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Influence of intrinsic soil factors on genotype-by-environment interactions governing micronutrient content of milled rice grains

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Abstract

Rice as the major staple food of the world although is high in calories but deficient in essential micronutrients. This deficiency can be tackled by biofortification of rice grains with enhanced micronutrient content. Field experiments were carried out across three locations involving 17 genotypes, with the objective of assessing the genotype × environment interaction for native soil micronutrient assimilation in rice kernels and the influence of intrinsic soil variables on micronutrient content in rice grains. Contents of Fe, Zn, Cu and Mn in the milled kernels were found to vary significantly among genotypes and locations. There was significant genotype-by-environment interaction (GEI) for all the nutrients. Further, it was found that soil pH and soil available P had significant influence towards grain Fe content. Soil Zn availability and the electrical conductivity of the soil solution were found closely associated with grain Zn content. The two factors that regulated Cu and Mn content of rice grains were soil organic carbon content and soil Cu availability. The factorial regression (FR) approach to assess the role of significant intrinsic soil factor (s) on enrichment of specific micronutrient in the milled rice kernels showed differential genotype responses that ultimately determined grain micronutrient accumulation in each genotype. These determinants enabled prediction of genotypes suited to different environments. In certain cases, further examination of environmental co-factors is advocated to bring out a comprehensive picture of the micronutrient assimilation in milled rice grains.

Keywords: Rice; biofortification; micronutrients; genotype × environment interaction; factorial regression.

Introduction

To meet daily metabolic needs, human beings require 23 nutrient elements, which include macro and micronutrients that comes from dietary supply. Being the staple food for more than half of the world population, rice (*Oryza sativa* L.) meets 27% of calorie supply and 20% of protein intake of the developing nations (Tollens, 2007), but a meagre quantity of essential nutrient elements. In Asia, where 95% of the world rice production is consumed, 40 to 80 per cent of the caloric intake comes from rice and its products. Although rice grains in general are deficient in essential nutrient elements, 50% of those nutrients available are found in the bran layer consisting of pericarp, seed coat, nucellus and aleurone layer and 10% in the embryo. Rice grains are extensively consumed in the form of milled (polished) rice, and the milling process removes both bran and embryo leaving only about 28% of grain element content in the polished (white) rice (Hunt et al., 2002). Globally, micronutrients malnutrition results in egregious societal costs including learning disabilities among children, increased morbidity and mortality rates, leading to lower labor productivity, high healthcare costs and all other factors diminishing human potential, felicity, and national economic development (Welch and Graham, 2004). Nearly two-thirds of all deaths in children are primarily associated with micronutrient deficiencies (Caballero, 2003). Mineral elements most frequently lacking in human diets are iron (Fe) and zinc (Zn), although other elements such as iodine (I), calcium (Ca), magnesium (Mg), copper (Cu) and selenium (Se) can be deficient in the diets of some populations (Welch and Graham, 2004). About three billion people in the world suffer from micronutrient deficiencies, particularly of Fe and Zn and the said ratio is still rising (Mason and Garcia, 1993; Welch et al., 1997, Welch and Graham, 2004, Nestel et al., 2006). Billions of people are at risk of Zn deficiency, and it is ranked as the 5th leading risk factor for diseases such as diarrhoea and pneumonia in children (Virk et al., 2007). These deficiencies are caused by habitual diets that lack diversity (over-dependence on a single staple food), situations of food insecurity where populations do not have enough to eat (FAO/WHO, 2001) and low intake of rich sources of minerals such as vegetables, fruits, and animal and fish products. Most of those afflicted with micronutrient

