Plant Environment Development 3(2):14-18, 2014 (July)

Print ISSN 1994-1501
Online ISSN 2311-3529

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Screening of Zn-efficient rice through hydroponic culture

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Abstract

Screening for Zn-efficiency in a number of Bangladeshi rice (Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29, BRRI 51, Parizat, BRRI 33 and BRRI 28) genotypes were studied based on different morphological and physiological parameters through hydroponic culture. Root length and Root fresh weight were not significantly reduced under Zn deficiency in Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51 compared to the plants grown on Zn sufficient conditions. In contrast, Parizat, BRRI 33 and BRRI 28 showed significantly decreased root length and root biomass under Zn deficiency compared to the controls. Again, Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29, and BRRI 51 did not show prominent decrease in shoot height and shoot fresh weight due to Zn deficiency. However, these parameters were significantly decreased in Parizat, BRRI 33 and BRRI 28 under Zn shortage compared to Zn-sufficient controls except for shoot fresh weight in BRRI 33. Furthermore, chlorophyll concentrations (a and b) were not significantly reduced in Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51 due to Zn deficiency compared to controls. In contrast, significant reduction in chlorophyll concentrations (a and b) were observed in Parizat, BRRI 33 and BRRI 28 under Zn deficiency compared to Zn-sufficient plants. Based on these findings, tolerance to Zn deficiency in these rice genotypes can be categorized as: tolerant (Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29, BRRI 51), and intermediate (BRRI 33) and highly sensitive (Parizat and BRRI 28). The ranking can be applied in plant breeding program and may have great advantage over conventional methods. This study also demonstrates the effectiveness of hydroponic culture as an efficient method to screen nutrient-efficient crop plants including rice.

Keywords: Zn-efficiency, hydroponic culture, Pokkali, morphological features.

INTRODUCTION

Zinc (Zn) is an essential element in all organisms. In oxidized Zn(II) form, it is found throughout biology, it acts as a catalytic or structural co-factor in a large number of enzymes and regulatory proteins (Maret, 2009). Well-known examples in plants include the enzymes carbonic anhydrase and alcohol dehydrogenase, and the structural Zn-finger domains mediating DNA-binding of transcription factors and protein–protein interactions (Sriram and Lonchyna, 2009).

Zn deficiency in cereal plants, including rice, is a well-known problem that causes reduced agricultural productivity all over the world. Exploiting genetic variability to breed staple crops with high Zn efficiency could offer a sustainable and cost-effective way to overcome Zn deficiency problems. Furthermore, there is a need to achieve maximum food production through increasing the productivity of the land by ameliorating nutritional stresses like Zn deficiency (Malakouti, 2008).

Therefore, it is indeed a priority work to determine the relative Zn efficiency of stable crops like rice. Screening of Zn deficiency tolerant lines would facilitate the breeding program having the high Zn (and Fe) rice. If this is successful, the majority of total production could eventually be biofortified. Certain factors should be considered when determining an appropriate screening protocol for nutritional characteristics. Firstly, because plant breeders usually evaluate large numbers of plants, screening procedures must be simple. If possible the procedure should be effective for plants at the seedling stage because of the ease with which seedlings can be produced in large numbers. Secondly, morphological and physiological features should be obvious under field as well as laboratory screening (Gerloff, 1987).

Among the different screening methods, hydroponic culture has often been used for screening for tolerance to mineral deficiency and toxicity. Screening in hydroponic culture allows for rapid screening, it overcomes seasonal effects and provides disease free conditions (Dragonuk et al., 1989). A number of different wheat genotypes have been screened for their response to low Zn in Zn-deficiency calcareous soil and significant different in Zn-efficiency have been consistently found among few genotypes in both field and growth chamber experiments (Cakmak et al. 1999; Hacisalihoglu et al., 2001). Despite the importance of Zn-efficient genotypes in food production and biofortification study, no efforts have been made to screen for Zn-efficient genotypes among Bangladeshi rice lines.

BRRI has recently released a Zn-enriched rice variety (BRRI-62) by crossing two different parents. Growing Zn enriched cultivars and Zn-efficient cultivars are equally important as it does not need application of any Zn compounds. Therefore, genotypic screenings of Zn-efficient rice on the basis of morphological and physiological features have long been the subjects of intensive studies. Thus, the present study was aimed at screening different rice genotypes mainly cultivated in Bangladesh tolerant to Zn deficiency. Further aim of this study was to establish the hydroponic method for screening rice plants under Zn deficiency.

