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Survey of Rice Genotypes of Southern India for Seed Zinc Concentration to Explore its Seed Endophytic Microbial Diversity

Yama Santhoshi Lavanya[1], Dhandapani Murugesan[2]
Anandham Rangaswamy[1], Kenas Vijila[1]

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ABSTRACT

Background: Rice is a vital staple food for a large part of the global population and zinc deficiency poses health risks, particularly in rice-dependent regions. Studying genotypic differences in rice seed zinc content is vital for breeding zinc enriched varieties to address malnutrition and enhance food security. In the plant microbe partnership exploring the role of seed bacterial endophytes in influencing the plant physiology holds promise for Zn accumulation contributing to the broader goals of enhancing food quality, nutrition and crop production in a world facing increasing agricultural demands and challenges.

Methods: Rice genotypes were surveyed and their seed zinc content was analysed using non-destructive X-ray fluorescence spectrometry. Surface sterilization of rice seed samples was performed to isolate endophytic bacteria, involving a series of treatments with distilled water, ethanol and sodium hypochlorite to remove surface microflora. The sterilized seeds were then macerated and diluted to obtain a countable number of colonies, which were subsequently spread-plated on nutrient agar, tryptic soy agar and soil extract agar to enumerate the different nutritional types of seed endophytic microflora.

Result: In this study, 34 different rice genotypes, comprising traditional landraces and modern cultivars, were collected and analysed for their inherent zinc content. One variety in each category of low, medium and high seed Zn content was selected. The occurrence of all nutritional types of bacterial and their population was highest in the genotype Karuppunel followed by CO51 and ADT 39. Nutrient agar was found to promote a higher count of culturable bacterial endophytes compared to Tryptic soy agar and Soil extract agar.

Key words: Bacterial endophytes, Rice genotypes, Seed zinc content.

INTRODUCTION

Three million people around the world rely heavily on rice as a significant food source, about 20% of their calorie intake. In Asia, nearly two billion individuals depend on rice for their daily calories, accounting for 60-70% of their diet and plays a pivotal role in addressing global food security (Maganti *et al*., 2020). However, the nutritional quality of rice, particularly its zinc (Zn) content, has gained significant attention in recent years due to its link with malnutrition. Zinc deficiency affects a substantial portion of the global population, particularly in regions where rice serves as a dietary staple. The Zn content of rice seed varies significantly among different rice genotypes, presenting an opportunity to address this nutritional concern through genetic diversity and breeding programs.

Plant seeds harbour a variety of microorganisms that play a vital role in maintaining the well-being of both seeds and the resulting plants (Grum *et al*., 1998; Gitaitis and Walcott, 2007). Endophytes refer to bacteria that reside within plants without causing any visible signs of disease (Luo *et al*., 2011). They can establish mutually beneficial partnerships with plants, thereby enhancing the plants' ability to withstand both biological and environmental challenges (Afzal *et al*., 2019; Santoyo *et al*., 2016). Additionally, endophytes can support the growth of their host plants through various mechanisms, such as improving nutrient acquisition, increasing chlorophyll levels and boosting resistance to oxidation and defence capabilities (Rashid *et al*., 2012; Li *et al*., 2019).

Microbial communities and endophytes found within seeds are of significant importance due to their ability to be passed down from one generation of plants to the next. As they are transmitted *via* seeds, these endophytes guarantee their presence in the succeeding plants, ensuring their continuity (Truyens *et al*., 2015). Seed microbiomes exhibit a wide range of interactions and are anticipated to be a valuable biological asset for the promotion of sustainable agricultural practices (Barret *et al*., 2015 and Lugtenberg *et al*., 2016). Moreover, emerging research has shed light

[1]Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[2]Department of Plant Breeding and Genetics, Tamil Nadu Rice Research Institute, Aduthurai-612 101, Tamil Nadu, India.