malnourishment are dependent on staple crops like rice. Dietary diversification is one of the main strategies to alleviate micronutrient malnutrition. Food-based approaches to fulfil micronutrient requirements of humans have been receiving strong global support (FAO, 1996 and 2001). Bio-fortification is a process of nutritional enrichment of staple food crop with essential micronutrients, aminoacids and vitamins through plant breeding (Nestel et al., 2006) in order to alleviate nutritional disorders. According to Bouis (2003) plant breeding holds great promise for making a major, low-cost and sustainable contribution to reducing micronutrient, particularly mineral deficiencies in man. Successful biofortification strategies must initiate with screening of diverse germplasm for desired micronutrient content followed by suitable breeding strategies (Poletti et al., 2004). Although identification of donors forms the fundamental, understanding of trait expression to the optimal levels requires exploring environmental conditions and elucidating underlying genotype-by-environment interactions (GEI). It is well established that environmental and cultural factors do interact with plant-gene expression to influence the amount of a micronutrient accumulated in a seed or storage organ (Bouis and Welch, 2010). Assimilation of micronutrients in rice grains is followed by the nutrient uptake by plants which in turn is determined by genetic factors governing nutrient absorption, transport and the availability of nutrient elements in the soil. Soil availability of nutrients is determined by various soil factors with inter- and intra-location variability. Liang et al. (2007) have shown significant GEI existed between phytic acid content and bioavailability of the micronutrients as influenced by growing conditions. Studies have also demonstrated high expression of Fe and high-Zn grain traits in all rice environments tested, with some evidence of significant GEI that ultimately affect Fe and Zn concentrations in extreme environments (Graham et al., 1999). Multi-environment trials (MET) are conducted for selecting cultivars suited to different locations by assessing the cultivar's stability across environments. Although GEI in crop plants has been extensively studied, described and interpreted by means of several statistical methods (Crossa, 1990), not much information is available on the contribution of external variables (co-factors) that specifically describe the target environment. When information on external variables such as intrinsic soil properties is available, it can be regressed on genotypic scores using factorial regression (FR) models. FR models are linear models that explain GEI by differential cultivar sensitivity to explicit external environmental variables (Vargas et al., 1999). In this study we have assessed the response of rice genotypes across three different target environments, for grain micronutrient contents, in order to understand the factors responsible for micronutrient assimilation in grains. The major objectives were (a) to assess the genetic variability of grain micronutrient content among the improved rice germplasm lines originating from different ecosystems, (b) associating the expression of these traits with soil properties, and (c) predicting the influence of independent soil factors on the expression of grain micronutrient content of genotypes.

Results

Soil pH at test locations varied between neutral (Authorial), slightly alkaline (Coimbatore) and slightly acidic (Ambasamudram). All three locations had very low level of salinity. Organic carbon status was the maximum at Aduthurai, followed by Ambasamudram and Coimbatore. Available soil P status was low in all three locations, while soil Fe, Zn, Mn and Cu contents were registered well above the critical values fixed for soils (Table 2). Combined analysis of variance showed significant variations for genotypes, environments and genotype-by-environments components for grain micronutrient contents (Table 3). Moreover, grain content of all four micronutrients varied significantly among the genotypes and across the environments (Table 4). The grain Fe content of genotypes varied from 2.30 to 5.03 µg g^{-1}, while that of Zn ranged from 2.26 to 8.53 µg g^{-1}. Further, Cu content ranged between 2.21 to 3.20 µg g^{-1}) and Mn content between 1.59 and 4.32 µg g^{-1}. Highest grain Fe content was observed in BPT5204 (5.03 µg g^{-1}) and ADT46 (4.98 µg g^{-1}) while CB01536 was found to have highest Zn (8.53 µg g^{-1}) and Mn content (4.32 µg g^{-1}). The highest Cu content was recorded in grains of TP1086 (3.2 µg g^{-1}). Genotypes grown at Coimbatore and Aduthurai accumulated significantly higher grain Fe content (4.13 and 3.84 µg g^{-1}) than at Ambasamudram, while average grain content of Zn and Cu remained unchanged across three environments. However, Mn content of the genotypes grown at Aduthurai was significantly higher (3.28µg g^{-1}) than those grown at Coimbatore and Ambasamudram. Correlation of rank orders of the genotypes between locations showed non-significant associations for all grain micronutrients. Stepwise regression coefficients for grain micronutrient content against environmental variables (soil properties) are furnished in Table 5. Among eight soil variables, pH (β = 0.47) and available P (β = -0.10) were found to be associated significantly with grain Fe content with the model explaining of 99.8% variation. Soil Zn was found to positively influence (β = 0.23) grain Zn content significantly in association with EC with a negative influence (β = -0.39). This model had a coefficient of determination of 76.9%. Organic carbon and soil Cu content were found to contribute to two grain micronutrients, Cu and Mn, explaining 97.8% of the variation in both the cases. While there was an increase in grain Mn (β = 0.16), and reduction in grain Cu (β = -0.40) content with increase in organic carbon content. ANOVA for factorial regression model for grain micronutrient content wherein the genotype (G), environment (E, soil factors), and their interactions (GE) showed varying levels of significant influences is furnished in Table 6. For grain micronutrients, G component of variation was significant, while E component was non-significant for grain Zn and Cu content. However, in all cases the GE was significant. Soil pH had significant variation than soil P, and influenced grain Fe content considerably. However, G × available P and G × pH variances were significant. For the grain Zn content, G × EC and G × soil Zn interactions showed significance. Similar observations were made for Grain Cu content where in G × soil organic carbon and G × soil copper variances revealed significance. Further, grain Mn content and soil organic carbon were significantly different among the environments, as well as their interaction with genotypes. Genotype × soil copper interaction was also significant for grain Mn content.

