

Original Research Article

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Effect of Zn Application on Rhizosphere Acidification in Rice and Wheat Varieties of Varying Zn Sensitivity

Deepa Rawat*, Santosh Chandra Bhatt¹, P.C. Srivastava² and S.P. Pachauri³

Department of Soil Science, College of Agriculture, GB Pant University of Agriculture and Technology, District: Udham Singh Nagar, Uttarakhand- 263945, India

*Corresponding author

ABSTRACT

Healthy seeds of different varieties of rice (PD 6, NDR 359, PD 16 and PS 5) and wheat (UP 262, UP 2628, PBW 175 and UP 2554) were germinated and fourteen days after germination, young rice/wheat seedlings were transferred to half strength of Hogland solution (pH 7.0) containing either recommended levels of Zn (0.05 mg Zn L⁻¹) or no Zn for four days. For solidification of medium 0.4% w/v agar- agar was mixed in the Hogland solutions of both -Zn and +Zn. Prior to solidification, 11.25 mL of 0.1% bromothymol blue dye solution prepared in 60 % ethanol and was mixed to agar nutrient solution. Twenty five mL of nutrient agar medium with +Zn or -Zn was poured in glass petri plates of 10 cm diameter and one seedling of each rice/wheat variety from +Zn or -Zn lot was transferred to the respective nutrient agar medium in duplicate. The colour change near roots under +Zn and -Zn were monitored and photographs were taken after 48 hrs in case of rice and 72 hrs in case of wheat for recording the extent of change in colour of the nutrient medium in the rhizosphere of all the rice and wheat varieties. In rice NDR 359 substantially reduced the pH in vicinity of roots (more yellow area around roots) than in Zn sufficient medium indicating possibly a better activity of the proton efflux pump to enhance Zn uptake under Zn deficient conditions. In Zn deficient conditions, Zn inefficient genotype UP 262 failed to substantially reduce pH in the vicinity of roots (less yellow areas around the roots) however, this variety under Zn sufficient conditions could reduce the pH of the growth medium (more yellowing around the roots).

Keywords

Zn Application,
Rhizosphere,
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Introduction

As an essential micronutrient, Zn is required for various physiological and metabolic processes in plants. It is the only metal which is present in the six classes of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Auld, 2001). Plant response to Zn deficiency occurs in

terms of decrease in membrane integrity, susceptibility to heat stress, decreased synthesis of carbohydrates, cytochromes, nucleotide, auxin and chlorophyll (Marschner, 1995). As Zn plays multiple roles in plant biochemical and physiological processes, even slight deficiency causes a decrease in growth, yield, and Zn content of edible plant parts. The ability of some crop genotypes to

tolerate low Zn availability over others is still not fully understood (Hacisalihoglu and Kochian, 2003). However, it may be partially related to rhizosphere acidification *via* organic acid release as root induced chemical changes in the rhizosphere have been shown to influence the phytoavailability of soil Zn (Marschner, 1995). Cultivars within a plant species differ in their ability to take up Zn, which may be caused by differences in absorption, translocation and utilization of Zn. Cakmak *et al.*, (1994) showed that a Zn-inefficient durum wheat cultivar exhibited Zn-deficiency symptoms earlier and more intensely than a Zn-efficient bread wheat cultivar even though the Zn tissue concentrations were similar in both lines, suggesting differential utilization of Zn in the two cultivars. Keeping in view the role of rhizosphere acidification in Zn uptake capacity of plant, the present investigation was performed to assess the mechanism of rhizosphere acidification in rice and wheat varieties of varying zinc sensitivity.

Materials and Methods

Healthy seeds of different varieties of rice (PD 6, NDR 359, PD 16 and PS 5) and wheat (UP 262, UP 2628, PBW 175 and UP 2554) varieties were surface sterilized using 0.01% solution of mercuric chloride. After thorough washing with distilled water, seeds were soaked in water and after 24 hours of soaking they were placed in loosely covered petri plates for germination and regularly watered with quartz distilled water. Fourteen days after germination, young rice/wheat seedlings were transferred to half strength of Hogland solution (pH 7.0) containing either recommended levels of Zn ($0.05 \text{ mg Zn L}^{-1}$) or no Zn for four days. For solidification of medium 0.4% w/v agar- agar was mixed in the Hogland solutions of both $-Zn$ and $+Zn$ by gently heating till dissolution of agar and then removed from hot plate for cooling. Prior

to solidification, 11.25 mL of 0.1% bromothymol blue dye solution prepared in 60 % ethanol and was mixed to agar nutrient solution. Twenty five mL of nutrient agar medium with $+Zn$ or $-Zn$ was poured in glass petri plates of 10 cm diameter and one seedling of each rice/wheat variety from $+Zn$ or $-Zn$ lot was transferred to the respective nutrient agar medium in duplicate. The petri plates were gently tilted so that the roots were covered by the nutrient agar medium. The desiccation of the nutrient agar medium was checked by daily adding 1.5-2.0 mL distilled water to each petri plate. The colour change near roots under $+Zn$ and $-Zn$ were monitored and photographs were taken after 48 hrs in case of rice and 72 hrs in case of wheat for recording the extent of change in colour of the nutrient medium in the rhizosphere of all the rice and wheat varieties.

