RESEARCH ARTICLE Agricultural Science Digest, Volume 44 Issue 6: 1090-1097 (December 2024)

Changes in Organic Acid Composition, Proton Efflux and Root Length in Rice Genotypes Differing in Grain Zinc Accumulation Efficiency

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ABSTRACT

Background: Rice genotypes exhibit notable diversity in grain zinc content, with wild types accumulating higher levels than cultivated varieties, even in zinc-deficient soils. This study focuses on the wild rice genotype Karuppunel (Zn tolerant), along with cultivated varieties CO51 (Zn responsive) and ADT39 (Zn sensitive). The research explores the relationship between the composition of organic acids in root exudates, proton efflux and root length at various growth stages under both +Zn and -Zn conditions.

Methods: The experiment was conducted by growing rice genotypes Karuppunel, CO51 and ADT39 under two different zinc applied conditions. Non target based metabolomics was done to study the alterations in organic acid composition in root exudates using LC-MS/MS. Proton efflux was studied by the methodology given by Bashan *et al.* (1989) and root length was measured from the plant's base to the tip of the longest root. The data was analyzed by using Metaboanalyst 5.0 software and Origin Pro 2023b, version 10.0.5.157.

Result: By non-target metabolomics we identified 14 organic acids were identified in rice root exudates across growth stages in response to zinc. Random Forest analysis's variable importance plot consistently highlights nicotinic acid in Karuppunel, regardless of zinc conditions. Karuppunel exhibits higher proton efflux of 4.23 and 5.12 µmole H^+ g fresh weight^{-1} h^{-1} followed by CO51 *i.e.,* 3.76 and 4.14 µmole H^+ g fresh weight^{-1} h^{-1} under +Zn and -Zn respectively, while ADT39 shows lower proton efflux. Despite varying zinc conditions, root length increases across stages, with Karuppunel consistently having the highest length, particularly at grain-filling of 16.65 cm under +Zn and 15.75 cm under -Zn. Understanding these physiological changes may contribute valuable insights for breeders in designing zinc nutrient acquisition-focused breeding programs.

Key words: Karuppunel, Organic acids, Proton efflux, Root exudates, Root length.

INTRODUCTION

Rice (*Oryza sativa* L.) is crucial for maintaining worldwide food security and serves as a fundamental dietary staple for over half of the global population (Birla *et al.,* 2017). Nonetheless, the productivity of rice often faces hindrances due to nutrient deficiencies, with zinc (Zn) being particularly essential as a micronutrient significant for optimal plant growth and development (Palmer *et al.,* 2009). Over 30% of the world's population experiences a deficiency of Zn in their diet, leading to challenges such as impaired immune system function, hindered growth and cognitive delays (Midya *et al.,* 2021).

A plant's total photosynthetic products are secreted as root exudates to an extent of approximately 50% (VanDam and Bouwmeester, 2016). Root exudates, rich in primary metabolites like sugars, organic acids and amino acids, play a crucial role in nutrient acquisition, plant-soil feedback on pathogen defense and reducing the reliance on fertilizers and pesticides (Preece and Penuelas, 2020). The composition of exudates is influenced by factors such as plant genotype, growth phases, root traits, nutrient availability and ecological conditions (Zhang *et al.,* 2019). Organic acids, predominant in root exudates, are intermediates of the tricarboxylic acid cycle and are induced by various environmental stresses, including micronutrient deficiencies such as iron, zinc, and manganese (Martínez-Cuenca *et al.,* 2013; Bandyopadhyay *et al.,* 2017; Rengel, 2015). Organic acids also play novel roles, such as malate influencing primary root growth during phosphorus deficiency in maize, acetic acid in drought stress tolerance in Arabidopsis, and oxalate in biotic stress tolerance (Lehner *et al.,* 2008; Kim *et al.,* 2017; Mora-Macías *et al.,* 2017).

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How to cite this article: Lavanya, Y.S., Murugesan, D., Chandrasekhar, C.N. and Vijila, K. (2024). Changes in Organic Acid Composition, Proton Efflux and Root Length in Rice Genotypes Differing in Grain Zinc Accumulation Efficiency. Agricultural Science Digest. 44(6): 1090-1097. doi: 10.18805/ag.D-5948.