Soil factors that significantly interact with different genotypes were graphically represented in Fig1. For instance, soil pH positively influenced grain Fe content in AD 02223, CB01001, ADT46, IWP and BPT5204, while negatively interacted with the rest of the genotypes. The genotypes, BPT5204 and ADT46 possessing high grain Fe accumulation, have shown strong negative contrasts for soil P and high positive contrasts for pH from other genotypes. A reverse of this situation was observed in ADT42 and AD01246 wherein the genotypes showed high negative contrasts for soil pH and positive contrasts for soil P. For those genotypes (CB01116 and CB21001), which had the

Table 1. List of genotypes studied for genotype × environment interactions for grain micronutrient contents.

Sl.No	Genotypes	Parentage	Source
1	TP1086	ADT 39 / IR 64	Thiruppathisaram, TN
2	CBMAS20001	IR50 / 9650	Coimbatore, TN
3	CB01116	IWP / ASD 16	Coimbatore, TN
4	CB01536	MTU 2067 / IR 64	Coimbatore, TN
5	CB21001	AD93019 / ADT 41	Coimbatore, TN
6	ADT42	AD9246 / ADT 29	Aduthurai, TN
7	ASD16	ADT31 / CO 39	Ambasamudram, TN
8	IR64	IR5657-33-2-1/ IR2061- 465 - 1- 5 - 3	IRRI, Philippines
9	AD01246	ADT 38 / IET13570	Aduthurai, TN
10	AD01252	CO 43 / IET13570	Aduthurai, TN
11	AD02223	CO 45 / IR 20	Aduthurai, TN
12	CB01001	CO 43 / ADT 38	Coimbatore, TN
13	CBMAS20005	Selected from IR 62266	Coimbatore, TN
14	CO43	Dasal / IR 20	Coimbatore, TN
15	ADT 46	ADT 38 / CO 45	Aduthurai, TN
16	Improved White Ponni	Taichung 65 / 2* Mayang Ebos 80	Aduthurai, TN
17	BPT5204	GEB 24 / TN 1 / Mahsuri	Bapatla, AP

Table 2. Characterization of environments.

Attributes	Ambasamudram	Aduthurai	Coimbatore
Geographic location	8°42'N 77°27'E	11°00'N 79°28'E	10°59'N 76°54'E
Altitude (m)	64.0	19.8	420.0
Soil pH	5.19	7.23	8.00
Soil electrical conductivity (EC) (dS m^{-1})	0.12	0.26	0.25
Soil total organic carbon (TOC) (%)	0.94	1.04	0.88
Soil phosphorus (P) (%)	8.20	9.60	10.80
Soil iron (Fe) (µg g^{-1})	41.67	12.00	40.67
Soil zinc (Zn) (µg g^{-1})	16.00	27.00	37.00
Soil copper (Cu) (µg g^{-1})	28.67	22.67	39.33
Soil manganese (Mn) (µg g^{-1})	38.33	36.67	13.00

lowest grain Fe content, differential contrast values were seen. Likewise for the individual genotypes, the magnitude and direction of the influence of the significant soil factors have been represented separately for four micronutrient content in the rice kernels.

