RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
48 (4) 425-433, 2016

GENETIC VARIABILITY STUDIES FOR ZINC EFFICIENCY IN AEROBIC RICE

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SUMMARY

To study variation in zinc efficiency (ZE) among rice genotypes under aerobic condition, a pot experiment was conducted with 60 rice genotypes at two Zinc (Zn) levels +Zn and -Zn. A Zn deficient clay soil was used in our screening. Zn deficiency resulted in a marked decrease in shoot and root dry matter production of most genotypes after 28 days of growth. Genotypes were ranked according to their tolerance to Zn deficiency based on ZE, expressed as the ratio of shoot dry weight at Zn deficiency over that at adequate Zn supply. Substantial genotypic variation in ZE (63.1 to 92.6%) was found among rice genotypes. Genotypes CB-07-701-128, CB-00-11-4 and CB-06-803-2 were most tolerant to Zn deficiency whereas genotypes CB-07-701-283 and CO51 were the most intolerant genotypes.ZE correlated significantly ($P< 0.05$) with shoot Zn concentration ($r^2 = 0.7$), Zn translocation from root to shoot ($r^2 = 0.4$) and root surface area ($r^2 = 0.3$). These results indicate that shoot Zn concentration, root surface area and Zn translocation may be an important determinant of ZE under aerobic condition in rice. Estimation of genetic parameters revealed that high GCV and PCV were observed for the traits *viz.*, shoot zinc content, root zinc content, shoot dry weight and root dry weight under +Zn and –Zn conditions. All these traits also exhibited high heritability and high genetic advance as percentage of mean indicating the presence of additive gene action. Directional selection for these traits would be more effective for desired genetic improvement.

Keywords: Genetic variability, zinc efficiency, aerobic rice

Key findings: The genetic evaluation of rice genotypes for zinc efficiency under aerobic condition at +Zn and –Zn levels showed that significant genotypic differences existed among the rice genotypes for zinc efficiency. Tolerant genotypes can be utilized as donors in the genetic improvement of aerobic rice genotypes for zinc efficiency.

Manuscript received: September 30, 2015; Decision on manuscript: February 2, 2016; Manuscript accepted: October 17, 2016.

Communicating Editor: Bertrand Collard

INTRODUCTION

Rice (*Oryza sativa* L.) is the heart of Indian culture and the staple food crop consumed by more than 50% of the world's population, which is cultivated on over 163 million hectares with the production of 718 milliontonnes (FAO, 2013). About 90% of the world's rice is produced in Asia, out of which 20% is produced in India. The area, production and productivity of rice in India are 42.7 million ha, 105.2 million M tons and 2,462 kg per hectare respectively in

2012-13 (DAC, 2014). Asia's food security depends largely on the irrigated rice fields, which produces 3 quarters of all rice harvested. But irrigated rice is a profligate user of water, consuming half of all available fresh water resources. The increasing scarcity of water threatens the sustainability of the irrigated rice production system. In Tamil Nadu, rice is cultivated in an area of 2 million ha. Among the several production constraints, availability of irrigation water is a major factor as rice crop consumes about 70% of the water available for agriculture (VibhuNayar and Ravichandran, 2012). Therefore, ways must be sought to reduce water requirements of rice and increase its productivity. Experimental results and evidence from China and Brazil show that among several technologies, aerobic rice proves to be a viable technology by reducing water losses through seepage, percolation, and evaporation which saves water up to 50%. In India, aerobic rice varieties are bred by crossing lowland and upland varieties. So that, an aerobic rice production is developed which is a fundamentally different approach where rice is grown like an upland crop, such as wheat, on non-flooded aerobic soils, thereby eliminating continuous seepage and percolation and greatly reducing evaporation (Bouman, 2001).However, due to transition from flooding of paddy fields to aerobic system of rice cultivation many factors that determine nutrient (Zinc) bioavailability changes in addition to water deficit. This includes change in bulk soil pH which may either increase or decrease depending on the original soil pH (Liu, 1996). Increase in redox potential causes Fe oxidation, with concomitant acidification, precipitation of Fe $(OH)_3$ and adsorption of Zinc (Zn) on these oxides. Increased nitrification may cause plants to take up NO_3^- instead of NH_4^+which also causes the rhizosphere pH to increase. Organic matter on to which Zn can be adsorbed will be oxidized (Gao *et al.*, 2006). As a consequence, zinc deficiency becomes predominant under aerobic conditions thereby limiting crop growth and yield in addition to water deficit. Fertilization is not always an option to resolve Zn deficiency because of agronomic and economic factors, such as the relatively high cost of fertilizer (Graham and Rengel, 1993). Alternatively, exploiting genetic variability to breed staple crops with high Zn efficiency (ZE) could offer a sustainable and cost-effective way to overcome Zn deficiency problems under aerobic rice and make it a successful one. The term ZE is defined as the capacity of a genotype to grow well under -Zn deficient conditions and ZE is usually expressed as the ratio of shoot dry weight under Zn deficiency over that under adequate Zn supply (Graham *et al*., 1992). So far, however, breeding efforts has mainly focussed on the yield of aerobic genotypes and improvement of zinc efficiency needs attention. Hence, it is relevant to investigate whether there is variation in ZE among rice genotypes for utilizing it in crop improvement programme.