Submitted: 20-01-2024 **Accepted:** 22-05-2024 **Online:** 04-07-2024

Proton efflux, the release of protons into the rhizosphere, is a fundamental aspect of plant nutrient mobilization and absorption, influencing rhizosphere pH. This process is closely linked to vital physiological processes, including mineral uptake and plant cell enlargement (Werner, 1992; Bashan and Levanony, 1989). Proton efflux from roots is associated with various conditions, such as root elongation, geotropism, iron or phosphorus deficiencies, nutrient availability and aluminum toxicity (Punpom *et al.,* 2022). Roots, capable of sensing environmental stimuli, dynamically adjust their growth strategies in response to biotic and abiotic stresses, involving changes in root exudates, length, branching, growth direction and cell wall compositions (Lamers *et al.,* 2020; Muthert *et al.,* 2020). Understanding these dynamic root growth strategies and their underlying mechanisms is crucial for enhancing crop yield in future agricultural strategies. This study focused on exploring the root exudation pattern of organic acids, rhizospheric proton efflux, and root length in three rice genotypes *i.e*., Karuppunel, CO51, ADT39 analyzing correlations under +Zn and -Zn conditions.

MATERIALS AND METHODS

Experimental setup for the growth of rice genotypes with response to zinc

The pot experiment was conducted at the Tamil Nadu Agricultural University in Coimbatore, India, using cement tanks with local paddy wetland farm soil. Rice genotypes, Karuppunel: 44 mg kg^{-1}, CO51: 18 mg kg^{-1} and ADT39: 12 mg kg^{-1}, with varying grain Zn content, were utilized (Lavanya *et al.,* 2024). The Soil characteristics were as follows pH (8.59), Electrical conductivity (0.21 Dsm^{-1}), Nitrogen (147.4 mg kg^{-1}), Phosphorus (27.2 mg kg^{-1}), Potassium (202.75 mg kg^{-1}), Zinc (2.02 mg kg^{-1}), Iron (2.51 mg kg^{-1}) and Organic carbon (0.49 %). Puddled soil in tanks was used for direct dibbling of paddy seeds, retaining two seedlings per hill after 15 days. The experiment comprised two conditions: (i) with basal application of zinc fertilizer at 25.00 kg ha^{-1} of $ZnSO_4$ and foliar spray with 0.5% $ZnSO_4$ after 30 days of sowing (+Zn) and (ii) without the application of $ZnSO_4$ (-Zn). At vegetative, panicle initiation and grain-filling stages plants were uprooted for sampling.

Collection of rice root exudates

Exudates of rice root were collected by uprooting and washing the roots, sterilization was done with sodium hypochlorite for 2 minutes, followed by a final rinse in sterile distilled water. Plants were placed in bottles with extraction buffer (0.01 M calcium sulfate) and exposed to sunlight for 2 hours. Collected exudates was filtered using Whatman No. 42 filter paper and a 0.45-µm membrane filter to remove debris. The filtrate of the exudates was then fractionated three times with equal parts of ethyl acetate. The combined ethyl acetate fractions were dehydrated, dissolved in HPLC grade methanol (Badri and Vivanco, 2009), and analyzed using liquid chromatography-mass spectrophotometry.

Metabolite analysis of root exudates by liquid chromatography-mass spectrophotometer

Root exudate samples were analyzed using a Liquid Chromatography Mass Spectrometer (LC/MS, Shimadzu, 8040, Japan) with Electrospray Ionization. Methanol-dissolved samples were introduced via a syringe pump at 0.3 mL min^{-1}. Scanning in positive and negative Q1 scan modes covered m/z 10–700, with a source voltage of 4.8 kV and a scan speed of 7500 u s^{-1}. Capillary temperature and sheath flow rate were maintained at 250 °C and 0.3 mL min^{-1}. The column was washed with water and methanol, equilibrated with acetonitrile and formic acid (pH 4.0), and set at a 50:50 (v/v) ratio, with a temperature of 35 °C. This solvent system served as the mobile phase at 0.3 mL min^{-1}, with a total run time of 30 minutes (Górka and Wieczorek, 2017). Bioactive metabolites were identified using the NIST mass spectral database library.

Measurement of proton efflux and root length

Proton efflux at different growth stages was measured by delicately uprooting three plants, cleaning them, and placing them in a nutrient solution. The solution contained elements (Mm) K_2SO_4 : 0.75; $MgSO_4$: 0.65; KH_2PO_4 : 0.1; $(NH_4)_2SO_4$: 0.5; $CaSO_4$: 0.5; H_3BO_3 : 1×10^{-2}; $MnCl_2.4H_2O$: 1×10^{-4}; $ZnSO_4.7H_2O$: 0.5×10^{-5}; $CuSO_4.5H_2O$: 0.5×10^{-5}; $H_2MoO_4.H_2O$: 0.5×10^{-6} (Marschner *et al.,* 1982). After a ten-hour period in a controlled environment at a temperature of 25±1°C, proton efflux was determined by titration with 0.01 M NaOH. The released protons were expressed as micromoles of H^+ per gram of fresh weight per hour (µmole H^+ g fresh $weight^{-1}$ h^{-1}). Root length (cm), measured from the base to the tip of the longest root, was assessed simultaneously.