Discussion

Improvement of nutritional health of people at high risk of micronutrient malnutrition can be achieved by increasing the micronutrient level in the edible product of staple foods through biofortification (Bouis and Welch 2010). Rice grains can be enriched with micronutrients using plant breeding efforts, since sufficient quantitative genetic variation exists within rice germplasm that can be exploited without negative impact on their productivity (Gregorio, 2002). Quantitative traits are complex in inheritance and are sensitive to the environments. Most contributing environments with regard to micronutrient accumulation in rice grains are the soils from which the grains are produced. It is well known that nutrient content and nutrient availability of soils differ spatially and temporally. Among these two types of variation, influences of spatial factors on the micronutrients are paramount. To understand the spatial influence of micronutrient accumulation, GEI studies using MET can be used to corroborate differential cultivar sensitivity to explicit external environmental variables (Vargas et al., 1999) thereby enabling breeders to select ideal cultivars for use in biofortification with essential micronutrients. In the routine GEI studies, performance of genotypes with respect to change in environments is assessed without providing any significance to individual variables that characterize environments. In the present investigation, for the first time, soil factors of three environments were integrated into GEI studies through a factorial regression approach (Denis, 1988; van Eeuwijk et al., 1996). The factorial regression models uniquely explains interaction effects in terms of genotype effects that are influenced by specific environmental factors, which can guide in explaining the influence of those external variables on GEI of grain micronutrient accumulation in different genotypes (Vargas et al., 1999). Locations of the present study had three different soil types ranging from acidic (Ambasamudram) to slightly alkaline (Coimbatore). Coimbatore soils are clay loamy (typic Haplustalf), while soils of Aduthurai are silty clay (typic Haplustert), whereas that of Ambasamudram were red clay. Soils of these locations were free from salinity and had sufficient levels of available micronutrient status. The variation in soil types and their differential feeding effect on micronutrients were evident from the observed significance for genotype -

