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Influence of Zinc on Functioning of Anti-oxidant Enzymes and Zinc content in Hogland Solution of Rice Genotypes

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A B S T R A C T

Hydroponic experiment was carried out to analyse the effect of Zn on anti oxidative enzyme activity, zinc content in shoot and root of rice genotypes and zinc efficiency. The experiment was comprised of 20 genotypes and two treatments *viz.*, T1: 0.01 µM (Zn-deficient); T2: 2.0 µM (Zn-sufficient/control). Results indicated 2.0 µM concentration of zinc sulphate significanty incresead the zinc content in shoot (10.6 ppm) and root (18.8 ppm), superoxide dismutase enzyme (12.8 g^{-1} protien^{-1}) and perioxidase enzyme activity (4.21µmol min^{-1} g^{-1} protein) were measured on 4-week old seedlings. Screening of Zn-efficient genotypes carried out in hydroponic experiment, Halga and Kalanamk and Dodigya was recorded as most Zn-efficient genotypes, however Koorigenellu was found as Zn-inefficient genotype with respect to shoot dry weight.

Introduction

India has a long history of rice cultivation. Globally, it stands first in rice area and second in rice production, after China. It contributes 21.5 per cent of global rice production. Within the country, rice occupies one-quarter of the total cropped area, contributes about 40 to 43 per cent of total food grain production and continues to play a vital role in the national food and livelihood security system (Anon, 2008).

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. Zinc (Zn) deficiency is major constraint to rice production. To overcome these nutritional constraints it comes at substantial cost to farmers and the efficiency of fertilizer use is low. Breeding crops that are efficient at acquiring Zn from native soil reserves or fertilizer sources has been advocated as a cost-effective solution.

Zinc (Zn) deficiency is one of the most critical global health problems because rice is the main staple food of Asia. Affecting nearly one-third of world population (Welch and

Graham, 2004; Hotz and Brown, 2004). Low dietary Zn intake is considered to be the major reason for widespread occurrence of Zn deficiency in human populations, especially in developing countries. Over 30% of the world's population may suffer from zinc deficiency (Welch *et al.,* 2005). Zn deficiency is especially prevalent among resource-poor women and children.

Zinc has multiple roles in basic cellular functions in all living organisms and is required for the normal development and functioning of non-specific and acquired immunity in humans (Shankar and Prasad, 1998). People who suffer from severe zinc deficiency show stunted growth, have slowly healing wounds, and become mentally retarded (Prasad and Bose, 2001). Yet, the most common deficiencies are of a less dramatic nature and lead to slight stunting, poorer mental development and poor immune system functioning. In China, average intake of zinc is 85.6% of its Recommended Dietary Allowance (RDA), and in Gansu province, the average intake of zinc is only 64.8% of the RDA (Ger *et al.,* 1996).

Genotypes of crop plants can vary widely in ZE, as reported for wheat (Cakmak *et al.,* 2001), common bean (Hacisalihoglu *et al.,* 2004) and rice (Sakal *et al.,* 1987). Mechanisms responsible for genotypic variation in ZE were thoroughly reviewed by Rengel (2001) and Hacisalihoglu and Kochian (2003). There seem to be many uncertainties on mechanisms that control tolerance to Zn deficiency. Most likely, there is no single mechanism in any crop species. The expression of high ZE in cereals including rice, wheat, rye, barley, triticale and oat was related to enhanced uptake and translocation capacity of Zn into shoots and higher amounts of physiologically active Zn in leaf tissues (Cakmak *et al.,* 1997).

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).

The overall aim of the study is to understand the effect of contrasting solution Zn concentrations on growth of rice genotypes, biophysical parameters and zinc uptake by shoot and root of rice genotypes.

Materials and Methods

The experiment was carried during 2016 at Department of Crop Physiology, College of Agriculture, UAS, Dharwad. Before growing, seeds were surface sterilised in 70 per cent ethanol and 5 per cent 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 (mM): KNO_3 (16000), Ca (NO3) $2.4H_2O$ (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 mM (Zn-sufficient/control) (Plate 1).