MATERIALS AND METHODS

Plant materials

Eight cultivars of rice (cv. Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29, BRRI 51, Parizat, BRRI 33 and BRRI 28), with different tolerance to Zn deficiency, were used in this study.

Germination and growth conditions for hydroponic culture

Before growing, seeds were surface sterilised in 70% ethanol and 5% sodium hypochlorite for 1 and 15 min, respectively. Seeds were then rinsed five times in deionised water. Seeds were germinated on moist filter paper wetted with deionised water for 3–4 days in the dark at room temperature. Only healthy and uniform seedlings were transplanted to solution culture. A basal nutrient solution (Hoagland and Arnon 1950; Pandey et al., 2012) was used with the following nutrient concentrations (µM): KNO_3 (16000), $Ca(NO_3)_2.4H_2O$ (6000), $NH_4H_2PO_4$ (4000), $MgSO_4.7H_2O$ (2000), KCl (50), H_3BO_3 (25), Fe-EDTA (25), $MnSO_4$. $4H_2O$ (2), $Na_2MoO_4.2H_2O$ (0.5), $CuSO_4.5H_2O$ (0.5) and Zn as $ZnSO_4$ at two levels viz. 0.01 (Zn-deficient) and 2.0 µM (Zn-sufficient/control). Target pH values (pH 6.0) were obtained by titrating the basal solution with KOH or H_2SO_4. Plants were grown in 2 L of aerated solution and the environment was strictly maintained under 10 h light and 14 h dark (550–560 µmol s-1 per µA).

Measurement of morphological characters

Shoot height, root length, shoot fresh weight and root fresh weight were measured on 2-week old plants grown on solution culture. Total roots developed by each plant sample were washed in distilled water to remove nutrient and then quickly blotted in tissue paper.

Determination of chlorophyll content in leaves

A chlorophyll content of leaves was determined spectrophotometrically as described previously by Lichtentaler and Wellburn with modifications (1985). Firstly, 100 mg leaf was weighted and placed in 95% acetone in a 5 ml falcon tube. The leaf sample was then grinded using mortar-pestle. The homogenate was filtered through whatman filter and was centrifuged at 2500 rpm for 10 min. The supernatant was separated and the absorbance were read at 662 (chlorophyll a) and 646 (chlorophyll b) on spectrophotometer. The amount of these pigments was calculated according to the formula given by Lichtentaler and Wellburn (1985).

Statistical analysis

Statistical analyses and graphs were performed using Genstat software (14^{th} Edition) and Graphpad Prism 5 software, respectively. Statistical significance was set at $P \leq 0.05$.

RESULTS

Root morphological features

Root length and root fresh weight were measured in all rice genotyes used grown in both Zn sufficient and Zn deficient hydroponic conditions. Though Zn deficiency caused slight decrease in root length, these decreases were not statistically significant in Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51 under Zn stress compared to the plants grown on Zn sufficient conditions (Table 1). Similar patterns were also observed for root fresh weight in the aforesaid genotypes in response to Zn deficiency. In contrast, Zn deficiency caused significant decline in root length and root fresh weight in Parizat, BRRI 33 and BRRI 28 compared to controls (Table 1).

Table 1. Root parameters in different genotypes of rice grown on Zn sufficient (Zn +) and Zn deficient (Zn -) hydroponic culture. There were three replications for each sample. Data were taken on three weeks old plants.

Cultivars	Root length			Root fresh weight (g)		
	Zn +	Zn -	t-test	Zn +	Zn -	t-test
Pokkali	9.16±1.34	9.155±1.60	*	0.050±0.0005	0.050±0.0001	*
BRRI 62	9.0±1.73	8.5±2.12	*	0.043±0.011	0.041±0.007	*
BRRI 3	13.25±0.35	12.6±0.84	*	0.042±0.0041	0.041±0.0015	*
BRRI 39	5.4±0.14	4.8±0.28	*	0.020±0.0033	0.019±0.0014	*
BRRI 29	4.3±1.13	3.95±0.63	*	0.026±0.0026	0.022±0.0012	*
BRRI 51	7.0±1.13	6.25±0.21	*	0.028±0.0042	0.024±0.0021	*
Parizat	11.45±0.06	6.25±0.07	**	0.044±0.0059	0.024±0.0046	**
BRRI 33	10.4±0.84	4.8±0.28	**	0.039±0.0025	0.023±0.0004	**
BRRI 28	7.55±0.07	4.75±0.35	**	0.032±0.0014	0.021±0.0001	**