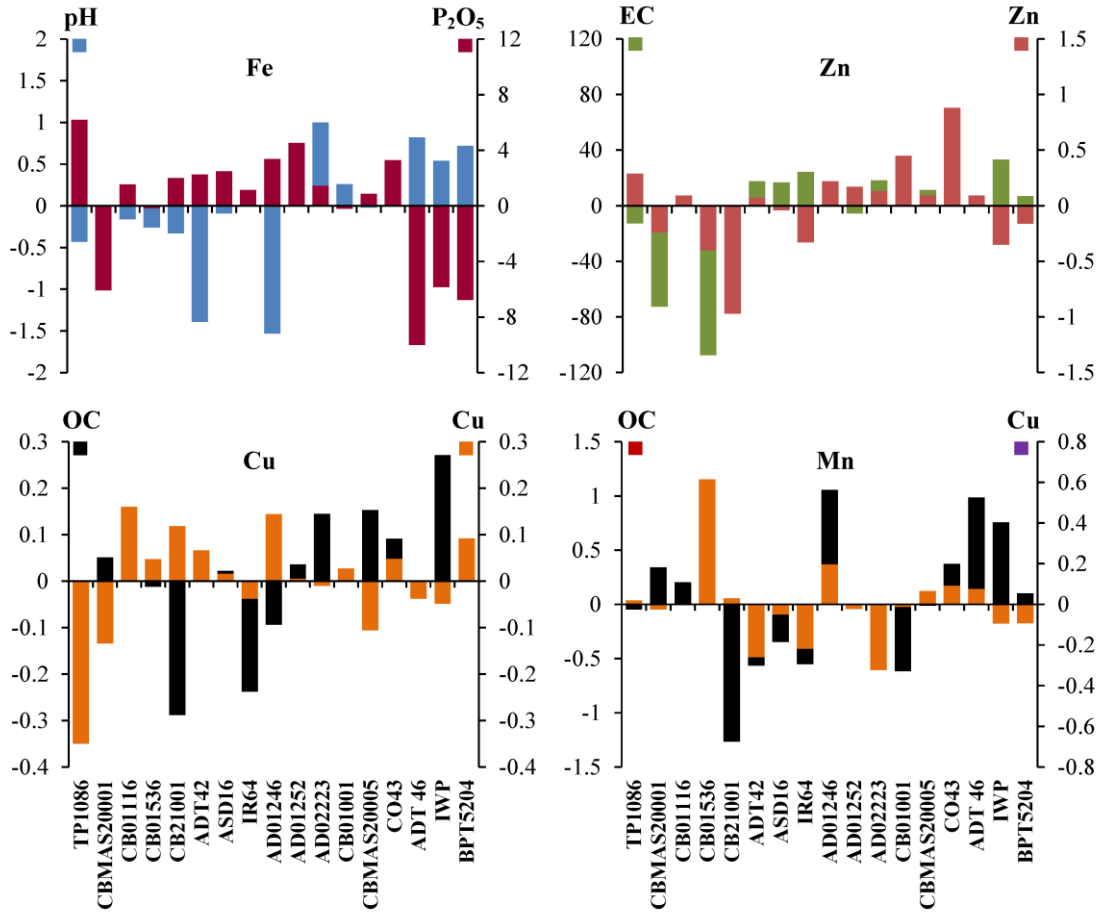

Fig 1. Genotype × environment contrasts of significant soil parameters defining grain micronutrient content in 17 rice genotypes for Iron (Fe) with relation to Soil pH and Soil P_2O_5; Zinc (Zn) with relation to Soil EC and Soil Zn content; Copper (Cu) and Manganese (Mn) with relation to Soil Organic Carbon (OC) and Soil Cu content.

by-environment component of variation. No significant association of rank orders between the genotypes under each locations indicated that GEI had a significant role in determining grain micronutrient content under each location. Slightly in contrast to some earlier studies that demonstrated micronutrient content in milled rice kernels to be relatively stable across environments albeit trivial influences from the soil factors (Graham et al., 1999), the present study could successfully apportion genotypic variations for realizable grain micronutrient content after milling, along with significant G × E interactions and soil factors accounting for it. This implies that considerable genetic variation lies within the gene pool of improved rice cultivars themselves, since no selection is being hitherto exercised on these traits. This forecast opportunities for biofortification of rice grains. Presence of multiple QTLs for Fe, Zn and phytate, which are under multigenic control were reported in rice (Stangoulis et al., 2007). They found that despite of having significant phenotypic correlations between micronutrients and phytate content, the QTLs of phytate were not located on the same chromosomal regions of Fe, Zn, and Mn related genes, underpinning the need for advanced breeding procedures such as marker assisted introgression. Several crop breeding works are in progress in order to biofortify the cereals and subtle characterization of the environment would help in predicting the expression of the trait. Among the micronutrients, iron (Fe) assimilation in rice grains was under the influence of two soil factors, pH and P fraction. The role of pH as the most significant factor in Fe availability in rice soils has been reported by many (Wenk and Bulakh, 2004; Thampatti et al. 2005; Su and Chen 2010). Although Fe is an element available in abundance in earth's crust, it often gets precipitated under natural or alkaline pH, limiting Fe availability to plants at elevated pH levels. However, in mostly flooded soils of rice ecosystems transient redox conditions in which temporal pH fluctuations occur, are a common feature since large amounts of acidity must be neutralized before pH values can approach neutrality (Moore and Patrick, 1993). These constant fluctuations in the redox environments may result in disequilibrium with respect to Fe

Table 3. Two-Way G ×E ANOVA for grain micronutrient content (µg g^{-1})

Source	Df	Mean squares			
		Fe	Zn	Cu	Fe
Block (A)	2	0.47	2.56	0.68	0.13
Location (B)	2	23.41**	4.38**	0.18***	3.03***
Genotype (C)	16	7.22***	29.22***	0.60***	5.31***
A×B	4	0.23	5.40	0.20	0.23
A×C	32	0.36	3.35	0.09	0.08
B×C	32	7.49***	40.61**	0.49***	3.44***
Error (A×B×C)	64	0.38	3.57	0.19	0.07
Total	152	-	-	-	-

, * Significant at 1% and <0.1% probability levels; Df – Degrees of freedom

Table 4. Genotypic and environmental means for grain micronutrient content.