Corresponding Author: Kenas Vijila, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Email: vijiladauphin@yahoo.co.in

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on the role of seed microflora, particularly endophytic bacterial populations, in influencing plant health (Gaiero *et al*., 2013) and nutrient uptake (Bajaj *et al*., 2018), further underscoring the complex interactions between the plant and its microbial partners. This is achieved through a variety of direct and indirect mechanisms, even in the face of various biotic and abiotic stress conditions, as evidenced by research (Santoyo *et al*., 2016; Shahzad *et al*., 2017; Rodrìýguez *et al*., 2018 and Shearin *et al*., 2018).

In this article, we delve into the multifaceted aspects of rice, including its significance as a food source, the challenge of Zn malnutrition, the genotypic variation in seed Zn content and the intriguing role of seed microflora endophytes in shaping rice plant health and nutrition. The present study was planned to survey the different rice genotypes and to determine their inherent seed zinc content. Also, the population density of different nutritional types of seed endophytic bacteria of selected representatives of rice genotypes was analysed and correlated with zinc content of seed.

MATERIALS AND AND METHODS

Survey and collection of rice genotypes

A survey was conducted in rice growing regions of South India and 34 different rice genotypes were collected from Tamil Nadu Agricultural University, Coimbatore; Tamil Nadu Rice Research Institute, Aduthurai and ICAR-Indian Institute of Rice Research, Hyderabad. The seeds of rice genotypes were collected and brought to the laboratory. The details of the samples are presented in Table 1.

Analysis of seed zinc content of rice genotypes

Zinc concentration in rice samples was estimated using non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model Hitachi X- supreme 8000; Oxford Instruments plc, Abingdon, UK) at TRRI, Aduthurai. Ten grams of well dried rice seed sample from each genotype was de husked using non-metallic de-husker (Krishi international 810 de-husker) having roller made of polymer to avoid zinc contamination. De-husked rice was cleaned by removing broken seed and debris and 5g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis in EDXRF. Concentration of seed Zn was expressed in milligram/ kilogram (mg kg^{-1}) or parts per million (ppm) seed.

Isolation and enumeration of seed endophytic microflora

Surface sterilization of seed samples

One gram of rice seeds of each Karuppunel, CO51 and ADT39 genotypes was taken in triplicate to isolate endophytic bacteria. To ensure the seeds were free from external contaminants, a surface sterilization process was applied to rice seeds. This involved rinsing the rice seeds with sterile distilled water, washing them with 70% ethanol for 30 seconds, treating them with 1% sodium hypochlorite for 150 seconds, followed by another 30-second wash with 70% ethanol. The sterilized seeds were rinsed three to four times with sterile distilled water to eliminate any remaining traces of the sterilizing agents. To validate the efficacy of the sterilization process, a 100 µl sample of the last rinse water was plated on nutrient agar and then incubated at 30±2°C for 48 to 72 hours.

Analysis of seed microbial endophytes

The surface-sterilized seed samples of three rice genotype were immersed in 10 ml of sterile water for 1 h to soften them and then macerated well with 10 ml of sterile water using a sterilized pestle and mortar, yielding a 10^{-1} dilution. To obtain a countable number of colonies, all the suspensions (10^{-1} dilutions) were thereafter serially diluted at the appropriate times. The suitable dilutions for each sample from all three genotypes were spread plated on Nutrient Agar (NA), Tryptic Soy Agar (TSA), Soil Extract Agar (SEA), Potato Dextrose Agar (PDA) and Rose Bengal Agar (RBA). The plates were incubated at 30±2°C for 4-5 days and observed each day for the appearance of bacterial colonies. The total number of colony-forming units (CFU g^{-1} of seeds) was counted for enumerating the population.

Statistical analysis

All the data were statistically analysed in Microsoft Excel and add-in with XLSTAT version 2022.1.1.1251 (M/s. Addinsoft Inc., USA). Each treatment was performed with at least three replications and the standard deviation was calculated and expressed in mean ± SD of three replicates. Significant differences among the treatments were statistically analysed using Tukey's test performed at a 5% significance level. Correlation analysis was done between seed endophytic bacterial population and zinc content by using Pearson correlation coefficient and all the graphs were constructed by using Origin Pro 2023b version 10.0.5.157 (Gomez and Gomez, 1984; Rodrigues *et al*., 2014).

