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Influence of Flooded and Aerobic Condition on Growth and Uptake of Zinc in Rice (*Oryza sativa* L.)

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ABSTRACT

In the present study, a field experiment was carried out to understand the effects of transition of rice cultivation from flooded to aerobic condition by growing different genotypes of rice under both the condition. Grain yield and other yield related parameters were significantly higher in aerobic condition compared to wetland condition. Zinc concentration was recorded highest for Chittimutyalu in aerobic condition while lowest was recorded for Burma Black under wetland condition. Molecular marker study using candidate gene specific marker OsZIP5 showed polymorphism for Chittimutyalu in both the condition. Grain yield was positively correlated with grain zinc content in both aerobic and wetland condition. These results indicate that transition from flooded to aerobic condition contributes to increased zinc accumulation in the grain without compromising grain yield.

Keywords: Aerobic rice, wetland rice, zinc

RICE is one of the main source of nourishment for most of the world's population and they directly supply more than 50 per cent of all calories consumed by the entire population (Jia-Yang *et al*., 2014). Rice is a very diverse crop which can grow in wider range from irrigated to upland condition. Irrigated rice seems to be the predominant cultivation system which occupies 90 per cent of rice growing areas and utilizes 50 per cent of fresh water resources, however with the decline in fresh water resources, production of rice under flooded condition is becoming a challenge.

One alternative way to reduce water use in rice production is to grow rice under aerobic condition. Aerobic rice is grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils. It includes growing of rice in aerobic soil with the use of external inputs like supplementary irrigation and fertilizers with the aim of achieving good yields in less water (Shashidhar, 2007). There are several reports regarding water use efficiency and yield of aerobic rice but only few studies are available on nutrient availability under aerobic rice cultivation (Chauhan and Johnson, 2011; Kadiyala *et al*., 2012).

It is not surprising that about 870 million people are suffering from chronic under nourishment globally (FAO, 2012) and vast majority of them are from developing countries where rice is closely associated with food security and political stability. Thus, improving the micronutrient status of rice is very important to tackle key nutrition and health related problems of these large populations, most notably in the developing countries.

Among these micronutrients, zinc is important for plant growth and human health. Stunted growth, chlorotic leaves, sterile spikes, leaves with brown blotches and streaks are few of the visible symptoms caused by severe zinc deficiency (Abdullah, 2015). Further, zinc deficiencies are reported to be the fifth highest health risk factor (Devi *et al*., 2014; Sharma *et al*., 2013) causing a high mortality rate and it is more prevalent among people which use rice as major grain source.

Transition from lowland to aerobic cultivation causes change in soil physiochemical properties like pH, redox status, bulk density which has benefit and risking effect on nutrient bioavailability. Flooding causes shifting of soil p^H towards neutral and hence aerobic condition can cause bulk soil p^H revert toward the original soil p^H (Gao *et al*., 2012). In an aerobic condition, Zn forms as insoluble zinc sulphide and insoluble carbonate mixtures which plant cannot uptake while increased oxidation under aerobic condition decrease Zn precipitation as ZnS and makes it available for plants (Bostick *et al.,* 2001).

Considering these, present investigation was carried out to understand effect of influence of flooded and aerobic condition on zinc uptake and growth in rice.

MATERIAL AND METHODS

Experimental sites

The experiment was conducted in *kharif* season during 2016-17 at the experimental site of Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore. Two cultivation method or treatments were used: aerobic and wetland cultivation. The aerobic plots used in the experiment were maintained with 4-5 times irrigation while wetland plots were under continuous flooded condition. A total of three genotypes containing one aerobic and two irrigated genotypes (ARB-6, Chittimutyalu, Burma Black) were grown under both conditions for further evaluation.

Chittimutyalu is a national check for zinc content (>20ppm in polished rice) under All India Coordinated Rice Improvement Programme (AICRIP) biofortification trial while ARB-6 is a promising aerobic rice genotype. Burma Black is cultivated in some parts of India for high quality and aroma.

Agromorphological traits

Different agro-morphological traits were evaluated at flowering stage including 50 per cent flowering and at maturity including plant height, panicle length, days to maturity, number of productive tillers, grain length, grain breadth, grain length to breadthratio, biomass yield and grain yield for all the genotypes under both the condition.