Factors	Grain micronutrient content (µg g^{-1})*							
	Fe		Zn		Cu		Mn	
Genotypes								
TP1086	3.45	d-f	4.51	c-g	3.20	a	4.12	ab
CBMAS20001	3.57	c-f	6.81	a-d	2.76	a-c	3.45	cd
CB01116	2.30	g	5.74	a-f	2.28	c	2.29	gh
CB01536	3.31	d-g	8.53	a	2.41	bc	4.32	a
CB21001	2.36	g	7.26	a-c	2.76	a-c	3.50	cd
ADT42	4.24	a-d	3.72	d-g	2.55	bc	2.59	f-h
ASD16	3.25	d-g	2.88	fg	2.43	bc	1.59	i
IR64	2.63	fg	6.03	a-e	2.72	a-c	2.67	fg
AD01246	4.81	ab	4.06	d-g	2.93	ab	3.59	cd
AD01252	4.51	a-c	4.38	c-g	2.45	bc	2.53	f-h
AD02223	3.89	b-d	3.40	fg	2.63	a-c	2.27	gh
CB01001	3.72	c-e	5.34	b-g	2.21	c	2.51	f-h
CBMAS20005	2.56	fg	5.54	a-f	2.65	a-c	2.82	ef
CO43	2.82	e-g	7.96	ab	2.33	c	3.19	de
ADT 46	4.98	a	2.26	g	2.35	c	3.64	cd
Improved White Ponni	3.72	c-e	4.74	c-g	2.32	c	3.84	bc
BPT5204	5.03	a	3.36	fg	2.53	bc	2.16	h
SE (G)	0.20		0.63		0.11		0.10	
Environments								
Ambasamudram (ASD)	2.84	c	5.39	a	2.58	a	2.92	b
Aduthurai (ADT)	3.83	b	4.80	a	2.50	a	3.28	a
Coimbatore (CBE)	4.13	a	5.08	a	2.61	a	2.82	b
SE (E)	0.08		0.26		0.05		0.04	
SE (G×E)	0.35		1.09		0.20		0.17	
Spearman's rank correlations (ρ) between environments								
ASD vs ADT	-0.064		0.154		0.123		0.034	
ASD vs CBE	0.216		-0.047		0.154		0.422	
ADT vs CBE	0.289		-0.145		-0.059		0.233	

* Means having same letters are significantly not different by Tukey's honestly significant difference test

availability to plants, which favour differential assimilation of Fe depending on the genotype efficiency. Therefore, those genotypes that can assimilate more Fe at a wider pH levels would ideally be suited for Fe fortification in irrigated rice ecosystems. In this context, it is pertinent here to keep focus on genotypes such as ADT46, IWP and BPT5204 which could accumulate Fe at higher pH, although pH negatively influenced Fe accumulation in most of the genotypes (Fig 1). These genotypes must be possessing genetic mechanisms to harness Fe ions from soil even when more Fe ions are getting fixed up in the soil through precipitation. With regard to influence of soil P fraction on Fe assimilation, it has been reported that when there is relatively high P content in the Fe-oxide fraction in soil, P fraction interferes the Fe uptake in plants by permitting simultaneous P uptake along with high amounts of iron following the reductive dissolution of P-rich Fe oxides (Prade et al., 1988). Furthermore, soil P is known to form insoluble complexes with Fe at pH levels lower than 6.0. Soil P, therefore exhibits a negative influence on grain Fe content. Furthermore, soil P fraction is believed to have a positive correlation with phytic acid content in plants. Phytic acid (inositol hexaphosphate) is a principal storage form of P in plant tissues, especially in rice bran, and is a known anti-nutritional factor in Fe enrichment (Liang et al., 2007). Rice grains contain on an average 120mg.100g $^{-1}$ of phytic acid content in unpolished brown rice, which is reduced to less than 30% on milling, phytic acid can chelate mineral element such as Fe and Zn, making them fixed and unavailable further (Mendonza, 2002), thereby it plays a significant role in making Fe content low in milled rice grains, which are devoid of bran layer. So to improve the bio-mineral availability of Fe in milled grains, genotypes that are either

Table 5. Stepwise regression coefficients for grain micronutrient content (μg g^{-1}) and environmental variables (soil properties).

Environmental variables	Stepwise regression coefficients (β)			
	Grain Fe	Grain Zn	Grain Cu	Grain Mn
Soil pH	0.47**	ns	ns	ns
Soil EC	ns	-0.39**	ns	ns
Soil organic carbon	ns	ns	-0.40**	0.16**
Soil phosphorus	-0.10**	ns	ns	ns
Soil iron	ns	ns	ns	ns
Soil zinc	ns	0.23**	ns	ns
Soil copper	ns	ns	0.29**	0.12*
Soil manganese	ns	ns	ns	ns
Coefficient of determination (R^2)	99.80	76.90	97.80	97.80

*, ** Significant at $p< 0.05$, and $p< 0.01$ respectively; ns - non-significant

Table 6. Analysis of variance (ANOVA) for factorial regression showing means squares of variation (MS) for the fitted models, describing the effect of soil factors in determining grain micronutrient content.

