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Shrikant Yankanchi
Department of Plant Molecular Biology Biotechnology, IGKV Raipur Chhattisgarh, India

Vinay Premi
Department of Plant Molecular Biology Biotechnology, IGKV Raipur Chhattisgarh, India

Dayanad Navrang
Department of Plant Molecular Biology Biotechnology, IGKV Raipur Chhattisgarh, India

Devidas Thomabre
Department of Plant Molecular Biology Biotechnology, IGKV Raipur Chhattisgarh, India

Ajit Kumar Mannade
Department of Plant Molecular Biology Biotechnology, IGKV Raipur Chhattisgarh, India

Corresponding Author:
Shrikant Yankanchi
Department of Plant Molecular Biology Biotechnology, IGKV Raipur Chhattisgarh, India

Comparative analysis of Zinc and Iron content estimated by destructive and non-destructive methods in selected rice genotypes

Shrikant Yankanchi, Vinay Premi, Dayanad Navrang, Devidas Thomabre and Ajit Kumar Mannade

Abstract
Iron and Zinc are the two essential micronutrients required in minute amounts for proper human nutrition as they take part in body's metabolism and immunity development. Nutrient analyses of plant samples are carried out by destructive and non-destructive methods. Iron (Fe) and Zinc (Zn) content of polished rice grains from twenty known rice genotypes was quantified using (Energy Dispersive X-ray Fluorescence) ED-XRF and the results were compared with standard Atomic Absorption Spectrometer (AAS). Average concentration, standard deviation and relative standard deviations were calculated for the comparison of both the methods. The mean grain Zn content of 20 rice genotypes analyzed by AAS and ED-XRF was 21.30 and 20.67 with standard deviation 4.739 and 4.417. Similarly the mean grain Fe content of 20 rice genotypes analyzed by AAS and ED-XRF was 4.54 and 4.4045 respectively with standard deviation 42.043 and 1.582. Results showed significant higher positive correlations between AAS and ED-XRF values for both Zn (0.921**) as well as Fe (0.940**) content. The Zn and Fe values obtained from AAS and ED-XRF for all 20 rice genotypes were close to each other with minor variations by 0.25 ± 2.45 ppm. Finally concluded that both analytical techniques, AAS and ED-XRF are very good and highly precise however, non-destructive ED-XRF is considered to be the easiest, fast, cost effective as well as eco-friendly as compared to destructive methods (AAS).

Keywords: Biofortification, micronutrients, atomic absorption spectrometer, energy dispersive x-ray fluorescence

1. Introduction

Micronutrients like Zinc (Zn), Iron (Fe), Copper (Cu), Boron (B), Manganese (Mn), Copper (Cu), Molybdenum (Mo) and Chlorine (Cl) are most essential in human diets, deficiency of which leads to reduced immunity and lower rate of recovery after infections [1]. Among these micronutrients Fe and Zn serves as the main source of growth, development and proper working of human's body immunity. Since rice is one of the important staple food crops that has been consumed by more than half of the global population it is targeted for biofortification programs for enhancing grain Zn and Fe contents [2].

The quantity of micronutrients is measured by two different methods, destructive and non-destructive. Destructive methods include Absorption Spectroscopy (AAS), Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) and Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) where the seeds samples are subjected to acid digestion prior to analysis. AAS, ICP-MS and ICP-OES are highly sensitive and precise techniques used for quantifying elemental concentrations in the liquid samples [3]. Energy Dispersive X-ray Fluorescence (ED-XRF) is a non-destructive method in which intact seed samples are used for the analysis without prior acid digestion [4]. AAS and ED-XRF have wide range of applications including determination of plant (roots, leaves, fruits, and seeds) elemental composition [5] micronutrients and trace elements level in soils [6, 7] heavy metal contents in ground water [8 9] and assessment food quality [3]. Both analytical methods are easy to operate and give accurate results. However, of these two methods, non-destructive method (ED-XRF) considered to be the quick, precise, easily operating, and economical method as compared to destructive methods (AAS, ICP-MS, ICP-OES) [10, 4]. Destructive methods analyze elements present in the liquid solutions due to which plant samples are subjected to acid digestion prior to analysis.

With this background current study was carried out with an objective to compare two standard analytical methods, AAS (destructive) and ED-XRF (Non-destructive) for their efficiency and economy in micronutrients analysis in plant samples. Zinc and Iron content of 20 known rice genotypes were quantified using ED-XRF spectrometry and the results were compared with standard AAS.