MATERIALS AND METHODS

A greenhouse pot experiment was conducted in Paddy Breeding Station, TNAU, Coimbatore to determine ZE for different rice (*Oryza sativa* L.) genotypes. Treatments include 2 Zn levels and 60 rice genotypes. Zn levels were -Zn (no Zn applied) and +Zn (12.5 mg Zn kg^1 of soil). Zn was applied together with the other nutrients as a solution of $ZnSO_47H_2O$, and was equivalent to 25 kg Zn ha^{-1}. A Zn-deficient soil (0.7 ppm) was collected from Wetland, TNAU, Coimbatore. The treatments were combined in a factorial randomized block design (FRBD) with 2 replicates. Twenty seeds of one genotype were sown per plastic pot containing 2 kg soil. At sowing time, each pot received a basal application of 110 mg N as CH_4N_2O, 390 mg P as KH_2PO_4 and 52.5 mg K as KCl. Deionized water was added in amounts sufficient to bring the soil water content to 80% of field capacity. After emergence, the plants were thinned to 12 seedlings per pot. The pots were watered daily with deionized water to 80% of field capacity. Plants were harvested 28 days after germination, because Zn deficiency problems are usually most severe in the first 2–4 weeks of growth (Doberman and Fairhurst, 2000). Shoots were cut off at ground level and soil was washed from the roots with tap water. Digital root images were made with a scanner (HP LaserJet). The resulting grayscale images were analyzed with imageJ root analysis software. All root images

were analyzed for root length and surface area. Shoots and roots were rinsed in deionized water, oven dried at 70°C for 48 h, and weighed. ZE was calculated as the ratio of shoot (root) dry weight under Zn deficiency over that under adequate Zn supply. Dried plant and root samples were digested in acid mixture (HNO_3+ H_2SO_4+ $HClO_4$) for Zn analysis (Hesse, 1971). Zn in plant digests was analyzed with an atomic absorption spectrophotometer (Varian SPECTRAA-200).

Data were recorded on characters *viz*. shoot length (cm), root length (cm), shoot zinc content (ppm), root zinc content (ppm), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g) and root surface area ($cm^2 plant^{-1}$).

Zn translocation was calculated as the ratio of Zn concentration in shoot toZn concentration in plant and expressed as percentage:

$$\text{Zn translocation} = \frac{\text{Zn in shoot}}{\text{Zn in plant}} \times 100$$

[Zinc Efficiency = (Dry weight at low Zn level/Experimental mean dry weight at low Zn)/(Dry weight at high Zn level/Experimental mean dry weight at high Zn)] (Graham, 1984; Fageria, 2001).

Statistical analysis of the data was performed using the GENRES statistical program (GENRES 1994) and SPSS (version 16) analytical software. Linear regression analysis was employed and LSD ($P < 0.05$) was used to test the difference among treatments. Analysis of variance was carried out as suggested by Panse and Sukhatme (1961). GCV and PCV were calculated using the formula suggested by Burton (1953). Broad sense heritability estimates were calculated by the method proposed by Lush (1949).