Results and Discussion

Changes in rhizospheric acidity under Zn deficient and sufficient conditions in rice

The data regarding the changes in rhizospheric pH of young seedlings of different rice genotypes raised in Zn-deficient ($-Zn$) or Zn-sufficient ($+Zn$) solutions are given in Figure 1 and the plate 1.

With the increase in soil pH, Zn availability is reduced due to adsorption on the hydrous oxides of aluminium, iron and manganese or precipitation as specific compounds (Marschner, 1995). The root-mediated processes that could decrease the rhizosphere pH might be capable of increasing the plant Zn availability by solubilizing Zn from organic and inorganic soil solid phases. In lowland rice, rhizosphere acidification is likely to improve the utilization of Zn (Kirk and Bajita, 1995). Also, rice roots take up nitrogen mainly as NH_4^+ , resulting in discharge of H^+ by roots which consequently

decreases the rhizosphere pH. In the present study, the solution pH which was initially 7, ranged from 6.46 to 5.78 in Zn sufficient condition after 2 days of growth whereas, under deficiency of Zn the pH of the solution ranged from 6.64 to 4.76. As compared to the Zn-sufficient medium the decrease in pH under Zn deficiency was more pronounced in NDR 359 than in PD 16 and PS 5. As shown in plate 1, the photographs of roots of young seedlings of different rice genotypes raised on Zn-deficient or -sufficient agar medium mixed with pH sensitive dye indicated that under Zn deficiency NDR 359 substantially reduced the pH in vicinity of roots (more yellow area around roots) than in Zn sufficient medium indicating possibly a better activity of the proton efflux pump to enhance Zn uptake under Zn deficient conditions. According to Kirk and Bajita (1995), under anaerobic conditions O_2 released from the roots lead to higher H^+ extrusion consequently increased Zn bioavailability for plants. Therefore, it was clear in the present investigation that NDR 359 was not able to take up sufficient Zn under deficient conditions and managed to take up Zn under deficient conditions by increasing the H^+ efflux and releasing low molecular weight organic acids. Hajiboland *et al.*, (2005) reported that citrate concentration in the fine roots with bicarbonate treatment (Zn deficient condition) peaked at about 5 mm from the tip in the Zn-inefficient genotype (IR 26), whereas no peak was found in the Zn-efficient genotype (IR 36). It is apparent from the photographs of PD 6 that as compared to Zn deficient condition more of the pH decrease (more yellow area around roots) around the rhizosphere took place under Zn sufficient medium but substantially lesser yellowness was observed in Zn deficient medium. This showed its lower tolerance and uptake capacity under Zn deficiency due to its lesser ability to reduce the rhizospheric pH when Zn was not present in sufficient amount in soil.

Hoffland *et al.*, (2006) observed that under Zn deficiency, lowland rice increased citric acid exudation which resulted in reduction of rhizospheric pH to enhance Zn uptake and it was suggested that the citrate exudation capacity of rice genotypes was related to their tolerance to Zn deficiency. Even with sufficient amount of Zn present in the medium, PD 6 managed to take up Zn by reducing the rhizospheric pH and the proton efflux pump in this genotype had lower activity under Zn deficiency. In case of PS 5, no substantial difference in the colour around the root surface was observed in Zn deficient and sufficient medium whereas, in PD 16 the yellowness around the roots was more visible under Zn deficient plate than in Zn sufficient condition however, the extent was not as prominent as visible in Zn deficient roots of NDR 359.

Changes in rhizospheric acidity under Zn-deficient and -sufficient conditions in wheat

The data regarding the changes in rhizospheric pH of young seedlings of different wheat genotypes raised in Zn-deficient (-Zn) or Zn-sufficient (+Zn) solutions after four days are given in Figure 2 and the photographs are given in plate 2.