2. Material and Methods

Seed materials were collected from the rice field experiment conducted at IGKV Raipur Chhattisgarh during the crop season *Kharif*- 2021. The plant materials comprised of a set of 20 rice genotypes (Table No.1) including released varieties, landraces and check varieties for grain Zn and Fe content. After harvesting 100 gram paddy samples were subjected to dehusking and polishing for 75 seconds in *Zaccaria* hulling and milling machine. Polished rice samples were washed with double distilled water and air dried for 24 hours under shade without any contamination. Air dried samples were separated into two halves in which one half kept for AAS and other for ED-XRF.

2.1 Zn and Fe estimation by AAS

One gram of polished rice samples from each rice genotype was subjected to 10 ml di-acid mixture (Nitric acid and Per-Chloric acid) digestion in *Kjeldahl* Digestion Unit at 280^0 C until the sample solution turns into colorless. After air cooling solution was filtered through Watsman’s No.40 filter paper and filtrate volume made up to 50 ml. Zn and Fe content was estimated as per Harvest Plus guidelines. *Thermo Scientifics’s* Standard AAS iOS 2000 series instrument located at IGKV Campus Raipur, Chhattisgarh was used to quantify Zn and Fe content.

2.2 Zn and Fe estimation by ED-XRF

25 gm of polished rice grains from each rice genotype was used for the estimation of Zn and Fe content by ED-XRF (*S2 Power Ranger*) located at ICRISAT Hyderabad.

3. Results and Discussion

The concentration of Zn and Fe content of 20 rice genotypes obtained from AAS and ED-XRFwere presentedin theTable 1.

Table 1: Concentration of Zn and Fe content of 20 rice genotypes obtained from AAS and ED-XRF methods

Sl. No	Genotypes	Zn-AAS (ppm)	Zn-ED-XRF (ppm)	Fe-AAS (ppm)	Fe ED-XRF (ppm)
1	CGZR-1	23.2	22.25	6.2	5.25
2	CGZR-2	22.6	23	4.5	4.5
3	ZincoRice-MS	25.2	24.5	6.7	4.25
4	Protezin	22.7	21.8	3.1	3.25
5	Madhuraj-55	15.4	14.5	3.1	3.25
6	MTU-1010	19.1	20.25	4.8	4
7	Chandrahasni	22	19.5	4.2	3.75
8	CR Dhan-310	17.1	19.5	2.6	1.5
9	DRR-48	22.2	21	2.3	2
10	SWARNA	17.1	15.25	3.8	3.5
11	IR64	19	19.5	3.5	3.75
12	Chitimutaliya	32.2	30.5	10.2	8.25
13	Chir-8	18.7	16.75	4.8	5.2
14	Makdo	19.5	17.25	3.7	4.2
15	Bangla gurmatia	28.3	25.5	6.5	5.5
16	RKVY 211	20.2	22.75	7.8	6.5
17	BAM698	28.5	27	4.7	3.75
18	IET-24780	16	18.25	2.8	3.25
19	DRR Dhan-45	23.4	22.25	3.2	3.5
20	Bas 8	13.6	12	2.3	1.75

3.1 Comparison of grain Zn content

The Zn content of 20 rice genotypes were analyzed by ED-XRF and compared with values obtained by standard AAS (Table 1) Figure 1A and 1B. The concentration of Zn estimated by AAS ranged from 13.6 ppm to 32.2 ppm with an average concentration of 21.30 and average standard deviation of 4.739. Similarly the concentration of Zn estimated by ED-XRF was ranged from 12 ppm to 30.5 ppm, with an average Zn concentration 20.67 ppm and average standard deviation 4.417 (Table 2).

Recorded grain zinc content values of all 20 rice genotypes from AAS are close to the values recorded by ED-XRF with some variations by ± 2 ppm Figure 1(A) and 1(B). Also the results show significant positive correlation (0.921) between AAS and ED-XRF (Table 3). Zn concentration of 68 rice genotypes were analysed by using ED-XRF. They reported Zn content of these rice genotypes 19.5 (IR64), 16.6 (Swarna), and 19.3 (MTU1010) were very close to our results [6]. CGZR1, CGZR2 and Zinco rice MS are the Zn biofortified varieties released by IGKV, Chhattisgarh with grain Zn content 22-24 ppm, 24 ppm and 24-26 ppm respectively. Our results from ED-XRF and AAS are on far with these biofortified rice varieties.