RESULTS

Out of 60 rice genotypes evaluated, mean performance of selected tolerant and susceptible genotypes for the zinc efficiency and related traits under +Zn and –Zn were given in Table 1 and 2. In this study, Zn deficiency resulted in a marked decrease in the dry weight of both shoot and root for most rice genotypes. Shoot based Zn efficiency varied from 63.1 to 92.6% among the rice genotypes. Under -Zn treatment root surface area ranged from 51.1 to 38.3 $plant^{-1}$ for zinc efficient genotypes and from 24.3 to 46.9 cm^2 $plant^{-1}$ for zinc inefficient genotypes (Table 1). Shoot Zn concentration at the -Zn treatment was below the marginal range of 10 – 20 mg kg^1 for all in efficient genotypes (Table 2) whereas zinc efficient genotypes had Zn content between 17.2–38.0%.

Results revealed that significant variation existed among the genotypes for all the traits studied under +Zn and –Zn. All characters showed low genotypic coefficient of variation than phenotypic coefficient of variation indicating the influence of environment on these traits. Skewness and kurtosis were studied for 10 characters (Table 3).

DISCUSSION

The ranking on shoot ZE was largely but not fully in accordance with that based on root ZE. Genotypes CB-08-702, CB-06-803, CB-08-701, CB-07-701-128, CB-07-701-129, CB-07-701-230, CB-07-701-181, CB-00-11-4, CB-00-11-21, CB-00-11-22, CB-06-803-2, Anna-4 and CB-00-15-44 were most tolerant to Zn deficiency with Zn values around 87.1 to 92.6% and showed slight or no deficiency symptom. In contrast, genotypes CB-07-701-115, CB-07-701-262, CB-07-701-264, CB-07-701-283, CB-00-11-7, CB-00-11-23, CB-00-755-2, PSBRC-83, CO51 and CB-07-701-265 showed severe deficiency symptoms and were the most intolerant genotypes with ZE values from 63.1% to 70.6%. Zn efficient genotypes showed a slight reduction in root and shoot biomass at the –Zn treatment compared to the +Zn treatment. But a severe reduction of root and shoot biomass was observed in Zn inefficient genotypes. Genotypic differences for Zn use efficiency for several crops have been reported by many workers (Cakmak *et al.*, 2001; Graham *et al.*, 1992; Rangel 2001).

Zn efficient genotypes showed a slight reduction in root and shoot biomass at the –Zn treatment compared to the +Zn treatment.

Table 1. Shoot dry weight, root dry weight and Zn concentration in shoots and roots, Zinc translocation ZE in shoot and root of selected tolerant genotypes grown in a Zn deficient soil with (+Zn) and without (-Zn) Zn application in pot experiment.