Statistical analysis

Experiments, conducted with a minimum of three replications, presented mean ± standard deviation of three replicates. Tukey's test at a 5% significance level determined significant differences among treatments. Metabolic patterns were visualized using a heatmap with Pearson distance measure and Ward clustering. Variable importance plot for random forest analysis was generated with Metabo-Analyst 5.0. Pearson correlation coefficient assessed the correlation between proton efflux and root length. Graphs were created using Origin Pro 2023b, version 10.0.5.157.

RESULTS AND DISCUSSION

Analyzing organic acids in rice root exudates through non-targeted metabolic profiling

A non-target based metabolomics was done to identify the different organic acids in root exudates of three rice genotypes *viz*., Zn tolerant Karuppunel, Zn responsive CO51 and Zn sensitive ADT39 during plant growth stages. We have identified 14 low molecular weight organic acids in the root exudates of rice genotypes which are in 2 clusters (Fig 1). The identified organic acids in this study are glutaric

Table 1: Metabolic profiling of the organic acids in rice genotypes during plant growth stages with response to applied Zn.

Compounds	Vegetative stage						Panicle initiation stage						Grain filling stage					
	+Zn			-Zn			+Zn			-Zn			+Zn			-Zn		
	KN	CO	ADT	KN	CO	ADT	KN	CO	ADT	KN	CO	ADT	KN	CO	ADT	KN	CO	ADT
Acetic acid	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
Aminobutyric acid	+	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	+	+
Benzoic acid	+	+	-	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-
Butyric acid	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cynauric acid	-	+	+	+	-	+	-	-	+	+	-	+	-	-	-	+	-	-
Fumaric acid	-	-	+	+	-	+	+	-	+	-	+	+	+	+	+	-	-	+
Glutaric acid	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	-
Isonicotinic acid	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Malonic acid	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
Nicotinic acid	+	+	-	+	-	-	+	+	-	+	-	-	+	+	-	+	-	-
Phosphoric acid	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Propanoic acid	+	+	-	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-
Sebacic acid	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-
Succinic acid	-	-	-	+	+	-	+	-	-	-	+	+	+	+	+	+	-	+

KN: Karuppunel, CO: CO51, ADT: ADT39, '+': Presence of compound, '-' : Absence of compound.

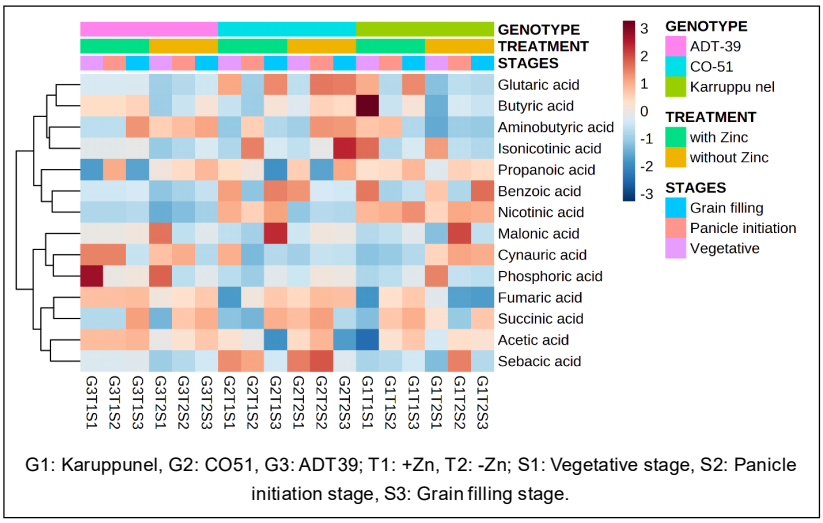
acid, butyric acid, amino butyric acid, isonicotinic acid, propanoic acid, nicotinic acid, benzoic acid, malonic acid, cyanuric acid, acetic acid, phosphoric acid, fumaric acid, succinic acid and sebacic acid (Table 1). All these 14 organic acids are present in exudates of Karuppunel under -Zn conditions at any stage of plant development. Most of the organic acids are absent in ADT39 under Zn deficiency conditions. Research report indicated that citrate, oxalate and malate as the major organic acids in rice root exudates. Many studies have been conducted to understand changes in citric, oxalic and malic acids in response to variations in plant physiological conditions. These fourteen organic acids have not received widespread documentation or analysis in the existing literature. Previous studies have recorded the occurrence of these organic acids in rice roots. The role of fumaric acid (Li *et al.,* 2018), succinic acid (Aulakh *et al.,* 2001) and acetic acid (Bhattacharya *et al.,* 2013) in TCA cycle is well explained. Role of glutaric acid in microbial sulphur and iron cycle in paddy is indicated by Xiao *et al.* (2023). Sebacic acid has been shown to present in root exudates in Nipponbare rice when the plant experiences physiological stress (Duan *et al.,* 2023). In *Arabadopsis thaliana* increased hormone levels such as indole acetic acid and gibberlic acid (El-bassiouny, 2005) and the release of phosphorus (Pantigoso *et al.,* 2022) by nicotinic acid have been reported. The role of Malonic acid is also reported in rice root exudates under conditions of phosphorus deficiency (Tawarya *et al.,* 2013).

