

Elemental Analysis of Popular Zinc rich Rice Varieties using ESEM and ICP-OES

Swapan Kumar Tripathy* and Sambit Patra

Department of Agricultural Biotechnology, College of Agriculture,
OUAT, Bhubaneswar-751 003, India.

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A comparative study was carried out for elemental composition of grains of eight popular rice genotypes. The Environmental Scanning Electron Microscope (ESEM) setup was used to explore type and quantity of surface elements in various layers of rice grains. The main constituents are carbon and oxygen, with elements like Al and Si being prominently found on the husk. Rice kernels serve as staple food. We found that the top surface layer of whole rice kernel is rich in carbon compared to oxygen, but oxygen content increased in the internal area of kernel (endosperm in broken kernel) in all test genotypes. Further, our ESEM results showed that the husk of grain consists of more elements than the seed part. Though most of the above rice varieties are Zn and/or Fe rich, the ESEM facility could not identify them. ICP-OES analysis revealed Nagina-22 and R-RHZ-7 as highly zinc and iron rich rice varieties respectively. The knowledge of elemental composition of husk and whole kernel may pave the way for their effective uses in biofortification programme and as such for use as food, animal feed and industrial purposes.

Keywords: Surface elemental composition, zinc rich rice, whole rice grain, whole kernel, broken kernel.

Rice is the staple food belonging to the genus *Oryza*. It contributes sizeable proportion to the national food production(43%) and cereal production (55%). India stands first in area under rice cultivation (44mill. ha) leading to second position in rice production. *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) are the two cultivated forms of rice. There are more than 40,000 varieties characterised by differences in shapes, sizes, texture, aroma, and colours; and they are named accordingly (www.riceassociation.org.uk). Apart from being well known for being the principal food for over 60% population of the

globe, it is also being used increasingly both for the properties of the grain and the husk. Besides, it is used commercially as food for livestock, as ingredient in cosmetics and also as dietary supplements and pills for health benefit.

Vitamin and/or mineral deficiency affects half of the world's population which increases the vulnerability to illness and premature death. Globally, micronutrient malnutrition is a chronic problem to more than three billion people. Death of children to the tune of 3.1 million each year is due to malnutrition alone¹. One in each three people in the globe, particularly children and

*Corresponding author E-mail: swapankumartripathy@gmail.com



women are associated with Zn deficiency related health problems including stunted growth, loss of appetite, reduced immunity to diseases, hair fall, diarrhoea, eye and skin lesions, weight loss, healing problem of wounds, and mental retardation².

Environmental scanning electron microscopy (ESEM) uses electron as source for imaging and due to variable pressure, it permits to carry out imaging without even coating a metallic layer for conductive purpose to reduce beam instabilities and poorer resolutions. With an energetic electron knocking out the target electrons and due to rearrangements of target atomic electronic configuration, a resultant secondary X-ray fluorescence is detected and further analysed to get elemental quantifications. An e-beam is focused to the specimen surface that generates a wide array of signals forming characteristic images. In fact, such images are the result of secondary electron (SE) and/or back-scattered electron (BSE) detection. Specimens can be resolved to 10 nm even in the simplest micrograph systems. ESEM offers two typical advantages. First, there is no requirement of a conductive carbon or metal thin film to be applied for non-conducting samples. In this case, the electrons generated from the beam/sample interaction enable ionization of the gas in the sample chamber and diffuse to the sample surface without dissipation of any negative charge. Secondly, if the sample in question is prone to out gassing volatile substances such as solvents and water, the near-ambient pressures used in the sample chamber can greatly diminish the likelihood of this occurring.

The elemental analysis of the rice grain can be done with various other methods. Inductive coupled plasma-mass spectrometry (ICP-MS) technique³ and inductively coupled plasma-optical emission spectrometry (ICP-OES) technique⁴ can diagnose the elements present in the rice grain besides quantifying the elements concerned. Similarly, the Particle Induced X-ray Emission (PIXE)⁵ is also a very powerful technique to detect the trace elements present in rice grains. This technique is based on emission of X-rays with energies characteristic of the target elements when charged particles (protons) are subjected to pass through matter. This clearly identifies the elements in the matter and their concentrations.