Tolerant genotypes	Shoot dry weight (g)		Root dry weight (g)		Shoot Zinc content (ppm)		Root Zinc content (ppm)		Zinc translocation (%)		Root surface area (cm^2plant^{-1})		ZE in shoot (%)	ZE in root (%)
	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)		
CB-08-702	1.6	1.5	1.0	0.8	63.2	36.7	76.2	36.5	48.0	50.1	49.2	45.1	89.4	85.6
CB-06-803	1.2	1.1	1.0	0.9	62.8	38.0	80.6	38.9	45.3	49.3	53.5	46.9	88.4	88.9
CB-08-701	1.0	0.9	1.1	0.9	63.1	37.8	81.8	34.9	44.8	52.0	54.2	51.1	89.2	81.5
CB-07-701-128	0.8	0.8	1.0	0.8	59.3	33.1	81.0	40.6	42.7	44.9	38.9	37.9	92.6	86.3
CB-07-701-129	1.1	1.0	0.9	0.8	61.9	30.5	79.0	39.5	45.6	43.5	35.3	34.1	88.7	88.2
CB-07-701-230	0.7	0.6	1.1	0.9	60.9	32.9	84.0	40.0	42.2	45.1	37.4	32.9	88.1	84.6
CB-07-701-181	0.9	0.8	0.8	0.7	53.0	17.2	71.4	35.0	43.4	33.0	39.0	36.3	87.1	85.3
CB-00-11-4	1.3	1.2	1.2	1.1	56.5	34.7	86.0	49.5	38.2	41.2	43.6	37.0	92.3	86.7
CB-00-11-21	1.2	1.1	1.4	1.2	57.6	32.4	84.0	47.8	39.9	40.4	47.5	35.1	89.6	84.3
CB-00-11-22	1.2	1.1	1.4	1.2	61.5	35.9	81.4	44.9	44.0	44.4	43.0	34.6	87.2	85.4
CB-06-803-2	1.2	1.1	1.0	0.9	62.5	37.2	79.2	42.4	45.8	46.7	46.4	39.7	92.1	88.1
Anna-4	1.0	0.9	1.0	0.8	61.5	36.9	83.5	46.4	42.9	44.2	52.3	44.7	87.3	84.4
CB-00-15-44	1.2	1.1	0.9	0.8	61.8	35.4	73.2	36.0	49.0	49.5	48.1	42.0	89.0	86.6

Table 2. Shoot dry weight, root dry weight and Zn concentration in shoots and roots, Zinc translocation ZE in shoot and root of selected susceptible genotypes grown in a Zn deficient soil with (+Zn) and without (-Zn) Zn application in pot experiment.

Susceptible genotypes	Shoot dry weight (g)		Root dry weight (g)		Shoot Zinc content(ppm)		Root Zinc content(ppm)		Zinc translocation (%)		Root surface area (cm^2plant^{-1})		ZE in shoot (%)	ZE in root (%)
	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)	(+Zn)	(-Zn)		
CB-07-701-115	1.1	0.7	0.9	0.6	35.9	9.0	60.1	20.0	30.3	31.0	41.2	38.4	66.8	67.7
CB-07-701-262	0.5	0.3	0.7	0.6	37.4	6.1	61.1	17.0	32.3	26.6	34.2	31.8	68.3	77.1
CB-07-701-264	0.3	0.2	0.4	0.3	39.2	7.7	64.2	15.0	32.8	33.9	34.7	30.9	68.7	71.2
CB-07-701-283	0.8	0.5	1.0	0.7	42.3	6.2	60.5	14.5	40.4	30.1	44.0	39.0	63.1	71.9
CB-00-11-7	1.2	0.8	1.4	0.9	56.5	8.6	70.8	20.7	46.8	29.4	40.8	41.0	66.6	65.8
CB-00-11-23	0.9	0.6	1.3	0.9	58.0	6.6	66.6	28.0	51.1	19.0	46.0	43.0	70.1	73.5
CB-00-755-2	1.2	0.8	1.1	0.7	44.0	10.8	71.3	23.1	34.3	31.9	35.4	33.1	66.4	66.2
PSBRC-82	0.8	0.6	1.2	0.9	52.9	10.5	71.5	19.5	43.3	35.0	41.3	38.0	70.6	73.7
CO51	0.7	0.4	0.5	0.3	54.3	6.4	65.5	13.0	49.0	33.0	31.4	27.1	64.1	64.6
CB-07-701-265	0.5	0.4	0.6	0.4	10.5	6.9	55.5	16.0	36.0	30.3	29.5	27.1	63.2	67.4

Table 3. Estimation of different genetic parameters for zinc deficiency tolerance component traits of 60 rice genotypes grown in a Zn-deficient soil with (+Zn) and without (-Zn) Zn application in pot experiment under aerobic conditions.