RESULTS AND DISCUSSION

Analysis of inherent seed zinc content of rice genotypes

Seeds of 34 different rice genotypes comprising landraces and modern cultivars were collected and their inherent zinc content was analysed (Fig 1). Of the total 34 rice genotypes analysed Zn content of 16 genotypes ranged from 7 to 14 mg kg^{-1} of seeds, in 11 genotypes the range was between 14 to 24 mg kg^{-1} and 7 genotypes contained 24-44 mg $kg^{-1}$of inherent seed Zn content and categorized as low, medium and high Zn varieties (Table 2). Under each category of rice genotypes based on seed Zn content one genotype was selected considering the prevalence of cultivation by farmers. The selected rice genotypes were Karuppunel with 44.33 mg kg^{-1}, CO51 with 18 mg $kg^{-1}$and ADT 39 with 12 mg kg^{-1} for further studies and characters of selected genotypes are presented in Table 3. Several authors reported the occurrence of wide variation in seed zinc content in rice genotypes and it was 15.9 and 58.4 mg kg^{-1} (Graham *et al*., 1999),

Table 1: Details of rice genotypes collected from different locations.

Genotype	Type	Location
ADT 36	Cultivated variety	TRRI, Aduthurai
ADT 39	Cultivated variety	TRRI, Aduthurai
ADT 43	Cultivated variety	TRRI, Aduthurai
ADT 48	Cultivated variety	TRRI, Aduthurai
Arupatham kuruvai	Landrace	TRRI, Aduthurai
Asandi	Landrace	ICAR-IIRR, Rajendhranagar
BPT 2615	Cultivated variety	ICAR-IIRR, Rajendhranagar
BPT 3173	Cultivated variety	ICAR-IIRR, Rajendhranagar
BPT 3291	Cultivated variety	ICAR-IIRR, Rajendhranagar
BPT 5204	Cultivated variety	ICAR-IIRR, Rajendhranagar
Chittiga	Landrace	ICAR-IIRR, Rajendhranagar
Chittimutyalu	Landrace	ICAR-IIRR, Rajendhranagar
CO 39	Cultivated variety	TNAU, Coimbatore
CO 51	Cultivated variety	TNAU, Coimbatore
JGL 1798	Cultivated variety	ICAR-IIRR, Rajendhranagar
Kakirekalu	Landrace	ICAR-IIRR, Rajendhranagar
Karunkuruvai	Landrace	TNAU, Coimbatore
Karuppunel	Landrace	ICAR-IIRR, Rajendhranagar
Karupu kavuni	Landrace	TNAU, Coimbatore
Kottanellu	Landrace	TNAU, Coimbatore
Kuruvai kalanchium	Landrace	TNAU, Coimbatore
Mappilai samba	Landrace	TNAU, Coimbatore
MTU 1121	Cultivated variety	ICAR-IIRR, Rajendhranagar
MTU 1156	Cultivated variety	ICAR-IIRR, Rajendhranagar
RNR 2354	Cultivated variety	ICAR-IIRR, Rajendhranagar
RNR-15048	Cultivated variety	ICAR-IIRR, Rajendhranagar
Savulu Samba	Landrace	TNAU, Coimbatore
Tella hamsa	Cultivated variety	ICAR-IIRR, Rajendhranagar
TKM-13	Cultivated variety	TRRI, Aduthurai
Vaagai	Landrace	ICAR-IIRR, Rajendhranagar
Warangal samba	Cultivated variety	ICAR-IIRR, Rajendhranagar
WGL 14	Cultivated variety	ICAR-IIRR, Rajendhranagar
WGL 32100	Cultivated variety	ICAR-IIRR, Rajendhranagar
WGL 44	Cultivated variety	ICAR-IIRR, Rajendhranagar