On the other hand, X-ray fluorescence (XRF) is also considered as a suitable alternative as it offers relatively quick, reliable, non-destructive and multi-elemental analytical advantages of a variety of food products. PIXE and XRF techniques have been reported for determination of fourteen elements (Na, Mg, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Rb and Sr) in rice plant samples⁶. In the present investigation, a comparative study of the elemental composition of some zinc rich rice varieties was attempted using ESEM and ICP-OES for their use as both food as well as commercial purpose.

MATERIALS AND METHODS

Eight popular nutrient rich rice genotypes including the mega variety Swarna were laid out in Randomized Block Design (RBD) with three replications in Kharif, 2019. Before planting, average pH of the experimental plot was 5.8 and the average iron and zinc content of soil were 450ppm and 0.52ppm respectively. After harvest of the crop, the rice grains were oven dried at 50°C for two hours to reduce the moisture content to 11-12%. The dried whole, manually dehulled and broken rice grains were studied for surface element composition using the Environmental Scanning Electron Microscope (ESEM) setup at Institute of Physics, Bhubaneswar, India. The ESEM under high vacuum conditions was used to get a better resolution of the rice grains.

For micronutrient (Zn and Fe) analysis, fine ground brown rice samples in three replicates were digested by di-acid mixture of nitric acid (HNO₃): and perchloric acid (HClO₄) in 3:2 ratio following the standard procedure⁷ with minor modification (i.e. 3:2 instead of 1:2 di-acid ratio). It is based on the principle that when excited electrons in zinc (Zn) and iron (Fe) atoms return to the ground state, they emit light at very specific wave lengths. The absorption spectrum of such radiation is used to calculate the concentration of Zn and Fe. Zinc and iron content was estimated in the aliquot of seed extract by using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) for Zn and Fe at 206.2nm and 238.20nm respectively at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The mean of the three replicates were worked out to indicate Zn and Fe-content of each genotype.

RESULTS AND DISCUSSION

The ESEM is an electron microscope which produces image of an object by scanning its surface with the electrons beam. The electron beam coming from the source of the electron gun in the ESEM has low intensity. That means it carries a low energy and penetrates into the surface layer of the target rice grain only. The electrons interact with the surface atoms and generate different signals depending on the interacting elements. By the excitation of the elemental atoms due to the electron beam, sometime it produces secondary electrons which are detected by an Everhart-Thornly detector. Of course, the number of secondary electrons and the signal intensity depends very much on the sample specimen. The samples can be observed in high/low vacuum or wet conditions of the environmental SEM. In our experiment, we performed the observation under high vacuum conditions to get a better resolution of the rice grains. Since the present SEM is an environmental set up, we place the target sample as such without any coating⁸. First of all, the whole grain with husk was used as a target. As a result, the electrons interacted only on the surface of the whole rice grain and revealed the information about the elements in the husk. Then the husk was gently removed and rice kernels were used as the target material to investigate the surface layer properties. Finally, the rice kernels were broken and the pieces were used as target materials to visualize and estimate the interior elemental composition. In the present investigation, the elemental composition of eight rice varieties e.g., BRRI Dhan-64, R-RHZ-7, Swarna (control), Chak Hao, URG-24, CGZR-1, Kalanamak and Nagina 22 was explored for above three different environments e.g., whole rice grain, whole rice kernel and broken kernel.

A systematic comparison of the major elements like C, O, Al, Si, Cl, Mg, S, P, K, Ca, Cu and Na has been presented for different varieties of rice (Fig 1 and Table 1). Although C and O are the major components in rice grains, we find sizeable percentage of Al, Si, Mg, Cl, K, Ca, P and S in many of the varieties. The husk is found to be rich in minerals which may be useful for commercial purposes.