*statistically non-significant **statistically significant

Shoot morphological features

Alike root parameters, shoot height and shoot fresh weight were measured on 2-week old all rice genotypes grown in both Zn sufficient and Zn deficient hydroponic conditions. In Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51, Zn deficiency caused slight (non-significant) decrease in shoot height and shoot fresh weight compared to the plants grown on Zn sufficient hydroponic culture (Table 2). However, shoot height and shoot fresh weight were severely affected by Zn deficiency in Parizat, BRRI 33 and BRRI 28 (Table 2).

Table 2. Shoot parameters in different genotypes of rice grown on Zn sufficient (Zn +) and Zn deficient (Zn -) hydroponic culture. There were three replications for each sample. Data were taken on three weeks old plants.

Cultivars	Shoot height			Shoot fresh weight (g)		
	Zn +	Zn -	t-test	Zn +	Zn -	t-test
Pokkali	14.3±1.69	13.5±0.84	*	0.047±0.0043	0.047±0.0022	*
BRRI 62	8.5±1.00	8.4±2.12	*	0.045±0.010	0.040±0.007	*
BRRI 3	8.65±1.48	8.5±0.70	*	0.041±0.0021	0.039±0.0014	*
BRRI 39	4.25±0.21	4.1±0.01	*	0.041±0.0014	0.037±0.0035	*
BRRI 29	8.85±0.2121	7.4±1.27	*	0.036±0.0071	0.027±0.0008	*
BRRI 51	7.2±0.98	5.9±0.56	*	0.030±0.0080	0.016±0.0012	*
Parizat	11.55±0.63	7.35±0.07	**	0.038±0.0007	0.015±0.0004	**
BRRI 33	8.9±0.070	7.05±0.07	**	0.030±0.0011	0.022±0.0155	*
BRRI 28	9.05±0.07	6.25±0.35	**	0.049±0.0014	0.031±0.0014	**

*statistically non-significant **statistically significant

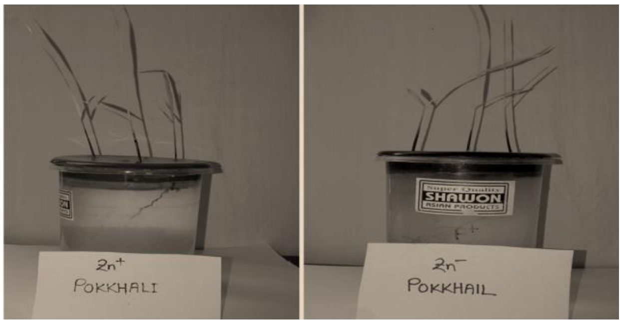

Fig. 1. Growth of Zn-efficient Pokkali on Zn-sufficient and Zn-deficient hydroponic conditions.

Chlorophyll concentrations in leaves

Concentrations of chlorophyll a and b were determined in leaves of all rice genotypes grown in Zn sufficient and Zn deficient conditions. It was found that chlorophyll concentrations (a and b) were not significantly reduced in Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51 due to Zn deficiency compared to controls. In contrast, significant reduction in chlorophyll concentrations (a and b) were observed in Parizat, BRRI 33 and BRRI 28 under Zn deficiency compared to Zn-sufficient plants (Fig. 2).

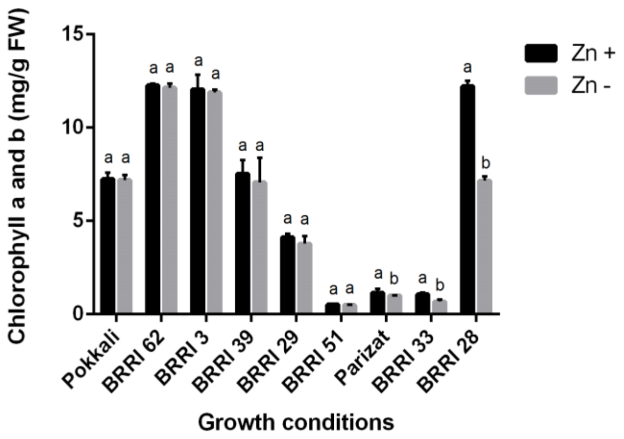

Fig. 2. Concentration of chlorophyll a and b in young leaves in a number of rice genotypes grown in Zn-sufficient (Zn+) and Zn-deficient (Zn-) hydroponic culture. Data were taken 3 weeks after Zn deficiency was imposed. Different letters indicate significant differences between means ± SD of treatments (n = 3), comparisons were done for Zn + and Zn - conditions.