The nutrient solution was aerated continuously and replaced at 5 days interval. Target pH values (pH 5.5) 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 mmol s^{-1} per mA).

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971). The reaction mixture contained 100 mL 1 mM riboflavin, 100 mL 12 mML-methionine, 100 mL 0.1 mM EDTA (pH 7.8), 100 mL 50 mM Na^2CO^3 (pH 10.2) and 100 mL 75 mM nitroblue tetrazolium (NBT) in 2,300 mL 25 mM sodium phosphate buffer (pH 6.8), with 200 mL crude enzyme extract in a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit by 50 per cent the photoreduction of NBT to blue formazan. The SOD activity of the extract was expressed as SOD unit g^{-1} protein.

Peroxidase activity was estimated following the method of Mahadevan and Sridhar (1986) with some modifications. Three ml of buffer solution, 0.05 ml guaiacol solution, 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide solution were pipetted into a cuvette. The absorbance was adjusted to zero at 436 nm in a UV-Vis spectrophotometer. The change in absorbance was noted at an interval of 20 seconds after adding 0.5 ml of two per cent H_2O_2 (Hydrogen peroxide). The enzyme activity was expressed as change in absorbance (DOD) µmol min^{-1} g^{-1} protein.

Zn concentration was analyzed in shoot and root. Samples were pre-digested by adding ten ml of concentrated nitric acid to 500 mg of powder sample and incubated in a digestion hood overnight. The next day, samples were wet digested (HNO3: $HClO_4$; 4:1) and in the extracts zinc concentration was measured by using atomic absorption spectrophotometer GBC Avanta Ver 2.02 Model. Zinc content was expressed in parts per million (ppm). Zinc efficiency can be determined as the ratio of shoot dry matter yield produced under Zn deficiency to that produced under Zn sufficient condition (Graham *et al.,* 1992).

Fisher's method of analysis of variance was applied for the analysis and interpretation of the experimental data as suggested by Panse and Sukhatme (1967). The level of significance used in 'F' and 't' test was P=0.01. Critical difference (CD) values were calculated at 1 per cent level, wherever 'F' test was significant.

Results and Discussion

Anti-oxidative enzyme

Graphical representation (Fig 1.) and from table 46 can be depicted that, SOD (ΔSOD g $protein^{-1}$) and Peroxidase activity (ΔOD µ mol $min^{-1}g$ $protien^{-1}$) differed significantly among the zinc treatments, genotypes and their interaction also differed significantly. Significantly higher SOD and Peroxidase activity was observed in zinc sufficient (Zn^+) hydroponic culture (12.8 and 4.21, respectively) over zinc deficient (Zn^-) hydroponic culture (10.7 and 3.55, respectively).

Among the genotypes, Koorigenellu resulted in significantly higher SOD activity (14.2)

whereas; Ambemohar-2 recorded significantly higher peroxidise activity (4.44) While, Dodigya (9.3) and Karibatta (3.21) observed to have significantly lower SOD and peroxidise respectively.

Similarly among interactions, Koorigenellu recorded significantly higher SOD activity in hogland solution with zinc sulphate (15.3) whereas; Dodigya was found with significantly lower SOD activity in zinc deficient (Zn^-) hydroponic culture (8.3) followed by MTU-1001 (9.1) and Hugibatta-1 (9.4). With respect to peroxidise activity, Koorigenellu (4.85) and Dambersali (4.85) recorded significantly higher peroxidise activity under zinc sufficient (Zn^+) hydroponic culture, however significantly lower peroxidise activity was resulted with Karibatta (2.95).

The results obtained in this study indicated that, leaf SOD and peroxidise enzyme activity decreased under Zn-deficient conditions, the reason for this is that Zn is required as a co factor in the functioning of SOD and peroxidase. Due to this reason a drop could be noticed under deficit conditions and improvement with its supply. Similar results were reported by Zeng *et al.,* (2010), found gradual increase in POD and SOD activity with the increasing plant tissues zinc concentrations. The induction of these enzymes due to high zinc content may play an important role in plant defence, aging and senescence. Which has been observed in overall better growth in zinc applied conditions.