Elemental analysis

Zinc concentration was estimated for all the genotypes grown under aerobic and wetland condition. Grains were harvested manually and hand threshed to avoid contamination. Seeds from all the genotypes were dehusked using a palm dehusker and cleaned using hydrochloric acid and double distilled water. Further seeds were dried in hot air oven at 70 ^{0}C for 72 hours and subjected to X-ray fluorescence (XRF) (OXFORD instrument X-supreme 8000), (Nicholas *et al.*, 2012) at MSSRF, Chennai for elemental analysis.

Molecular characterization

DNA was isolated using CTAB method (Doyle and Doyle, 1990). A total of twenty-five gene specific markers for zinc transport belonging to ZIP family (Palmer and Guerinot, 2009; Waters and Sankaran, 2011) were considered to check the polymorphism.

PCR for candidate gene marker OsZIP5 was carried out in Mastercycler® Nexus Gradient, Eppendorf in a total volume of 10 µl containing 1X PCR buffer, 0.25µM of each forward and reverse primers (Sigma Aldrich, USA), 50ng rice genomic DNA, 0.5mM dNTPs mix and 1.2 units of *Taq* polymerase (Bangalore Genei, India). Agarose electrophoresis was done with 3 per cent gels to visualize the amplicons.

Statistical analysis

Mean values of five replication was used from different plant traits of all the genotypes. The results were analyzed by analysis of variance (ANOVA). When ANOVA values were significant, means were compared using Duncan Multiple Range test at 5 per cent level probability level. Student t-test was administered to compare zinc concentration in aerobic and wetland condition. Further phenotypic correlation study was also done for all the traits under both the condition using SPSS version 15.0.

RESULTS AND DISCUSSION

In the present study, a significant change was observed for all the agro-morphological traits as compared to wetland with aerobic condition (Table I). Grain yield under aerobic condition was found highest for ARB-6, while under wetland condition, Chitimutyalu showed the highest. Grain length was not significantly different among the genotypes except for Chittimutyalu. Number of productive tillers, panicle number and other traits were found significantly different across genotypes under both the conditions (Table III). These results indicated that cultivation system has profound effect on yield and yield related parameters. Evaluation for improved yield under both the condition is important to select

TABLE I

Analysis of variance for plant morphological traits under aerobic and wetland condition

Sources	D F	DTF	PHT	DTM	NPT	P L	G L	G B	LBR	B Y	G Y
Aerobic	2	996.82	1171.06	252.70	73.70	2.4	5.7	4.05	1 .66	1309.54	78.72
P value		<.0001	<.0001	<.0001	.05	.07	.001	.03	.01	.002	.001
Wetland	2	2280.4	705.65	2243.44	22.57	3.05	.012	.044	.156	35.86	18.95
P value		.002	.001	<.0001	.05	.01	.25	.03	03	.07	.03

DTF : Days to 50% flowering, PHT: Plant Height, DTM: Days to Maturity, NPT: Number of Productive Tillers, PL: Panicle Length, GL: Grain Length, GB: Grain Breadth, LBR: Grain Length Breadth Ratio, BY: Biomass Yield, GY: Grain Yield

the suitable genotype since same genotype is expected to perform well in both the condition (Mwadzingeni *et al.*, 2016).

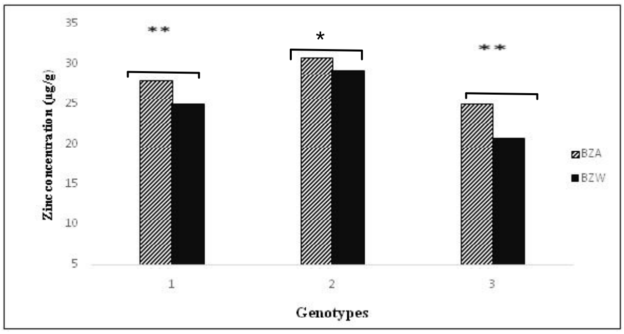
Zinc concentration of brown rice was measured for the selected genotypes in both aerobic and flooded condition. As interaction effect of aerobic and flooded condition was not significant (Table II), main effects were considered after calculating mean values of zinc concentration of selected genotypes in both the condition. Among the genotypes, Chittimutyalu was recorded to be highest for zinc concentration *i.e.*, 30.8 µg/g and 29.2 µg/g under aerobic and wetland condition respectively, while performance of Burma Black was recorded lowest with 25.04 µg/g and 20.77 µg/g of zinc under both the condition (Fig. 1) in brown rice. Interestingly, for all the genotypes, aerobic condition showed higher zinc concentration compared to wetland condition which may be due to decrease