Sources of variation	Grain Fe		Grain Zn		Grain Cu		Grain Mn	
	MS	*p*	MS	*p*	MS	*p*	SS	*p*
Genotype (G)	7.22	<0.001	29.23	<0.001	0.60	<0.001	85.00	<0.001
Environment (E)	23.41	<0.001	4.38	0.298	0.18	0.188	6.07	<0.001
Soil pH	46.75	<0.001	-	-	-	-	-	-
Soil P	0.08	0.648	-	-	-	-	-	-
EC	-	-	7.14	0.160	-	-	-	-
Soil Zn	-	-	1.62	0.502	-	-	-	-
Organic C	-	-	-	-	0.36	0.071	5.96	<0.001
Soil Cu	-	-	-	-	0.01	0.805	0.11	0.255
G × E	7.49	<0.001	40.61	<0.001	0.49	<0.001	110.02	<0.001
G × Soil pH	6.08	<0.001	-	-	-	-	-	-
G × Soil P	8.90	<0.001	-	-	-	-	-	-
G × EC	-	-	50.79	<0.001	-	-	-	-
G × Soil Zn	-	-	30.43	<0.001	-	-	-	-
G × Organic C	-	-	-	-	0.30	0.001	78.64	<0.001
G × Soil Cu	-	-	-	-	0.68	<0.001	31.38	<0.001
Residual	0.37	-	3.57	-	0.11	-	0.08	-

high Fe efficient or having low phytic acid content are desirable. Two genotypes of the present investigation, ADT46 and BPT5204, in spite of having negative influence of soil P fraction on grain Fe content, were looking efficient enough to accumulate more Fe ions, and were ranked the best genotypes with respect to grain Fe content. These varieties are good candidates worth further examination and utilization. Zinc (Zn) nutrition is very important both in terms of nutritional quality and metabolic health of rice plants and with respect to its role in ion transport. Although many soil factors affect the availability of Zn such as total Zn content, pH, organic matter content, clay content, calcium carbonate content, redox conditions, microbial activity in the rhizosphere, soil moisture status, concentrations of other trace elements, concentrations of macro-nutrients, especially phosphorus (Alloway, 2008), soil Zn concentration and EC of the soil solution were found to be the most determining factors leading to the grain accumulation of Zn. Self-regulation of Zn uptake in plants have been demonstrated by Zhou et al. (2005), who found that Zn accumulation had improved with increase in soil content and EC. Zn is an essential nutrient that plays important roles in numerous physiological processes in plants, such as serving as cofactors for many enzymes such as ZIP transporters and as the key structural motifs in transcriptional regulatory proteins such as Zn finger protein (ZFP) transcription factors. Rice is known to possess 12 putative ZIP transporters. The ZIP (Zn-regulated transporter (ZRT) /Fe-regulated transporter (IRT)-like protein) transporter proteins comprise of a large family of transition metal transporters in plants that have diverse functions to transport zinc, iron, copper, etc. (Chen et al., 2008). Hence, Zn must be available in soil in sufficient quantities as well as in absorbable form to trigger self-regulation of Zn ion transport in rice plants. Positive relationship of plant uptake of Zn and soil EC and soil Zn content has been reported earlier by Sahoo et al. (2003). Considerable deviations are observed in the genotypic responses for EC and soil Zn with accumulation of grain Zn content (Fig 1), allowing the selection of Zn efficient genotypes, amenable to biofortification. Those genotypes which are capable of assimilating more Zn in presence of high available soil Zn concentrations, increasing soil-available supply can result in significant increases in their concentrations in edible plant products (Graham et al., 2007; Welch, 1995). Furthermore, in the present study varying genotype responses were observed with respect to soil Zn content and EC, indicating that effect of other factors hitherto not studied in determining the ultimate grain Zn content in rice genotypes. Relevance of soil organic carbon in micronutrient nutrition is well established (Plaza et al., 2004: Tejada and Gozlavez, 2006), because high amount of easily decomposable organic matter leverages more intensive and rapid bacterial reduction processes (Ottow and Glathe, 1973; Munch and Ottow, 1983) making micronutrients easily available for absorption by plants. As observed in the present study, influence of OC on copper (Cu) and manganese (Mn) assimilation was reported earlier (Gao et al., 2003; Mousavi et al., 2010). Positive influence of soil Cu content on grain Cu content suggests that soil amendment of Cu can boost the assimilation into grains as seen in the case of Zn. Like that of