Biochemical

Table 01 depicted that shoot zinc (ppm) and root zinc (ppm) differed significantly among the treatments, genotypes and similarly interaction between zinc treatments and genotypes was resulted significant.

Zinc sufficient (Zn^+) treatment recorded significantly higher shoot zinc content (10.6) and root zinc (18.8) were resulted by Zinc sufficient condition over zinc deficient (Zn^-) (9.1 and 15.9, respectively). Among the genotypes, Dambersali was recorded significantly higher shoot zinc content (11.9), however higher root zinc content resulted with Ambemohar-1 (19.5) and Koorigenellu (19.3).

Among interactions, Ambemohar-1 resulted significantly higher shoot zinc content (13.3) and root zinc content (22.0) with zinc sufficient hogland solution. Whereas, significantly lower shoot zinc content was found with genotype BPT-5204, whereas SIRI-1253 (14.7) resulted lower root zinc content in hogland solution without zinc sulphate (6.6).

Sufficient amount of Zn in solution, could be reason of higher zinc content in shoot which could be attributed to its synergistic effects on the enhancement of root development and facilitated greater absorption of Zn (Chaudhary and Sinha, 2007). Similar result was reported by Naik and Das (2007) also found similar result. Similarly genotypes showed significant difference with respect to root zinc content. Apart from this, genotypes with higher root length and root weight *viz.*, Dambersalib and Koorigenellu showed higher zinc content in root and shoot. Hence, root traits of these genotypes also contribute for zinc content. The increase in root zinc content may be attributed to increase in root proliferation due to greater availability of the cation zinc which enhanced its uptake from solution through diffusion and mass flow from the immediate vicinity of plant roots. Mehdi *et al.,* (1990) also reported that increase in level of Zn increases the zinc content of roots.

Table.1 Effect of zinc on growth parameters in 04 week old seedlings of rice genotypes in the hogland solution culture