The study conducted on rice genotypes Karuppunel, CO51 and ADT39 demonstrated a clear separation of 14 organic acids into two groups, revealing metabolite differences during growth stages. The organic acids in cluster1 were found at elevated levels in -Zn conditions in CO51. In the cluster-1, glutaric acid, butyric acid, aminobutyric acid, isonicotinic acid in sub cluster-1 whereas propanoic acid, benzoic acid and nicotinic acid in subcluster-2. Under cluster-2 the cyanuric acid, malonic acid, phosphoric acid, in subcluster-1 whereas acetic acid, sebacic acid, fumaric acid, succinic acid, in sub cluster-2 (Fig 1). Under Zn deficiency stress, Karuppunel exhibited higher organic acid secretion, indicating its adaptation to activate slowly available Zn for increased absorption. In both +Zn and -Zn conditions, Karuppunel surpassed CO51 and ADT39 in organic acid composition in rhizosphere soil, suggesting a role in releasing slowly available Zn. This aligns with previous findings on age-related discrepancies in exudate quality and quantity in rice and other plants. Such exudates contribute to nutrient acquisition strategies (Li *et al.,* 2018; Iannucci *et al.,* 2021).

The random forest importance plot identified the key metabolites with nicotinic acid having high mean decrease accuracy value of 0.05 indicating the most influenced metabolite followed by sebacic acid and glutaric acid (Fig 2) in all the 3 rice genotypes. The significance of nicotinic acid is suggested in literatures as its role in stress responses and as a precursor for the biosynthesis of coenzymes involved in various metabolic pathways are demonstrated (Dutilleul *et al.,* 2003; Pantingoso *et al.,* 2022). Plants release root exudates to facilitate either the direct solubilization of nutrients or the growth of particular microbial communities during specific developmental stages. These exudates play a role in enhancing the function of beneficial microbes like phosphate solubilizing bacteria and siderophore-releasing bacteria, aiding in the mobilization of essential nutrients in the soil (Banion *et al.,* 2020). The presence of organic acids, such as nicotinic acid identified in this study, may specifically attract microbial communities that assist in releasing siderophores, thereby aiding in the mobilization of zinc in soil that would otherwise be unavailable to plants.

Proton efflux in rice genotypes with response to zinc

Proton efflux patterns in rice genotypes (Fig 3) was assessed and among the genotypes Karuppunel showed highest proton efflux during all growth stages followed by



G1: Karuppunel, G2: CO51, G3: ADT39; T1: +Zn, T2: -Zn; S1: Vegetative stage, S2: Panicle initiation stage, S3: Grain filling stage.

Fig 1: Heat map analysis showing abundance of organic acid metabolites with response to zinc in rice genotypes during stages of growth.