Zn and Fe, accumulation of Cu could also be under regulatory mechanisms of transporter proteins like ZIP transporters (Pedas et al., 2008), which are self-regulatory depending on the soil availability of concerned nutrient ions. Further, influence of soil Cu content on Mn assimilation implies that soil-nutrient interactions can also affect micronutrients uptake by crops (Aulakh and Malhi, 2005), either in positive or negative directions. In conclusion, the present investigation revealed significant effects of genotype, environment and genotype-by-environment in determining the food realizable micronutrient content of milled rice grains. This is slightly in contrast to the some earlier findings that micronutrient concentration remains stable across environments. Evaluating the genotype responses across environments in relation to soil factors further revealed most determining soil factors that regulated grain micronutrient accumulation. Nevertheless, variation across genotype response was observed towards the determining co-factors for different micronutrients, enabling for the prediction of genotypes suited to different environments. In some cases, further examination of environmental co-factors is needed to bring out a comprehensive picture of the micronutrient assimilation in milled rice grains.

Materials and methods

Genotypes and environments

Seventeen improved rice genotypes (Table 1) originated from various rice breeding programmes of Tamil Nadu Agricultural University, Coimbatore, India and International Rice Research Institute (IRRI), Philippines were used for this study. Multi-environment testing of the genotypes were conducted at three locations of Rice Research Station, Ambasamudram (Environment 1), Tamil Nadu Rice Research Institute, Aduthurai (Environment 2) and Paddy Breeding Station, Coimbatore (Environment 3) (Table 2). Experiments were conducted under native field conditions laid out in randomized block design replicated thrice. Uniform plot size of $10m^2$ per genotype was used in all locations with 20cm between rows and 10cm between plants. Standard agronomic practices were adopted in all the locations. Soil properties of the test locations were characterized by testing of soil from the fields wherein the genotypes were raised. Random soil samples were collected from 10 test points in the corresponding field from each locations and analyzed for soil variables such pH, electrical conductivity (EC), organic carbon content, available phosphorus (P) and micronutrients (Fe, Zn, Cu and Mn).

Soil analysis

Soil samples collected were dried under shade, powdered and passed through 2mm sieve. Ten grams of processed soil samples was mixed with 25ml of distilled water and pH and EC of the soil-water suspensions (1:2.5) were measured after 30 min (Jackson, 1973). Organic carbon content was determined using chromic acid wet digestion method (Walkey and Black, 1934). Soil available phosphorus (P) content was determined by Olsen's bicarbonate extraction method (Olsen et al., 1954) for soils having pH 6.0 and above and by Bray 1 method (Bray and Kurtz, 1945) for soils below pH 6.0. Micronutrient contents was determined by extraction with DTPA (Diethylenetriamine pentaacetic acid; 1.967 g DTPA and 1.47 g $CaCl_2$ mixed together, made upto 1 liter and pH adjusted to 7.3) shaken for 2 hours in a mechanical shaker. Filtered extract was fed to Atomic Absorption Spectrophotometer (Spectra AA 220, Varian, Australia) to read the micronutrient contents.

Grain analysis

Rice grain samples were air dried to 12-14 % moisture content and hand hulled by rubbing gently using wooden plank on a plain unpolished granite stone to obtain brown rice. Brown rice was hand milled by gentle sand paper (No. 100) abrasion to avoid any contamination from the metallic mills. The milled rice kernels were then finely powdered using a mortar and pestle. 500mg of powdered grain sample was treated with 12 ml of triple acid mixture (9:2:1 nitric: sulphuric: perchloric acid) and kept overnight for cold digestion and boiled on a hot plate till extract turned colourless. The extract was diluted to 50 ml and fed to Atomic Absorption Spectrophotometer to determine micronutrient concentration.

Data analysis

Analysis of variance (ANOVA) was performed to partition the genotype, environment and genotype-by-environment component of variation for grain micronutrient content across three locations. Taking grain micronutrient content as dependent target trait, and soil parameters as independent traits, a forward stepwise procedure was implemented to assess the multiple linear regressions to identify the component(s) that significantly describe the dependent variable. Further, using these variables a factorial Regression (FR) was conducted as per Vargas et al. (1999). All the analyses were done using GENSTAT v.9 (Payne, 2006).

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