Genotypes	SOD (g^{-1} protien^{-1})			POX (µmol min^{-1} g^{-1} protein)			Shoot zinc (ppm)			Root zinc (ppm)			Zinc efficiency for shoot dry matter (ZEs)
	T_1	T_2	Mean	T_1	T_2	Mean	T_1	T_2	Mean	T_1	T_2	Mean	
Ambemohar 1	11.5	15.2	**13.4**	3.41	4.36	**3.88**	10.3	13.3	**11.8**	17.0	22.0	**19.5**	
Koorigenellu	13.0	15.3	**14.2**	3.96	4.85	**4.40**	10.4	12.9	**11.6**	17.0	21.6	**19.3**	0.82
Dambersali	11.6	15.1	**13.4**	3.85	4.85	**4.35**	10.7	13.1	**11.9**	15.7	20.0	**17.9**	0.79
Kempunellu	11.9	13.9	**12.9**	3.05	3.97	**3.51**	10.4	12.6	**11.5**	14.9	18.5	**16.7**	0.84
Dodda Batta	12.3	14.8	**13.6**	3.88	4.69	**4.28**	9.5	11.8	**10.6**	17.7	20.8	**19.2**	0.85
Ambemohar 2	10.3	13.2	**11.7**	4.06	4.81	**4.44**	9.4	11.3	**10.3**	16.5	20.7	**18.6**	0.88
Dodigya	8.3	10.3	**9.3**	3.06	3.52	**3.29**	10.5	12.1	**11.3**	15.0	17.6	**16.3**	0.90
Laldodki	10.1	12.2	**11.2**	3.77	4.73	**4.25**	9.8	11.0	**10.4**	16.5	18.8	**17.6**	0.94
Budda	10.0	12.6	**11.3**	3.39	4.16	**3.78**	10.5	12.4	**11.4**	16.2	19.8	**18.0**	0.90
Wari M. S.	12.0	14.2	**13.1**	3.86	4.65	**4.25**	9.4	10.4	**9.9**	17.0	19.1	**18.1**	0.88
Champakali	9.5	11.8	**10.6**	4.00	4.79	**4.39**	8.4	9.6	**9.0**	16.5	19.4	**17.9**	0.90
Improved chitimutayalu	11.0	12.9	**11.9**	3.37	3.91	**3.64**	10.3	11.5	**10.9**	16.2	18.4	**17.3**	0.85
Karibatta	11.5	13.1	**12.3**	2.95	3.47	**3.21**	9.3	10.7	**10.0**	15.4	17.4	**16.4**	0.90
Chandibatta	10.3	11.1	**10.7**	3.60	3.96	**3.78**	8.5	9.9	**9.2**	15.0	18.0	**16.5**	0.88
Halga	12.0	13.2	**12.6**	3.12	3.41	**3.27**	8.3	9.0	**8.7**	15.5	16.6	**16.0**	0.90
Siri1253	9.6	10.7	**10.2**	3.32	3.69	**3.51**	7.4	8.1	**7.7**	14.7	16.2	**15.4**	0.94
Kalanamak	9.6	10.4	**10.0**	3.21	3.49	**3.35**	7.9	8.5	**8.2**	15.1	16.3	**15.7**	0.92
Hugibatta-1	9.4	10.7	**10.1**	3.96	4.41	**4.19**	7.0	7.6	**7.3**	15.5	17.0	**16.2**	0.94
MTU1001	9.1	10.5	**9.8**	3.66	4.15	**3.91**	7.8	8.7	**8.3**	15.0	17.0	**16.0**	0.92
BPT5204	11.6	14.5	**13.1**	3.53	4.38	**3.96**	6.6	7.9	**7.2**	16.4	20.2	**18.3**	0.90
Mean	**10.7**	**12.8**	**11.8**	**3.55**	**4.21**	**3.93**	**9.1**	**10.6**	**9.9**	**15.9**	**18.8**	**17.4**	0.82
For comparing means of	**S.Em. ±**	**C.D. @ 5 %**		**S.Em. ±**		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**	
Genotypes (G)	**0.2**	**0.8**		**0.1**		**0.3**	**0.2**		**0.7**	**0.3**		**1.2**	
Treatments (T)	**0.1**	**0.3**		**0.0**		**0.1**	**0.1**		**0.2**	**0.1**		**0.4**	
G x T	**0.3**	**1.2**		**0.1**		**0.4**	**0.3**		**1.0**	**0.5**		**1.8**	

T_1: Zinc deficient (Zn -) hydroponic culture T_2: Zinc sufficient (Zn +) hydroponic culture