The changes in rhizosphere pH occur as a result of excretion of protons (H^+) and hydroxyl (OH^-) or bicarbonate (HCO_3^-) ions due to a cation–anion imbalance in the plant, the evolution of CO_2 by respiration, and the excretion of low-molecular-weight organic acids (LMWOAs) (Gao *et al.*, 2012). The pH of medium initially 7 in which young seedlings of different wheat varieties were grown decreased after 4 days under both Zn deficient and sufficient conditions. The decrease in pH under Zn deficient medium was slightly more pronounced (ranging from 6.66 to 5.72) than in Zn sufficient condition (ranging from 6.71 to 5.80). Zinc deficient

roots of two varieties i.e. Durati and Warigal, released correspondingly, five and three times more phytosiderophores and resulted a reduction in the rhizosphere pH than under Zn sufficient conditions (Rengel *et al.*, 1998). However, in UP 262 the pH noted under Zn sufficient condition was slightly less than in Zn deficient medium. As shown in Plate 3, the photographs of roots of young seedlings of different wheat genotypes raised on Zn-

deficient or -sufficient agar medium mixed with pH-sensitive dye indicated that under Zn deficient conditions, Zn inefficient genotype UP 262 failed to substantially reduce pH in the vicinity of roots (less yellow areas around the roots) however, this variety under Zn sufficient conditions could reduce the pH of the growth medium (more yellowing around the roots).

Fig.1 Changes in rhizospheric pH in rice varieties in Zn-sufficient and -deficient nutrient agar media

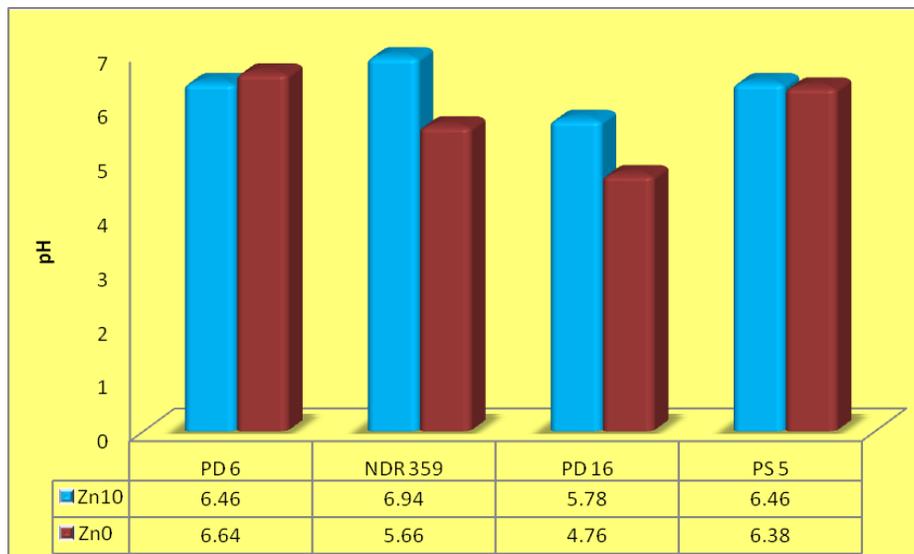
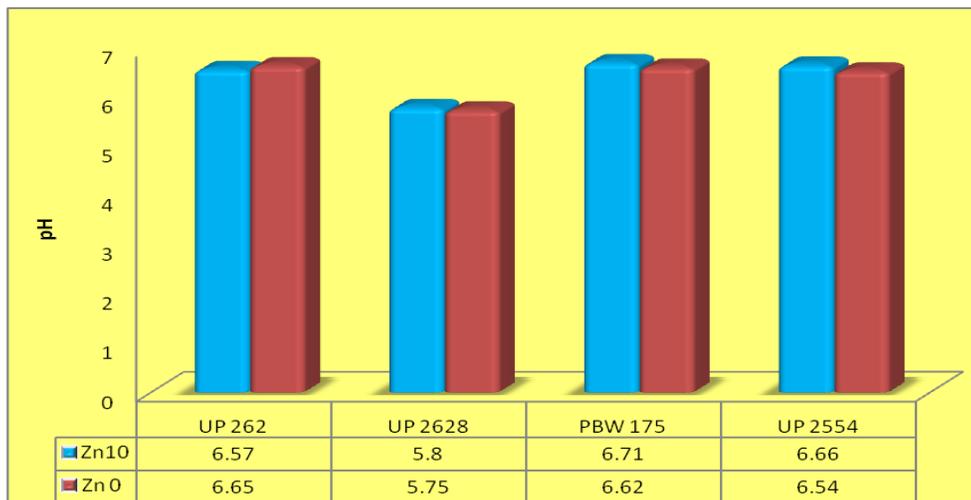


Fig.2 Changes in rhizospheric pH in wheat varieties under Zn-deficient and sufficient conditions