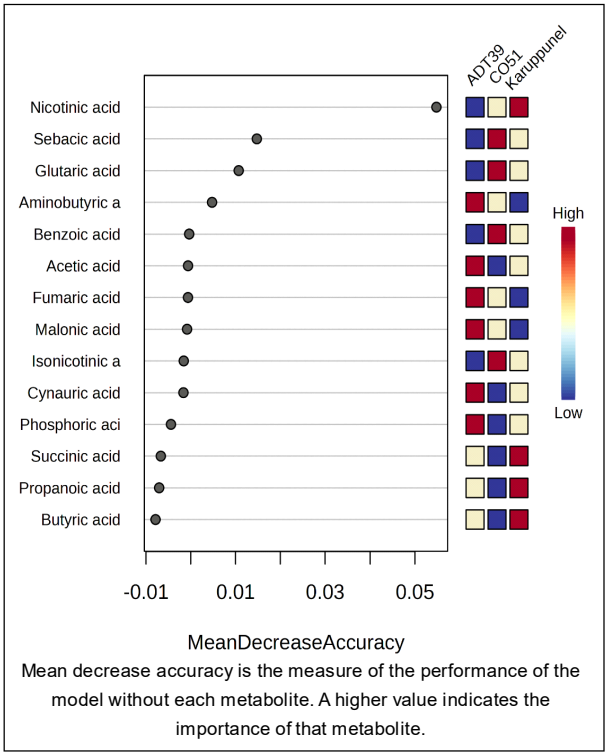
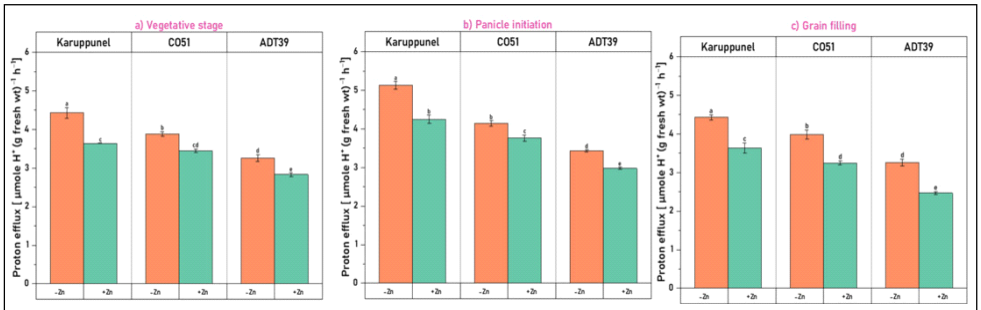


Fig 2: Random forest variable importance plot of organic acids.

CO51 and ADT39. The -Zn condition showed highest proton efflux. Also, proton efflux increased until panicle initiation stage and a declined subsequently during grain filling stage. Karuppunel showed higher proton efflux of 4.23 and 5.12 μ mole H^+ g fresh weight^{-1} h^{-1} during panicle initiation stage, under +Zn and -Zn conditions respectively. Similar findings have been reported in cassava, where the Fe and Al-tolerant genotype KU50 released more protons compared to the Fe and Al-sensitive RY9 cultivar (Armatmontree *et al.,* 2022 and Punpom *et al.,* 2022).

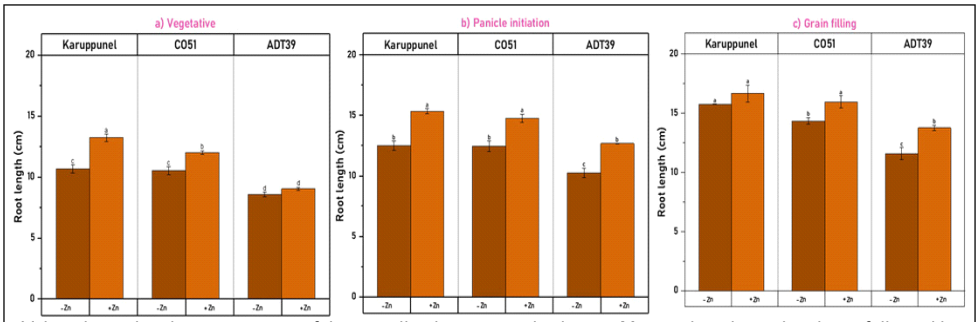
Root length measurement in rice genotypes with response to zinc

The measurements of root elongation are illustrated in Fig 4. Across all genotypes, there was a rise in the length of roots as the plants underwent growth. When compared with Zn nutritional status the root length was higher in +Zn conditions than -Zn conditions. The Karuppunel genotype extended the root length from vegetative stage to grain filling stage under +Zn and -Zn conditions *viz.,* 13.23 cm to 16.65 cm and 10.66 cm to 15.75 cm followed by CO51, 12.02 cm to 15.94 cm and 10.54 cm to 14.34 cm. The lowest root length was shown by ADT39 *i.e.,* 9.03 cm to 13.75 cm and 8.56 cm to 10.25 cm. These differences of root length suggest the difference in Zn nutrient acquisition pattern