TABLE II

Analysis of variance for zinc concentration in brown rice under aerobic and wetland condition

Sources	DF	MSS	F Value	P value
Cultivation	1	64.10	50.25	<.0001
Genotype	2	49.89	39.10	<.0001
Cultivation X	2	.73	.58	.52

Cultivation: Aerobic and wetland



1:ARB-6, 2:Chittimutyalu, 3:Burma Black

Fig. 1: Variation in grain zinc concentration in aerobic (BZA) and wetland (BZW) condition

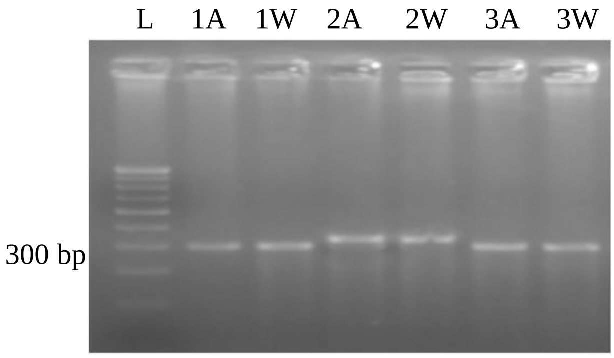
TABLE III

Mean values of plant morphological traits under aerobic and wetland condition

Genotype	Cultivation	PHT	DTF	DTM	NPT	PL	GL	GB	LBR	GY
ARB-6	Aerobic	65.60 b	106.77 a	145.66 a	30.74 c	15.44 a	2.22 b	.83 a	2.16 a	21.34 c
	Wetland	66.16 b	71.66 a	110.66 a	13.26 b	16.73 b	2.48 a.	90 a	2.30 a	15.07 a
Chittimutyalu	Aerobic	53.87 a	116.68 b	144.33 a	28.53 ab	15.66 ab	1.36 a	.78 a	1.74 a	16.87 ab
	Wetland	51.4 a	75.0 a	115.66 a	14.06 b	14.8 a	2.36 a	1.13 b	2.21 a	19.57 b
Burma Black	Aerobic	92.41 c	142.81 c	174 b	21.26 a	17.88 b	2.27 b	1.03 b	2.74 b	12.63 a
	Wetland	82.06 c	121.0 b	160.33 b	8.9 a	15.26 a	2.44 a	1.07 b	2.68 b	13.15 a

PHT: Plant Height, DTF: Days to 50% flowering, DTM: Days to Maturity, NPT: Number of Productive Tillars, PL: Panicle Length, GL: Grain Length, GB: Grain Breadth, LBR: Grain Length Breadth Ratio, GY: Grain Yield. Values in a row followed by same letters are not statistically significant (p<.005) of Zn precipitation as ZnS or exudation of Zn chelators which increase its availability. Similar observation was also recorded by Yin *et al*. (2016) where zinc concentration of rice was recorded in a range from 10-22 mg/kg in United States.

Among the gene specific marker related to zinc transportation, OsZIP5 showed polymorphism among the genotypes (Fig. 2). For ARB-6 and Burma Black,



L=Ladder 1:ARB-6, 2:Chittimutyalu, 3:Burma Black

Fig 2: Gel picture depicting polymorphism for OsZIP 5 gene specific marker among the genotypes under aerobic (A) and wetland(W) condition

amplification was similar to product size (300 bp) but for Chittimutyalu, it showed polymorphism and significant marker trait association at 0.05 probability level. As Chittimutyalu has recorded highest zinc concentration among the genotypes, it can be inferred that the marker can be used to screen zinc rich genotypes. However, further validation is needed in this regard with other zinc rice genotypes.There was no difference observed in product size for gene specific marker between aerobic and wetland genotypes. This is due to the reason that genomic DNA was used which is stable in both the condition. Further expression level of transporter gene has to be studied by using RT-PCR in both the condition to understand the effect of cultivation on zinc content of selected genotype.