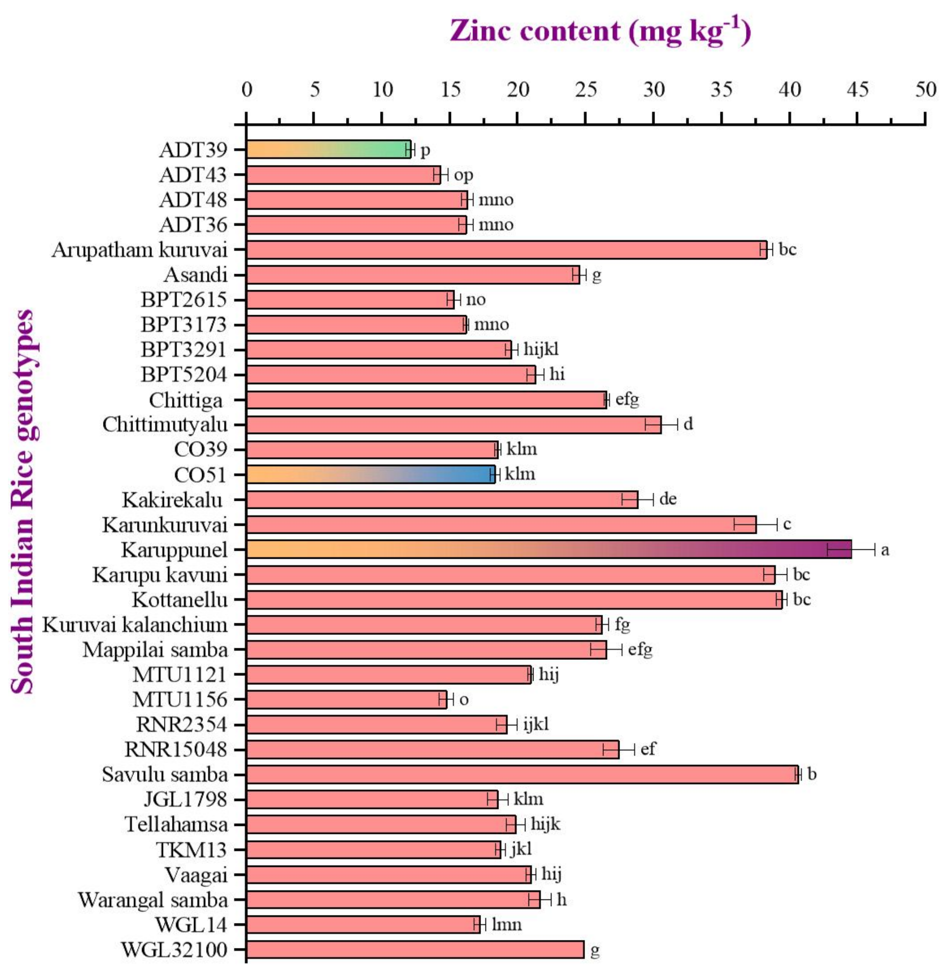
Table 2: Variation in seed zinc content among selected rice genotypes.

Seed zinc content range	Number of rice genotypes (Out of 34 genotypes analyzed)	Classification of rice genotypes
7-14 mg kg^{-1}	16	Low
14-24 mg kg^{-1}	11	Medium
24-44 mg kg^{-1}	7	High

15.3 to 58.4 mg kg^{-1} (Gregorio, 2002), 16.2-21.2 mg kg^{-1} (Pathak *et al*., 2017), 14.5 to 35.3 mg kg^{-1} (Maganti *et al*., 2020). Zinc in rice seed is distributed all through the endosperm. Hence, estimates of zinc in brown rice are effective indicators of zinc in polished rice (Maganti *et al*., 2020). The zinc micronutrient density in rice seeds across genotypes is influenced by a complex network of interconnected metabolic processes. These processes encompass uptake from the soil, transportation to source tissues and mobilization or remobilization to developing seeds (Grusak, 2002; Chandel *et al*., 2010).