Character		Maximum	Minimum	Mean	CD (5%)	PCV	GCV	h^2	GAM	Skewness		Kurtosis	
ZS	+Zn	7.0	1.0	2.5	0.8	71.1	68.9	93.9	16.3	0.9	*	-0.0	
	-Zn	7.0	1.0	4.0	0.9	53.3	52.1	95.4	15.3	-0.0		-1.1	*
SL	+Zn	63.4	30.5	40.8	2.6	28.7	20.1	49.2	29.0	0.9	**	1.1	**
	-Zn	62.1	24.4	37.1	2.8	26.5	21.1	59.0	40.9	0.8	**	0.4	**
RL	+Zn	37.5	23.0	30.8	3.4	34.9	21.3	37.0	26.8	0.0		-0.9	**
	-Zn	36.9	20.1	27.5	2.7	35.7	25.8	52.0	38.4	0.2	**	-0.1	
SZC	+Zn	67.4	10.5	51.8	3.1	35.0	31.7	82.0	59.1	-0.2	**	-1.1	**
	-Zn	39.3	6.1	17.7	2.2	68.3	66.0	88.4	31.4	0.7	**	-1.2	**
RZC	+Zn	90.1	50.5	70.2	2.3	44.3	41.4	87.4	79.8	0.3	**	-0.7	**
	-Zn	50.1	13.0	26.7	3.0	28.4	24.4	74.0	43.3	0.7	**	-0.9	**
SFW	+Zn	16.7	8.3	11.6	1.8	22.6	16.8	55.2	25.7	0.5	**	-0.3	**
	-Zn	14.9	7.2	10.0	1.2	23.4	14.5	38.4	18.5	0.9	**	0.1	
SDW	+Zn	1.6	0.3	1.0	2.9	35.0	28.8	67.6	48.8	-0.1	**	-0.2	
	-Zn	1.5	0.2	0.8	3.6	28.6	23.7	69.0	40.7	0.1	**	-0.3	
RFW	+Zn	4.8	2.3	3.7	0.5	21.9	12.1	30.7	13.9	-0.2	**	-0.9	
	-Zn	3.9	1.2	2.8	0.5	25.6	17.6	47.4	25.0	-0.3	**	-0.2	
RDW	+Zn	1.5	0.4	1.0	0.7	33.9	27.8	67.1	46.9	0.0		-0.4	
	-Zn	1.2	0.2	0.8	1.1	28.7	22.4	60.8	36.0	-0.0		-0.4	
RSA	+Zn	54.2	26.2	41.7	1.8	20.7	14.3	47.9	20.5	-0.1	**	-0.7	
	-Zn	51.1	24.3	37.6	1.5	19.2	12.1	39.7	15.7	-0.1	**	-0.71	

**,* significant at 1%, 5% levels respectively.ZS – Zinc Score, SL – Shoot Length (cm), RL – Root Length (cm), SZC – Shoot Zinc Content (ppm), RZC – Root Zinc Content (ppm), SFW – Shoot Fresh Weight (g), SDW – Shoot Dry Weight (g), RFW – Root Fresh Weight (g), RDW – Root Dry Weight (g), RSA – Root Surface Area (cm^2 $plant^{-1}$), GCV-Genotypic Coefficient of Variation, PCV-Phenotypic Coefficient of Variation, h^2-Heritability, GAM – Genetic Advance as per cent of Mean.

However, a severe reduction of root and shoot biomass was observed in Zn inefficient genotypes (Table1). The dramatic decline in shoot and root biomass in Zn inefficient genotypes when compared to the efficient ones as witnessed in this present study was earlier reported for lowland rice genotypes by Sudhalakshmi *et al.* (2007). This is because, utilization efficiency in terms of dry matter production per unit of Zn uptake may be linked to differences in the ability of a genotype to maintain an optimal activity of the important Zn regulated enzymes, *viz.* superoxide dismutase (SOD) and carbonic anhydrate (CA). There is also large number of enzymes in which Zn is an integral component of the enzyme structure. Activity of these enzymes has been correlated with Zn availability to the plants. Differences in internal utilization or mobility of Zn have been shown to be involved in expression of Zn efficiency (Hafeez *et al.*, 2009).