Correlation studies for various phenotypes is presented in Table IV and V. Correlation study of grain yield with other traits revealed that it has significant and positive correlation with number of productive tillers (0.58), while it was non-significant and positively correlated with panicle length (0.30), grain length (0.03), grain length breadth ratio (0.47) and grain zinc content (0.44) under aerobic condition. Under wetland condition, grain yield showed significant and positive correlation with number of tillers (0.51) and grain zinc content (0.74) while it

TABLE IV

Estimates of phenotypic correlation coefficients for morphological characters in selected rice germplasm under aerobic condition

Traits	DTF	PH	NPT	PL	DTM	GL	GB	LBR	BZN	BY	GY
DTF	1.00	0.82 **	-0.74 *	0.53	0.93 **	0.24	0.81 **	-0.33	-0.66	0.89 **	-0.93
PH		1.00	-0.66	0.51	0.93 **	0.73 *	0.85 **	0.26	-0.88	0.94 **	-0.65
NPT			1.00	-0.73	-0.61	-0.22	-0.73	0.28	0.42	-0.58	0.58 *
PL				1.00	0.48	0.25	0.64	-0.20	-0.48	0.49	0.30
DTM					1.00	0.51	0.85 **	-0.01	-0.83	0.98	-0.83
GL						1.00	0.58	0.79 *	-0.79	0.54	0.03
GB							1.00	-0.03	-0.83	0.79	-0.59
LBR								1.00	-0.34	0.07	0.47
BZN									1.00	-0.82	0.44
BY										1.00	-0.79
GY											1.00

DTF: Days to 50% flowering, PHT: Plant Height, DTM: Days to Maturity, NPT: Number of Productive Tillers, PL: Panicle Length, GL: Grain Length, GB: Grain Breadth, LBR: Grain Length Breadth Ratio, BY: Biomass Yield, GY: Grain Yield, BZN: Brown Rice Zinc content

Table V

Estimates of phenotypic correlation coefficients for morphological characters in selected rice germplasm under wetland condition

Traits	DTF	PH	NPT	PL	DTM	GL	GB	LBR	BZN	BY	GY
DTF	1.00	0.79 *	-0.82 *	-0.29	0.98 **	0.15	0.27	-0.26	-0.67	0.21	-0.62
PH		1.00	-0.80 *	0.21	0.81 **	-0.09	-0.23	0.16	-0.85 *	0.36	-0.74
NPT			1.00	-0.02	-0.83 *	0.14	0.04	0.03	0.76 *	-0.47	0.51 *
PL				1.00	-0.33	-0.61	-0.74	0.49	-0.29	0.22	0.15
DTM					1.00	0.11	0.26	-0.25	-0.68	0.15	-0.59
GL						1.00	0.13	0.23	0.38	0.04	-0.16
GB							1.00	0.93 **	0.83	-0.16	0.11
LB								1.00	0.14	0.21	-0.13
BZN									1.00	-0.34	0.74 *
BY										1.00	-0.08
GY											1.00

DTF: Days to 50% flowering, PHT: Plant Height, DTM: Days to Maturity, NPT: Number of Productive Tillers, PL: Panicle Length, GL: Grain Length, GB: Grain Breadth, LBR: Grain Length Breadth Ratio, BY: Biomass Yield, GY: Grain Yield, BZN: Brown Rice Zinc content

was non-significant and positively correlated with panicle length (0.15) and grain breadth (0.11).

Zinc content of grain in aerobic condition showed positive correlation with number of productive tillers (0.42) and grain yield (0.44), while it was negatively correlated with other traits. In wetland condition, it showed significantly positive correlation with number of productive tillers (0.76) and grainyield (0.74) while it was non-significant and positively correlated with grain length (0.38), grainbreadth (0.83) and grain length breadth ratio (0.14).

The correlation between yield attributing traits and their association with the target variable was studied. As number of tillers and zinc concentration showed significant and positive correlation with grain yield, these traits can be utilized in future breeding programme to improve grain yield. These results are in corroboration with the earlier finding (Kalyan *et al.*, 2017) for number of productive tillers per plant. For grain zinc concentration, Jeomho *et al.* (2008), Gangashetty *et al.* (2013) has reported positive correlation with grain yield which showed the possibility of developing high yielding variety with high zinc content.

From the above study, it can be concluded that for both the lowland and aerobic genotype, zinc concentration and grain yield were higher in aerobic condition compared to wetland condition. Molecular marker study with candidate gene specific marker OsZIP5 showed polymorphism for zinc rich genotype Chittimutyalu. Further, a significant and positive association was found between grain yield and zinc concentration. Therefore, there is a possibility that transition from wetland to aerobic condition cannot only solve the problem of water shortage but can give zinc rich rice grain without compromising yield of the crop.

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