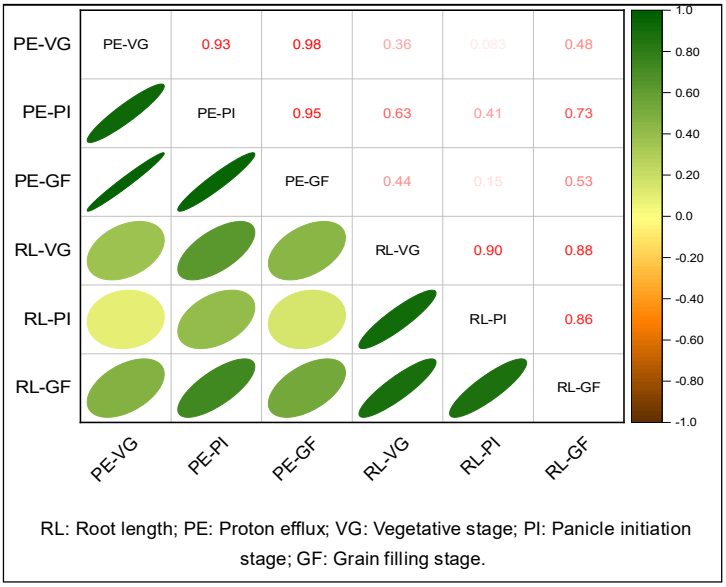
Values in each column are mean of three replications ± standard error. Mean values in each column followed by same letter(s) are not significantly different at 5% level.

Fig 3: Proton efflux measured in the root zone of Karuppunel, CO51 and ADT39 during stages of growth under -Zn and +Zn conditions.



Values in each column are mean of three replications ± standard error. Mean values in each column followed by same letter(s) are not significantly different at 5% level.

Fig 4: Root length of Karuppunel, CO51 and ADT39 during stages of growth under -Zn and +Zn conditions.



RL: Root length; PE: Proton efflux; VG: Vegetative stage; PI: Panicle initiation stage; GF: Grain filling stage.

Fig 5: Correlation between the proton efflux and root length of the rice genotypes during different growth stages with response to applied zinc.

among the rice genotypes. Similar results were reported in rice (Liu *et al.,* 2006; Gu *et al.,* 2023). These distinctions underscore genotype-specific responses to zinc availability, indicating that genetic factors play a crucial role in root length modulation under different zinc regimes.

Correlation between proton efflux and root length

The correlation analyses investigating proton efflux and root length indicated a positive association between the length of roots and proton efflux at every growth stage in rice genotypes (Fig 5). The correlation coefficient (r value) ranged from 0.083 to 0.98. A distinctly pronounced positive correlation was identified specifically in the panicle initiation stage of plant growth. This correlation underscores the complex connection between rhizospheric ionic properties, as indicated by proton efflux and the development of roots. The proton efflux of roots has been found to correlate with plant age, ATPase inhibitors, and various other factors (Bashan *et al.,* 1989). Amooaghaie *et al.* (2002) highlighted a positive relationship between the release of protons and the elongation of wheat roots. A similar interrelationship was reported by Wang *et al.* (2020) in wheat, where H^+ influx influenced root activity and pH, impacting wheat plant growth.

Conclusion

In conclusion, this study delves into the intricate interplay of underground plant traits, focusing on organic acids in root exudates, proton efflux and root length in response to varying zinc conditions in rice genotypes (Karuppunel, CO51, ADT39). Variations in metabolite composition across growth stages suggest genotypic differences in zinc accumulation efficiency due to inherent variations in zinc availability. The heightened organic acid secretion in low zinc conditions, particularly in wild-type Karuppunel, indicates an adaptive strategy for enhanced zinc absorption, with nicotinic acid playing a significant role in stress responses and coenzyme biosynthesis. Nicotinic acid secretion by plants could potentially facilitate the attraction of specific microbial communities involved in both the mobilization and remobilization of zinc, thereby making it accessible for uptake by the plant. Patterns in proton efflux and root length highlight inherent genotypic disparities in ion transport regulation and root development, with increased proton efflux under zinc deficiency suggesting a compensatory mechanism for nutrient uptake. The direct correlation between proton efflux and root length underscores the impact of rhizospheric ionic properties on root growth. While further studies are needed for a comprehensive explanation, these findings contribute to understanding the variation in zinc use efficiency among rice genotypes, aiming to enhance zinc nutrition in cultivated rice varieties through plant breeding programs.

Acknowledgement

The authors express their gratitude to Dr. Neeraja and Dr. P.C. Latha, ICAR-Indian Institute of Rice Research, Rajendhranagar, Telangana, for providing the Karuppunel seeds. The Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, is recognized for providing facilities for carrying out the research work.

Conflict of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

Amooaghaie, R., Mostajeran, A. and Emtiazi, G. (2002). The effect of compatible and incompatible *Azospirillum brasilense* strains on proton efflux of intact wheat roots. Plant and Soil. 243: 155-160.