Population dynamics of seed endophytic bacteria of the selected rice genotypes

Seeds of rice genotypes were used for the enumeration of total bacterial population using different growth media which include Nutrient Agar (a nutrient-rich medium for the enumeration of non-fastidious microorganisms), Tryptic Soya Agar (suitable for fastidious and non-fastidious microorganisms), Soil Extract Agar (which supports the growth of variety of soil microorganisms), Potato Dextrose Agar and Rose Bengal Agar (which supports the growth of variety of fungi) and results were presented in Table 4 and Fig 2. The total number of culturable bacteria obtained was found to be the highest in the Karuppunel followed by CO51 and ADT 39. More population of bacteria was enumerated using NA followed by TSA and SEA from three rice genotypes. Comparing the population among the rice genotypes on NA plates *i.e.*, 76 × 10^4 CFU g^{-1} in Karuppunel, followed by 69 × 10^4 CFU g^{-1} in CO51 and 61 × 10^4 CFU g^{-1} in ADT 39. Of the total number of bacterial endophytes enumerated a portion of 38%, 33% and 29% were obtained using NA. Over all, the concentration of endophytic bacterial



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

Fig 1: Inherent seed zinc content of rice genotypes of South India.

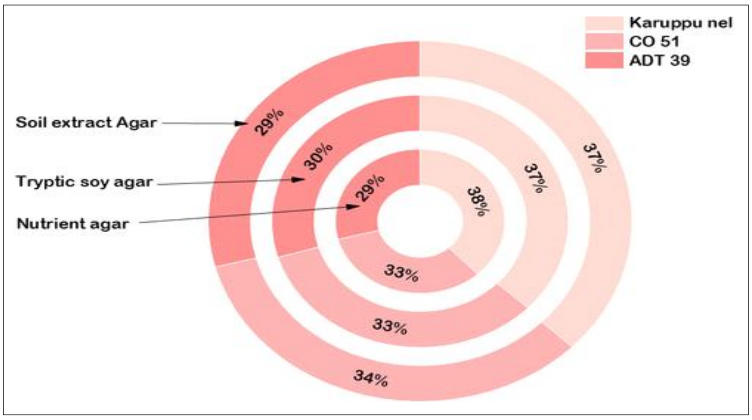
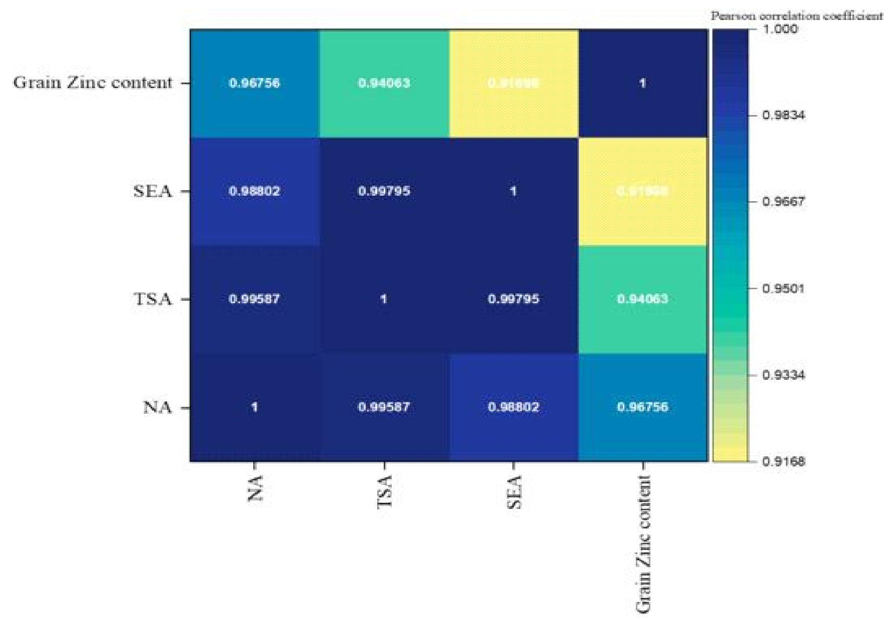


Fig 2: Percentage of bacterial population in the seeds of rice genotypes obtained using different growth media.