Zn application resulted in an increase to on average 51.8 mg Zn kg^{-1} dw, which is considered above sufficiency level. In the +Zn treatment, no Zn toxicity symptoms were found and all genotypes grew well. This indicates that plant growth in the -Zn treatment was limited by Zn availability indeed. Shoot Zn concentration and ZE were positively and significantly (P = 0.05) related, with shoot Zn concentration explaining 71.3% (adj. r^2) of variation in ZE (Figure 1). It was contrary to what was reported for wheat (Cakmak *et al.*, 1997) and common bean (Hacisalihoglu *et al.*, 2004).

The uptake of the relative immobile Zn^{2+} by plant roots can be determined by root uptake surface area and so root surface area of genotypes were determined. Root surface area correlated significantly with Zn efficiency and explained 38% of variation in Zn efficiency (Figure 2). Root surface area correlated significantly with Zn efficiency and explained 38% of variation in Zn efficiency which was consistent with the results on rice (Gao *et al.*, 2005). To assess whether the translocation of Zn from root to shoot is a factor that is involved in ZE, the percentage of Zn present in the shoot was calculated. It varied from 13.8 to 63.3% in the -Zn treatment (Table 1).

The Zn efficient genotypes CB-07-701-128, CB-00-11-4 and CB-06-803-2 translocated around 50% to the shoot. The most inefficient genotype CB-07-701-283 translocated only 17.9% of its Zn to the shoot. The regression between Zn translocation as independent and ZE as dependent variable was positive and significant and explained 41.9% of variation in Zn efficiency (Figure 3). Therefore it is evident that zinc efficiency under aerobic condition in rice is determined by shoot zinc concentration, root surface area and zinc translocation. Zn absorbed by roots can be rapidly transported to the shoots. Higher Zn translocation was thought to be a mechanism to explain the genotypic differences in ZE among wheat genotypes by Cakmak *et al.* (2001).

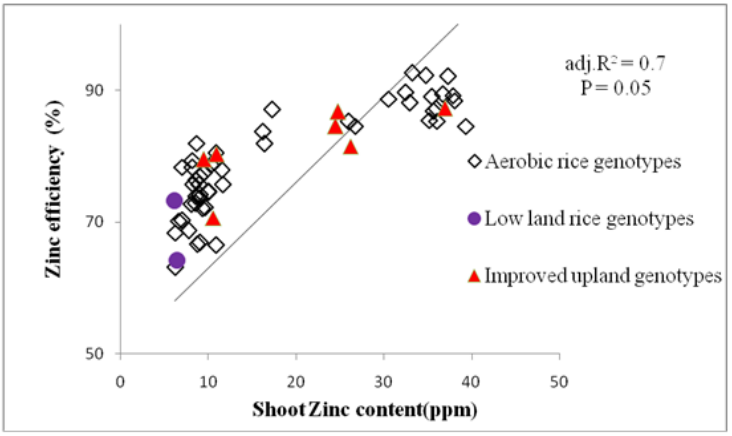

Figure 1. Relationship between Zn efficiency (%) and shoot zinc content(ppm) for 51 aerobic rice (◊),2 lowland rice genotypes (●) and 7 improved rice genotypes (Δ).

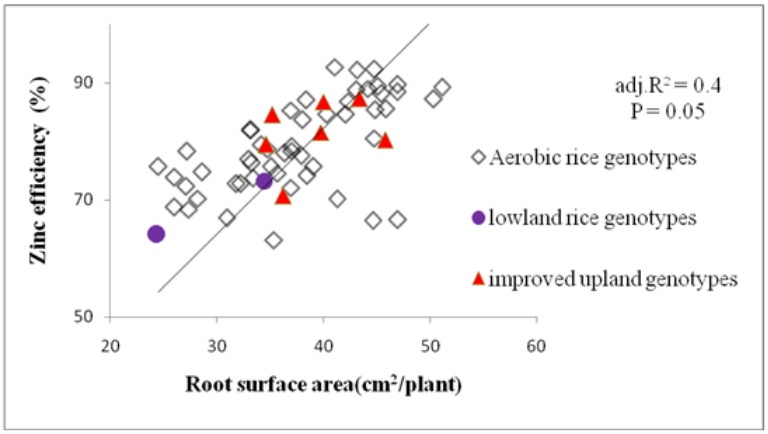

Figure 2. Relationship between Zn efficiency (%) and root surface area(cm^2/plant) for 51 aerobic rice (◊),2 lowland rice genotypes (●) and 7 improved rice genotypes (Δ).