DISCUSSION

Screening of Zn-deficiency tolerant line has been mainly carried out *in vivo* by field tests. Moreover, screening of the rice genotypes for Zn deficiency was never studied among Bangladeshi genotypes. Zn deficient plants grown hydroponically showed the typical chlorosis within few days after the beginning of the experiments. Different root and shoot parameters were affected by Zn-deficiency induced hydroponic conditions. However, genotypic variations were observed among the rice genotypes in Zn-efficiency.

Results suggest that Parizat, BRRI 33 and BRRI 28 are unable to tolerate Zn deficiency or in other word, they are not efficient to operate mechanisms conferring Zn deficiency tolerance as evident by their significant reduction in root and shoot parameters. In general, plants survive under Zn deficiency by operating a number of Zn-efficient mechanisms in roots. In contrast, Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51 were not significantly affected by Zn deficiency in their root and shoot parameters. It suggests that Zn-efficient mechanisms are actively present in root systems that eventually let these genotypes to continue normal growth and development. In contrast, these root parameters are negatively affected in Parizat, BRRI 33 and BRRI 28 resulting stunned root and poor shoot growth. Chlorophyll (a and b) concentrations in leaves of all genotypes were studied in both Zn sufficient and Zn deficient hydroponic conditions. Pokkali, BRRI 62, BRRI 3, BRRI 39, BRRI 29 and BRRI 51 showed Zn-efficiency showing no significant reduction in chlorophyll a and b; whereas, Parizat, BRRI 33 and BRRI 28 found to be Zn-inefficient.

Based on these investigations, it is evident that genotypic variation exits in Bangladeshi rice genotypes for Zn deficiency tolerance. Comparatively, Pokkali and BRRI 62 showed the best Zn-efficiency among other genotypes used in this study. In contrast, BRRI 33 found to highly sensitive to Zn deficiency and unable to survive or maintain normal growth and development under Zn deficiency. Previously, Jamalomidi et al. (2006) reported the Zn-efficiency of Pokkali in response to bicarbonate induced Zn deficiency. Bicarbonate tolerance and Zn efficiency have been developed in rice simultaneously during natural or artificial selection for plant growth on alkaline and calcareous soils with low Zn availability.

Pokkali which is known for its greater salt tolerance is also efficient in tolerating Zn deficiency in soil. Recently, The Bangladesh Rice Research Institute (BRRI) breeders developed high zinc rice variety, BRRI-62. HarvestPlus, supported this biofortification project under the Washington-based global agro-science coordinating body Consultative Group for International Agricultural Research (CGIAR). Our findings further establish that BRRI 62 is not only rich in Zn but also tolerant to Zn deficiency.

Zinc uptake by higher plants appears to be mostly controlled by the transport of zinc across the plasma membrane, which is largely metabolism-dependent and genetically controlled. Zn-efficient genotypes may be able to maintain structural and functional stability of their root-cell plasma membranes better than Zn-inefficient genotypes under Zn deficiency (Rengel and Graham, 1995). Pokkali is known to have higher hydrogen peroxide (H_2O_2)-scavenging enzyme activities in non-treated seedlings, including ascorbate peroxidase, catalase, and peroxidase activities (Lee et al., 2013). These mechanisms may contribute to the greater Zn-efficiency of this rice line. This study also confirms the efficiency of hydroponic culture for screening rice genetic line for screening Zn or other mineral deficiency tolerance germplasm. This method overcomes the difficulty associated with the use of calcareous soils under field, greenhouse, and growth chamber conditions.

This paper provides physiological evidences on the genotypic variations in rice plants in response to Zn deficiency in Bangladeshi rice lines. Results also enrich the knowledge for varietal characteristics of rice and can be used by farmers and plant breeders where Zn deficiency is a major obstacle for rice production. Efficiency of hydroponic culture for the successful screening of plant genetic lines may also be followed by future researchers.

Acknowledgements

The work was financially supported by the Ministry of Science and Technology, Bangladesh (Grant No. 39.009.006.01.00.049.2013-2014/BS-102/534).

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