	Tillering Stage			Flowering Stage			Harvest Stage		
Shoot									
T_1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
T_2	0.0084	0.0116	0.0100	0.0385	0.0403	0.0394	0.0585	0.0863	0.0724
T_3	0.0310	0.0125	0.0218	0.0568	0.0696	0.0632	0.0902	0.1357	0.1130
T_4	0.0367	0.0112	0.0240	0.0685	0.0576	0.0631	0.1258	0.1221	0.1240
T_5	0.0329	0.0110	0.0220	0.0819	0.0477	0.0648	0.1701	0.0972	0.1337
Mean	0.0218	0.0093	0.0155	0.0491	0.0430	0.0461	0.0889	0.0883	0.0886
Root									
T_1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
T_2	0.0032	0.0035	0.0034	0.0437	0.0123	0.0280	0.0395	0.0249	0.0322
T_3	0.0048	0.0047	0.0048	0.0969	0.0169	0.0569	0.0485	0.0388	0.0437
T_4	0.0056	0.0051	0.0054	0.1048	0.0207	0.0628	0.0527	0.0478	0.0503
T_5	0.0069	0.0036	0.0053	0.1121	0.0154	0.0638	0.0680	0.0363	0.0522
Mean	0.0041	0.0034	0.0037	0.0715	0.0131	0.0423	0.0417	0.0296	0.0357

V : CD (P = 0.05) : 0.0004   V : CD (P= 0.05) : 0.0014   V : CD (P= 0.05) : 0.0023

T : CD (P= 0.05) : 0.0009   T : CD (P= 0.05) : 0.0030   T : CD (P= 0.05) : 0.0052

V x T : CD (P= 0.05) : 0.0129   V x T : CD (P= 0.05) : 0.0043   V x T : CD (P= 0.05) : 0.0074

Table 3. Genotypic divergence on the per cent zinc derived from soil (%Zndfs) in rice

Treatments	PMK 3	Norungan	Mean	PMK 3	Norungan	Mean	PMK 3	Norungan	Mean
	Tillering Stage			Flowering Stage			Harvest Stage		
Shoot									
T_1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
T_2	98.0	97.8	97.9	92.3	97.3	94.8	96.9	95.4	96.2
T_3	93.4	97.8	95.6	91.5	95.8	93.7	92.9	93.5	93.2
T_4	93.0	98.3	95.7	90.8	96.5	93.7	91.6	94.1	92.9
T_5	96.9	98.0	97.5	92.0	96.8	94.4	91.9	94.4	93.2
Mean	96.3	98.4	97.3	93.3	97.3	95.3	94.7	95.5	95.1
Root									
T_1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
T_2	97.9	97.6	97.8	92.2	98.2	95.2	97.3	97.3	97.3
T_3	95.8	98.9	97.4	91.7	98.4	95.1	95.0	96.9	96.0
T_4	95.6	99.0	97.3	90.8	98.3	94.6	94.8	95.4	95.1
T_5	96.3	99.4	97.9	93.9	98.5	96.2	94.5	94.9	h94.7
Mean	97.1	99.0	98.1	93.7	98.7	96.2	96.3	96.9	96.6

V : CD (P = 0.05) : 1.98   V : CD (P = 0.05) : 1.97   V : CD (P= 0.05) : NS

T : CD (P = 0.05) : NS   T : CD (P = 0.05) : 4.41   T : CD (P= 0.05) : 4.36

V x T : CD (P = 0.05) : NS   V x T : CD (P = 0.05) : NS   V x T : CD (P= 0.05) : NS

From the present study it is concluded that in the inefficient genotype PMK 3, the percent zinc derived from fertilizer was higher by 50% than that of the efficient genotype which implies that the nutrient mobilization capacity from the soil is higher for zinc efficient genotype. The Zn – efficient genotype Norungan had the capacity to mobilize Zn from native source whilst Zn-inefficient genotype utilized only the applied zinc. Zinc inefficient genotype viz., PMK 3 is fertilizer responsive whilst Zn efficient genotype – Norungan showed negative response to excessive dose of fertilizer. Utilization of zinc from unavailable pool and better translocation from root to shoot differentiates a zinc efficient genotype from an inefficient one as established by tracer technique.

Table 4. Genotypic divergence on zinc uptake from soil (mg plant^{-1}) in rice

Treatments	PMK 3	Norungan	Mean	PMK 3	Norungan	Mean	PMK 3	Norungan	Mean
	Tillering Stage			Flowering Stage			Harvest Stage		
Shoot									
T1	0.2533	0.4710	0.3622	0.4628	1.3489	0.9059	0.4895	0.9893	0.7394
T2	0.4084	0.5204	0.4644	0.6072	1.4264	1.0168	0.7762	1.7087	1.2425
T3	0.4357	0.6260	0.5278	0.8087	1.5710	1.1899	0.9773	1.8406	1.4090
T4	0.4914	0.5615	0.5265	0.8782	1.5683	1.2233	1.2591	1.9499	1.6045
T5	0.7213	0.5468	0.6341	0.9432	1.4454	1.1943	1.8591	1.7712	1.8151
Mean	0.4620	0.5451	0.5036	0.7400	1.4720	1.1060	1.0722	1.6579	1.3651
Root									
T1	0.0745	0.1282	0.1014	0.7874	0.4244	0.6059	1.1098	0.6926	0.9012
T2	0.1443	0.1475	0.1459	1.1264	0.6847	0.9056	1.1111	0.7831	0.9471
T3	0.1091	0.1530	0.1311	1.3357	0.8667	1.1012	1.4302	0.9763	1.2033
T4	0.1040	0.1733	0.1387	1.3823	1.0202	1.2013	1.4912	1.2425	1.3669
T5	0.1542	0.1895	0.1719	1.5039	1.1864	1.3452	2.4398	1.4266	1.9332
Mean	0.1172	0.1583	0.1378	1.2271	0.8365	1.0318	1.5164	1.0242	1.2703

Tillering Stage	Flowering Stage	Harvest Stage
V : CD (P= 0.05) : 0.1095	V : CD (P= 0.05) : 0.0279	V : CD (P= 0.05) : 0.0335
T : CD (P= 0.05) : 0.0244	T : CD (P= 0.05) : 0.0624	T : CD (P= 0.05) : 0.0749
V x T : CD (P= 0.05) : 0.0346	V x T : CD (P= 0.05) : 0.0882	V x T : CD (P= 0.05) : 0.1059

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Received after revision : March 24, 2017; Accepted : April 21, 2017