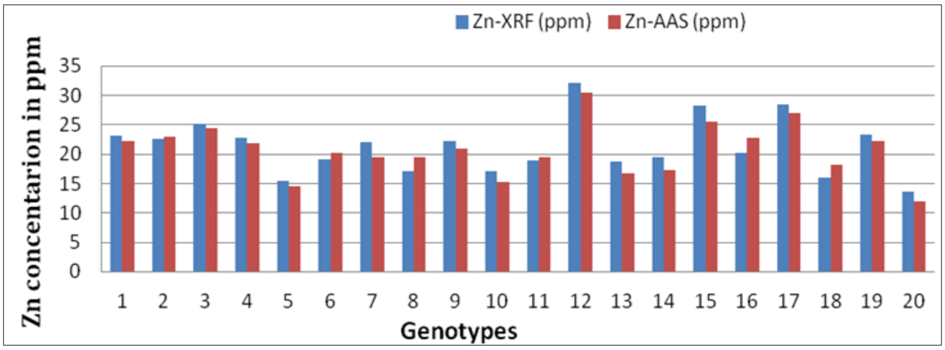

Fig 1A: Bar Graph representing the variations of grain Zn Content in 20 rice accessions analyzed by AAS and ED-XRF.

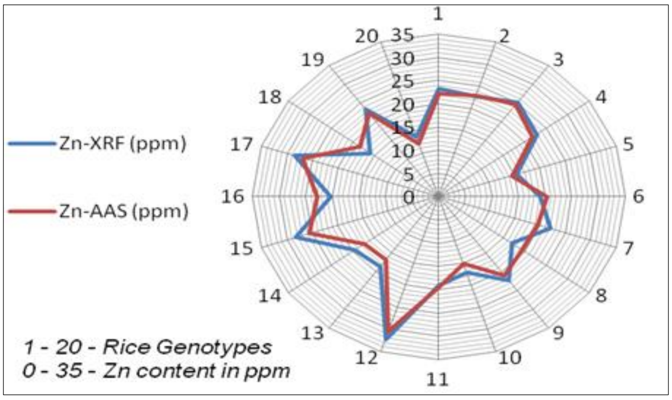

Fig 1B: Spider Graph-Comparison of rice grain Zn content analyzed by AAS and ED-XRF

3.2 Comparison of grain Fe concentration

The Fe content of 20 rice genotypes were analyzed by ED-XRF and compared with values obtained by standard AAS (Table 1) Figure 2A and 2B. The concentration of Fe estimated by AAS ranged from 2.3 ppm to 10.2 ppm with an average concentration of 4.54 and average standard deviation of 2.043. While, the concentration of Fe estimated by ED-XRF was ranged from 1.5 ppm to 8.25 ppm, with an average Fe concentration 4.045 ppm and average standard deviation 1.582 (Table. 2). Except CGZR-2 (4.5 ppm Fe in both methods), remaining genotypes showed minor variations in ED-XRF values as compared to AAS values by ± 2.45 ppm. Results found significant and very high positive correlation (0.940**) between the values of these two analytical methods for grain Fe content (Table 3) [11]. Screened a set of 96 rice genotypes including released varieties, advanced breeding lines (ABLs) and landraces for grain Fe and Zn content using ED-XRF. The reported grain Fe contents of GGZR1, RKVY211, IR64 and Swarna were 7.2 ppm, 9.5 ppm 6.5 ppm and 8.3 ppm respectively. However, these results are not on far with our results obtained from both ED-XRF as well as AAS.

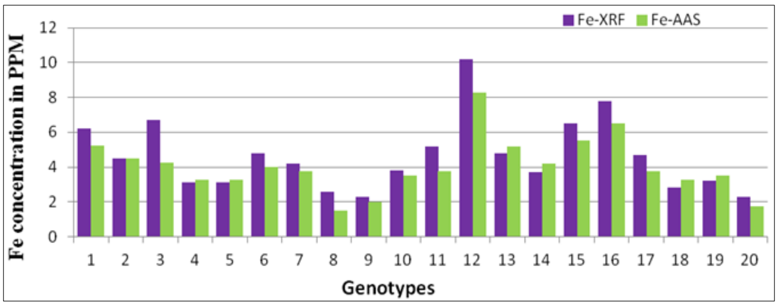

Fig 2A: Bar Graph representing the variations of grain Fe Content in 20 rice accessions analyzed by AAS and ED-XRF.