Armatmontree, C., Leksungnoen, P., Nansahwang, A., Aramrak, S., Kongsil, P. and Wisawapipat, W. (2023). Iron toxicity downregulates root-proton efflux and decreases zinc accumulation in cassava. Annals of Agricultural Sciences. 68(1): 97-104.

Aulakh, M. S., Wassmann, R., Bueno, C., Kreuzwieser, J. and Rennenberg, H. (2001). Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biology. *3*(2): 139-148.

Badri, Dayakar, V, and Jorge, M. Vivanco. (2009). Regulation and function of root exudates. Plant Cell and Environment. 32 (6): 666-681.

Bandyopadhyay, T., Mehra, P., Hairat, S. and Giri, J. (2017). Morpho-physiological and transcriptome profiling reveal novel zinc deficiency-responsive genes in rice. Functional and Integrative Genomics. 17: 565-581.

Banion, O.B.S., Neal, O.L., Alexandre, G., Lebeis, S.L. Bridging the gap between single-strain and community-level plant-microbe chemical interactions. Molecular Plant-Microbe Interactions. 33 (2): 124-34. https://doi.org/10.1094/MPMI-04-19-0115-CR PMID: 31687914.

Bashan, Y. and Levanony, H. (1989). Effect of root environment on proton efflux in wheat roots. Plant and Soil. 119: 191-197.

Bashan, Y., Levanony, H. and Mitiku, G. (1989). Changes in proton efflux of intact wheat roots induced by *A. brasilense*. Canadian Journal of Microbiology. 35: 691-697.

Bhattacharyya, P., Das, S. and Adhya, T.K. (2013). Root exudates of rice cultivars affect rhizospheric phosphorus dynamics in soils with different phosphorus statuses. Communications in Soil Science and Plant Analysis. 44(10): 1643-1658.

Birla, D.S., Malik, K., Sainger, M., Chaudhary, D., Jaiwal, R. and Jaiwal, P.K. (2017). Progress and challenges in improving the nutritional quality of rice (*Oryza sativa* L.). Critical Reviews in Food Science and Nutrition. 57(11): 2455-2481.

Duan, R., Lin, Y., Yang, L., Zhang, Y., Hu, W., Du, Y. and Huang, M. (2023). Effects of antimony stress on growth, structure, enzyme activity and metabolism of Nipponbare rice (*Oryza sativa* L.) roots. Ecotoxicology and Environmental Safety. 249: 114409.

Dutilleul, C., Garmier, M., Noctor, G., Mathieu, C., Chétrit, P., Foyer, C.H. and de Paepe, R. (2003). Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity and determine stress resistance through altered signaling and diurnal regulation. The Plant Cell. 15(5): 1212-1226.

El-Bassiouny, H.M. (2005). Physiological responses of wheat to salinity alleviation by nicotinamide and tryptophan. International Journal of Agriculture and Biology. 7(4): 653-659.

Górka, B. and Wieczorek, P.P. (2017). Simultaneous determination of nine phytohormones in seaweed and algae extracts by HPLC-PDA. Journal of Chromatography B. 1057: 32-39.

Gu, H.Z., Xian, W.A., Zhang, M.H., Jing, W.J., Hao, W.U., Xiao, Z.L., Zhang, W.Y., Gu, J.F., Liu, L.J., Wang, Z.Q, Zhang, J.H. (2023). The response of roots and rhizosphere environment to integrative cultivation practices in paddy rice. Journal of Integrative Agriculture. DOI: 10.1016/j.jia.2023.06.031.

Iannucci, A., Canfora, L., Nigro, F., De Vita, P. and Beleggia, R. (2021). Relationships between root morphology, root exudate compounds and rhizosphere microbial community in durum wheat. Applied Soil Ecology. 158: 103781.

Kim, J.M., To, T.K., Matsui, A., Tanoi, K., Kobayashi, N.I., Matsuda, F., Habu, Y., Ogawa, D., Sakamoto, T., Matsunaga, S. and Bashir, K. (2017). Acetate-mediated novel survival strategy against drought in plants. Nature Plants. 3(7): 1-7.

Lamers, J., Van Der Meer, T. and Testerink, C. (2020). How plants sense and respond to stressful environments. Plant Physiology. 182(4): 1624-1635.

Lavanya, Y.S., Rangaswamy, A., Murugesan, D. and Vijila, K. (2024). Survey of Rice Genotypes of Southern India for Seed Zinc Concentration to Explore its Seed Endophytic Microbial Diversity. Indian Journal of Agricultural Research. doi: 10.18805/IJARe.A-6193.

Lehner A, Meimoun P, Errakhi R, Madiona K, Barakate M, Bouteau F. (2008). Toxic and signalling effects of oxalic acid. Plant Signaling and Behavior. 3: 746-748.