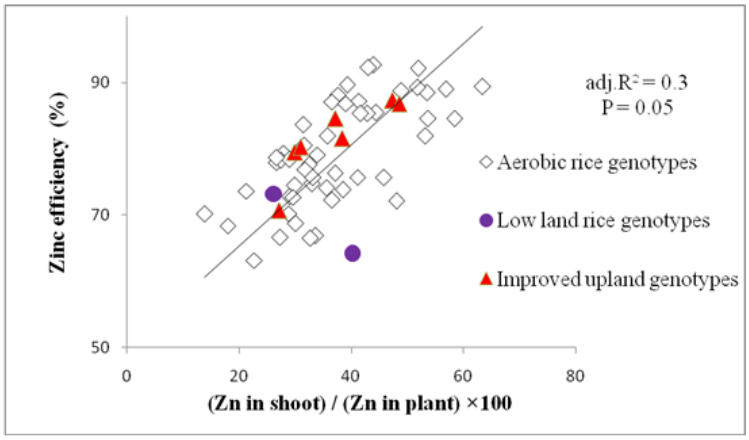

Figure 3. Relationship between Zn efficiency(%) and zinc translocation for 51 aerobic rice (◊),2 lowland rice genotypes (●) and 7 improved rice genotypes (Δ).

As the success of crop improvement depends upon the extent of genetic variability present in a crop, genetic parameters were estimated for the various traits determining zinc efficiency under aerobic condition in rice. However, a close proximity between GCV and PCV for all the characters under study indicates less influence of environment on these characters. High GCV, PCV, high heritability and high genetic advance (GAM) as percentage of mean were observed for the traits viz., shoot zinc content, root zinc content, shoot dry weight, root dry weight under control and stress conditions. High GCV, PCV, moderate heritability and high genetic advance as percentage of mean was observed for the traits namely shoot length and root length under control and stress condition. All these traits also exhibited high heritability and high genetic advance as percentage of mean indicating the presence of additive gene action. Directional selection for these traits would be more effective for desired genetic improvement. Information on heritability of root traits are still very limited (Gowda *et al.*, 2011). Ekanayake *et al.* (1985) has reported the heritability of more than 50% for root length and root dry weight. Bekele *et al.*

(2013) reported high GCV, PCV, heritability and GAM for zinc content.

The study of distribution of quantitative traits using skewness and kurtosis provides information about nature of gene action and number of genes controlling the traits respectively. The skewed distribution of a trait in general suggests that the trait is under the control of non-additive gene action and is influenced by environmental variables. Positive skewness is associated with complementary gene interactions while negative skewness is associated with duplicate (additive x additive) gene interactions. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait. Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions. The traits with leptokurtic and platykurtic distribution are controlled by fewer and large number of genes respectively. Among the traits, positive skewness was observed for root zinc content and root surface area. Negative skewness was observed for root fresh weight and root surface area in both conditions. Hence directional selection could be made for root surface area. In case of kurtosis, the character shoot length in normal condition recorded leptokurtic nature. Due to narrow variability, selection may not improve the *per se* performance for these traits. The traits shoot dry weight, root fresh weight, root dry weight and root surface area had platykurtic nature. Due to wider variability, directional selection could be made to improve the per se performance of these traits.

Thus, genetic evaluation of rice genotypes for zinc efficiency under aerobic condition at +Zn and –Zn levels showed that significant genotypic differences existed among the rice genotypes for zinc efficiency. This variation among rice genotypes for zinc efficiency offers opportunities for breeding as a tool to resolve Zn deficiency problems in aerobic rice. Genotypes CB-07-701-128, CB-00-11-4 and CB-06-803-2 were most tolerant to Zn deficiency whereas genotypes CB-07-701-283 and CO51 were the most intolerantgenotypes. Tolerant genotypes can be utilized as donors in the genetic improvement of aerobic rice genotypes for zinc efficiency.

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