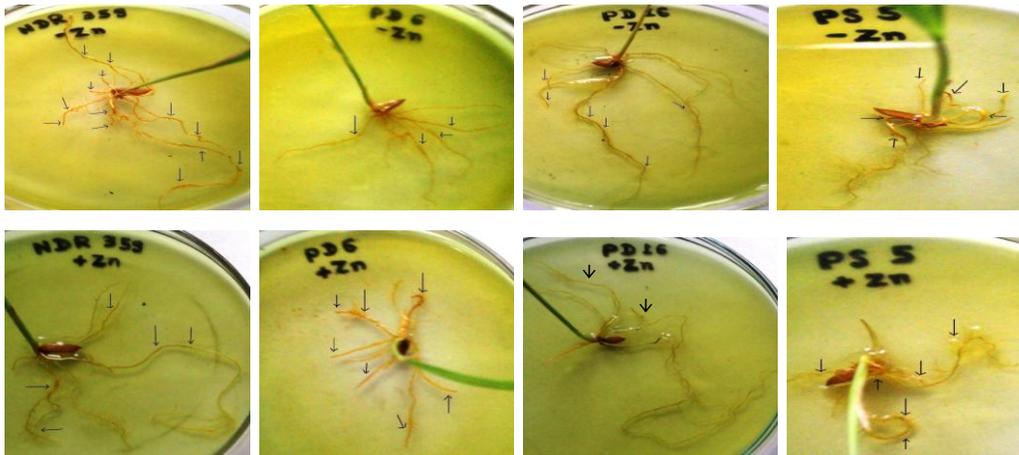


Plate 1: Changes in rhizospheric acidity under Zn deficient and sufficient conditions in rice

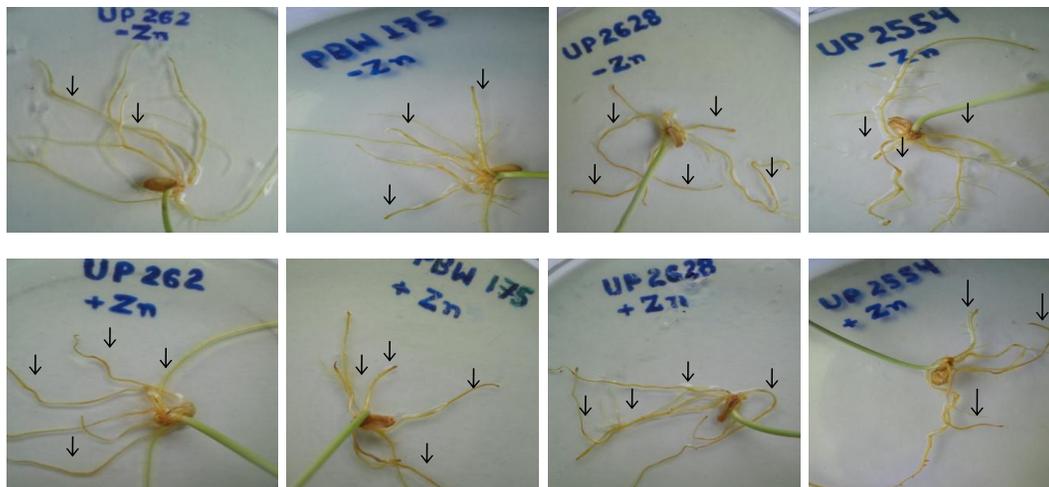


Plate 3: Changes in rhizospheric acidity under Zn deficient and sufficient conditions in wheat

This possibly indicated poor activity of proton efflux pump in this Zn inefficient genotypes under Zn deficiency. Under Zn deficient condition, an alternation of plasma membrane lipids had been reported to cause impairment in trans-plasma membrane proton gradient (Pinton *et al.*, 1993). On the other hand, Zn efficient genotype (UP 2628) showed almost similar pH decrease in the growth medium under both Zn-deficient or sufficient condition and a similar extent of yellowing

around the roots occurred under both Zn-deficient and Zn-sufficient conditions. Rengel (1999) compared the responses to Zn deficiency in two wheat genotypes (*Triticum aestivum*, cv. Aroona and *T. turgidum* L. conv. Durum, cv. Durati) grown in conventional and chelator-buffered nutrient solutions. With continuation of Zn deficiency stress, the Aroona plants grown in the conventional nutrient solution exuded increasing amounts of phytosiderophores into

the medium thereby decreasing the pH in the root vicinity, while the plants grown in the Zn-deficient chelator-buffered nutrient solution generally could not sustain a high rate of phytosiderophore exudation at day 22 compared to younger plants. The variation in Zn uptake ability between bread and durum wheat cultivars was ascribed to differential release of phytosiderophores (Cakmak *et al.*, 1996). The performance of genotypes PBW 157 and UP 2554 was intermediate between UP 262 and UP 2628.

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