Li, X., Dong, J., Chu, W., Chen, Y. and Duan, Z. (2018). The relationship between root exudation properties and root morphological traits of cucumber grown under different nitrogen supplies and atmospheric CO_2 concentrations. Plant and Soil. 425: 415-432.

Liu, Z.H., Wang, H.Y., Wang, X.E., Zhang, G.P., Chen, P.D. and Liu, D.J. (2006). Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron and zinc contents in wheat (*Triticum aestivum* L.). Journal of Cereal Science. 44(2): 212-219.

Marschner, H., Romheld, V. and Ossenberg-Neuhaus, H. (1982). Rapid method for measuring changes in pH and reducing processes along roots of intact plants. Zeitschrift für Pflanzenphysiologie. 105(5): 407-416.

Martínez-Cuenca M.R., Iglesias D.J., Talon M, Abadía J, López-Millán A.F., Primo-Millo E, Legaz F. (2013). Metabolic responses to iron deficiency in roots of Carrizo citrange [*Citrus sinensis* (L.) Osbeck. × *Poncirus trifoliata* (L.) Raf]. Tree Physiology. 33: 320-329.

Midya, A., Saren, B.K., Dey, J.K., Maitra, S., Praharaj, S., Gaikwad, D.J., Gaber, A., Alhomrani, M. and Hossain, A. (2021). Crop establishment methods and integrated nutrient management improve: Part II. nutrient uptake and use efficiency and soil health in rice (*Oryza sativa* L.) field in the lower indo-gangetic plain, India. Agronomy. 11(9): 1894. doi: 10.3390/agronomy11091894.

Mora-Macías, J., Ojeda-Rivera, J.O., Gutiérrez-Alanís, D., Yong-Villalobos, L., Oropeza-Aburto, A., Raya-González, J., Jiménez-Domínguez, G., Chávez-Calvillo, G., Rellán-Álvarez, R., Herrera-Estrella, L. (2017). Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. Proceedings of the National Academy of Sciences. USA 114: E3563-E3572.

Muthert, L.W.F., Izzo, L.G., Van Zanten, M. and Aronne, G. (2020). Root tropisms: Investigations on Earth and in space to unravel plant growth direction. Frontiers in Plant Science. 10: 1807.

Palmer, C.M. and Guerinot, M.L. (2009). Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nature Chemical Biology. 5(5): 333-340.

Pantigoso Guevara, H.A., Vivanco, J.M., Fonte, S., Davis, J. and Manter, D. (2022). Role of rhizosphere bacteria and root exudates on the assimilation of phosphorus. https://hdl.handle.net/10217/235267.

Preece, C. and Penuelas, J. (2020). A return to the wild: Root exudates and food security. Trends in Plant Science. 25(1): 14-21.

Punpom, T., Leksungnoen, P., Aramrak, S., Kongsil, P. and Wisawapipat, W. (2022). Triggering root proton efflux as an aluminum-detoxifying mechanism in cassava. Annals of Agricultural Sciences. 67(2): 173-180.

Rengel, Z. (2015). Availability of Mn, Zn and Fe in the rhizosphere. Journal of Soil Science and Plant Nutrition. 15: 397-409.

Tawaraya, K., Horie, R., Saito, A., Shinano, T., Wagatsuma, T., Saito, K. and Oikawa, A. (2013). Metabolite profiling of shoot extracts, root extracts and root exudates of rice plant under phosphorus deficiency. Journal of Plant Nutrition. 36(7): 1138-1159.

Van Dam, N.M. and Bouwmeester, H.J. (2016). Metabolomics in the rhizosphere: Tapping into belowground chemical communication. Trends in Plant Science. 21(3): 256-265.

Wang, G., Feng, S., Ding, W., Hu, T. and Ru, Z. (2020). Winter wheat adapts to environmental pH by changing H^+ net flux in roots at the seedling stage. BioRxiv, 2020-10.

Werner, D. (1992). Symbiosis of Plants and Microbes (No. SB731 W49). London: Chapman and Hall.

Xiao, W., Zhang, Q., Zhao, S., Chen, D., Gao, N., Huang, M. and Ye, X. (2023). Citric acid secretion from rice roots contributes to reduction and immobilization of Cr (VI) by driving microbial sulfur and iron cycle in paddy soil. Science of the Total Environment. 854: 158832.

Zhang, J., Liu, Y.X., Zhang, N., Hu, B., Jin, T., Xu, H., Qin, Y., Yan, P., Zhang, X., Guo, X. and Bai, Y. (2019). NRT1. 1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nature Biotechnology. 37(6): 676-684.