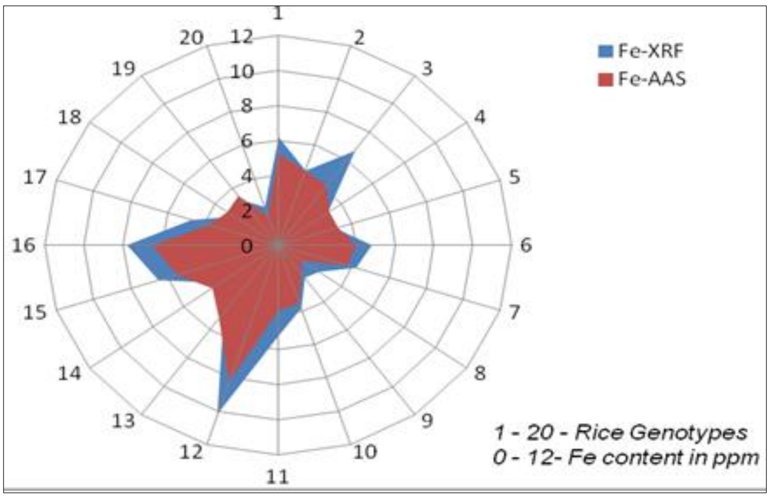

Fig 2B: Spider Graph-Comparison of rice grain Fe content analyzed by AAS and ED-XRF.

Table 2: Descriptive Statistics for rice grain Zn and Fe content analyzed by AAS and ED-XRF

Character	Range (ppm)	Mean (ppm)	Standard Deviation	Std. Error
Zn-AAS	13.6 - 32.2	21.30	4.739	1.027
Zn-ED-XRF	12 – 30.5	20.67	4.417	0.972
Fe-AAS	2.3 – 10.2	4.54	2.043	0.959
Fe-ED-XRF	1.5 – 8.25	4.045	1.582	0.787

Table 3: Spearman's Rank Correlation Matrix for rice grain Zn and Fe content analyzed by AAS and ED-XRF

	Zn (AAS)	Zn (ED-XRF)	Fe (AAS)	Fe (ED-XRF)
Zn-AAS	1			
Zn-ED-XRF	0.921**	1		
Fe-AAS	0.589**	0.625**	1	
Fe-ED-XRF	0.554*	0.570**	0.940**	1

Availability of micronutrient to plants are affected by several factors like soil micronutrients levels, crop seasons, fertiliser doses, soil properties such as redox potential, pH, soil organic matter content, other soil nutrient, type of plant or variety, and environmental factors, such as temperature, light and soil moisture levels [12]. Apart from these factors grain micronutrient levels also affected by post-harvest processes like grain polish percentage, presence and absence of broken, diseased and immature seeds in the samples, type of water used for washing, dust particles on seeds, rust and methods used for nutrient analysis. In particularly iron is most affected by these post-harvest processing factors in contrast to other elements.

4. Conclusion

The results from both analytical methods for Zn and Fe content of 20 rice genotypes found that there were no much differences among the values of all rice genotypes. Grain Zn content of Zn biofortified varieties (CGZR1, CGZR2, Zincorice-MS) showed variations upto ± 1 ppm as compared to ED-XRF. Highest variation of grain Zn content found in case of Bangla gurmatia where the recorded ED-XRF value was 22.75 ppm which is 2.55 ppm higher than the AAS value and RKVY-211 recorded with 22.75 ppm which is -2.55 ppm lesser than AAS value. Similarly grain Fe content of 20 rice genotypes obtained by ED-XRF showed variations ranging from -0.5 ppm in Mokdo to 2.45 ppm in Zincorice-MS as compared to AAS. Between AAS and ED-XRF values for both grain Zn and Fe contents high positive correlation was found indicating both methods are significant to use. Both analytical methods are simple and easy to operate and their results are more or less accurate to each other. However, non-destructive ED-XRF spectrometry takes first place when it comes to large-scale screening of plant samples to choose the best genotypes with high grain Zn and Fe contents and when there is a low budget for sample analysis because of its simple working principle, ease of operation, and speed as compared to destructive methods (AAS, ICP-MS, ICP-OS).

5. Acknowledge Ements

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