Figure.1 Influence of zinc on shoot growth of rice genotypes in Hogland solution

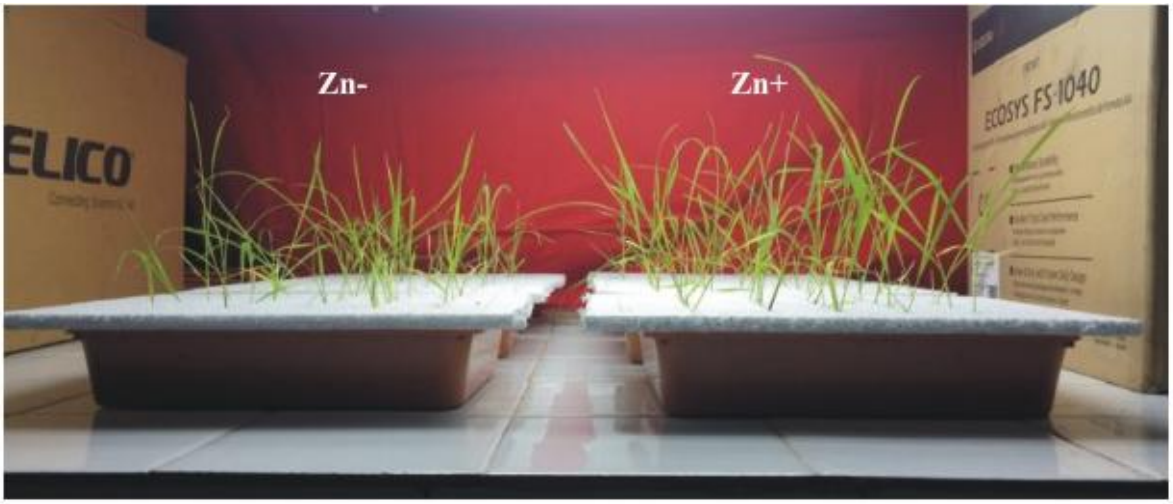
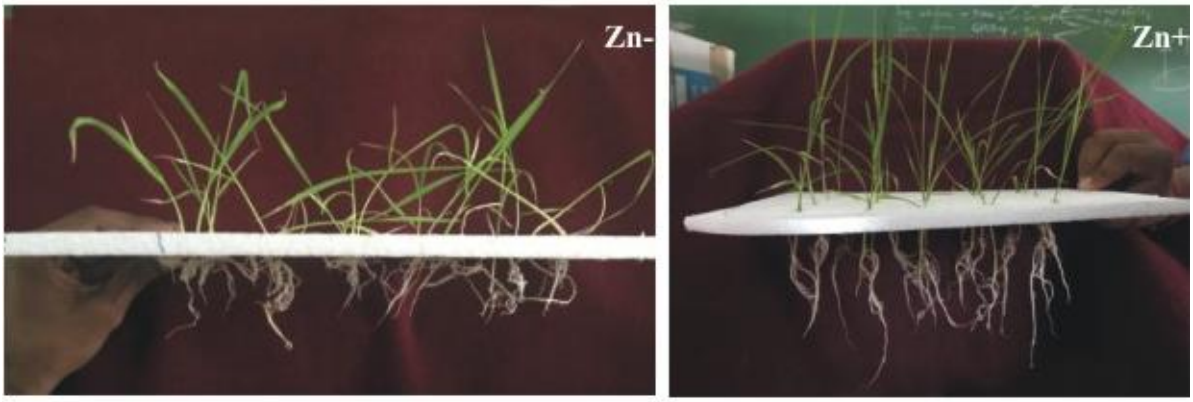


Figure.2 Influence of zinc on root growth of rice genotypes in Hogland solution



It was concluded from the experiment that 2.0 µM Zn-sufficient solution culture found to have beneficial effects on increasing the growth parameters, physiological and zinc content of rice plant.

Zinc efficiency

Zinc efficiency is defined as the ability of a plant to grow and yield well under zinc deficient condition (Erenoglu *et al.*, 2000). Higher the value of zinc efficiency higher is the growth of the genotype in zinc deficient condition. Response to Zn deficiency and Zn fertilization differs greatly among cereals species and genotypes of a given species.

Halga and Kalanamk and Dodigya was recorded as most Zn-efficient genotypes, however Koorigenellu was found as Zn-inefficient genotype with respect to shoot dry weight. Zn-inefficient genotypes 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. Whereas, zinc efficient genotypes survive under Zn deficiency by operating a number of Zn-efficient mechanisms in roots that eventually let these genotypes to continue normal growth and development.

Genotypes, Kalanamak with higher root length were zinc efficient. Hence, from this it can be concluded that higher root length is one of the root trait by which genotypes able to tolerate zinc deficient condition. The genotype, kempunellu which has been found zinc efficient might have root based biochemical mechanisms to survive under zinc deficient condition. 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).

From this we can conclude that Zinc is very important nutrient for functioning of anti-oxidative enzymes which necessary for scavenging ROS which are harmful for plant normal functioning. The result of Zinc efficiency showed that genotypes which are zinc efficient survive under Zn deficiency by operating a number of Zn-efficient mechanisms in roots that eventually let these genotypes to continue normal growth and development, so from this we can improve the root characteristics of genotypes which have traditionally grown under zinc deficiency condition.

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