Values in each plot represent the Pearson correlation coefficient (r, n=3). Blue indicates positive high correlation and yellow indicates positive low correlation. NA: Nutrient agar, TSA: Tryptic soy agar, SEA: Soil extract agar.

Fig 3: Correlation between the seed endophytic bacterial population and zinc content of rice genotypes.

Table 3: Characters of the rice genotypes used in the present study.

Characters	Karrupunel	CO 51	ADT 39
Special trait	Zn Tolerant	Zn Responsive	Zn Sensitive
Type	Traditional, Wild genotype, Tall	High yielding, widely cultivated in Tamil Nadu, Semi-dwarf	
Seed Zn content (mg kg^{-1})	40-44	18-20	10-12
Days	75-80	105-110	120-125

Table 4: Seed endophytic bacterial population of rice genotypes differing in their seed zinc content.

Genotypes	Endophytic bacterial population (10^4 CFU g^{-1})		
	NA	TSA	SEA
Karuppunel	76 (±1.28)^a	65 (±1.45)^a	41 (±0.87)^a
CO51	69 (±1.44)^b	54 (±0.76)^b	37 (±0.67)^b
ADT39	61 (±0.76)^c	48 (±0.30)^c	32 (±0.23)^c

Values in each column are mean of three replications ± SE (standard error). Mean values in each column followed by same letter(s) are not significantly different at 5% level. NA: Nutrient agar, TSA: Tryptic soy agar, SEA: Soil extract agar.

population was in the order Karuppunel>CO51>ADT39 obtained using bacterial growth media which supported different nutritional types of bacteria. No fungal population was detected on standard fungal growth media. The results were in parallel with Hardoim *et al.* (2015) who detected up to 3.5×10^5 CFU g^{-1} of fresh tissue in rice seeds and Aswini *et al.* (2023) enumerated seed bacterial endophytes population in wheat seeds in which NA media held the highest population ranging from 12×10^2 to 57×10^2 CFU g^{-1} of seeds. Similarly, Singh *et al.* (2018); Sai Prasad *et al.* (2021); Manias *et al.* (2020) and Sharma *et al.* (2023) who used diverse growth media for the study of different nutritional types of endophytes.

Correlation between grain zinc concentration and endophytic bacteria density

A correlation analysis was conducted to examine the relationship between the population of seed endophytic bacteria and zinc content (Fig 3). All the endophytic bacterial population grown on different media showed positive correlation with the inherent seed zinc content of seed. The positive correlation between seed endophytic bacterial population and seed Zn content is likely due to the role of these bacteria in facilitating zinc uptake, transport within the plant, enhance nutrient availability, resulting in increased zinc accumulation in seeds (Wang *et al*., 2019; Makar *et al*., 2021). This symbiotic relationship may contribute to higher seed Zn content, ultimately benefiting the plant's nutrition and growth.

CONCLUSION

In conclusion, this study examined the seed zinc content of wild and cultivated rice genotypes, in South India highlighting significant variation in Zn concentration *i.e.*, 7 to 44.33 mg kg^{-1} of seeds among the genotypes. Furthermore, our investigation into seed bacterial endophytes in three selected genotypes revealed the occurrence of various types of seed endophytic bacteria in high number. The landrace, Karuppunel exhibited the highest abundance, followed by the medium Zn content CO51 and low Zn content ADT 39. The positive correlation between seed endophytic bacterial population and seed zinc content across different growth media suggests a consistent and potentially significant biologically relationship. Since this high Zn concentration invites varying types and more population of microflora to become partner with host, their interaction with host plant would be helpful to the plant during its growth through improved nutrient uptake and biotic and abiotic stress resistance.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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