Husk is the hard protective covering of rice grains. Rice hulls/husk can be put to use as

building materials, cardboards, Portland cement particle boards⁹, fertilizer (soil ameliorants / ammendments), insulating material, absorbent for oil and chemicals, or fuels. In the present study, carbon content of husk varies with a wide range starting from 22.55% to 67.11%. The CGZR-1 showed maximum Carbon content (67.11%) while, BRRI-64 had the least with 22.55% C. Similarly, the husk of BRRI DHAN-64 has the maximum Oxygen content followed by R-RHZ-7, Kalanamak, Swarna, Chak Hao, URG-24, CGZR-1 and Nagina-22. The Nagina -22 has an exceptionally high Aluminium (Al) percentage (17.95%), but all others revealed less than 1% Al. Silicon (Si) is an important ingredient in rice husk of most of the rice varieties except Nagina 22. Except CGZR-1 which has only 0.50% Si, all others have a comparable percentage in the range 14.5 to 24.45%. The percentage of Chlorine (Cl) was detected as 0.11, 0.59 and 2.49% only for Chak Hao, CGZR-1 and Nagina-22 respectively. While, the availability of Magnesium (Mg), Sulphur (S), Potassium(K) and Calcium(Ca) was shown to be very small in some of the varieties (Table 1, Fig 1).

The data in Table 1 is also displayed in Fig. 1. The major elements present in the husk are clearly visible in the histogram, though the trace elements having crucial role for food value are not conspicuous. To get a feeling for these trace elements, we have enlarged the portion of the trace elements in the insert of the figure.

————Carbon is relatively low in the rice husk, but it is higher in the whole kernel (brown rice) ranging from 59.56 % in CGZR-1 to 71.86% in BRRI DHAN-64. Oxygen content is lower in the grains, and it is 25.71% in -Nagina 22 at the lowest spectrum to 40.07% at the highest in CGZR-1. Kalanamak and URG-24 had not revealed any trace elements. In contrast, Chak Hao showed all elements like Al, Si, Mg, Cl, K, Ca, P and S (except Cu), followed by both Swarna and R-RHZ-7 showing Si, Mg, Cl, K, Ca, and S. As compared to other varieties, Swarna, Chak Hao and R-RHZ-7 had shown similar composition. The Manipuri popular black rice “Chak Hao” had shown highest number of minerals followed by Swarna, R-RHZ-7 and BRRI DHAN-64 (Table 1). The major components present in the surface of kernel were also remarkably visible in Fig 1. However,

for better visualization of the trace elements, we have shown these in a enlarged insert of Fig 1.

From Table 1, it is immediately clear that the Carbon content in all the varieties goes down whereas the presence of Oxygen increases as compared to top surface layer (aleurone) of whole rice kernel. From this observation, one can infer that the Carbon percentage may be reduced by polishing the rice grains. The presence of Al is seen on the surface of Chak Hao, whereas the reverse is true for other grains where its presence was visualized in the interior. It is worth mentioning

that by polishing the Chak Hao variety, Al, P and Si may be removed. Further, it is interesting to note that though there is no Al on the surface of Kalanamak, a lot of Al (5.49%) is present in the interior. Conversely, Nagina-22 contains Al to the tune of 2.15% on the surface, whereas it is reduced to 0.24% in the endosperm. We find some unexpected elements like Cupper(Cu) in the interior (endosperm) of Swarna and Kalanamak; and Na in Chak Hao. From the above table, we find that Chak Hao is the most element-rich rice variety, but on the other hand URG-24 is deficient

Table 2. Zinc (mg/kg) and iron content (mg/kg) in whole kernel (brown rice) of different test genotypes using ICP-OES

Micro-nutrient	Swarna	Chak Hao	CGZR 1	Nagina 22	Kala-namak	R-RHZ7	BRR1 Dhan 64	URG 24
Zinc	17.3	13.8	21.7	28.1	22.2	24.7	23.1	24.0
Iron	36.8	45.8	53.5	58.7	53.4	61.53	36.6	48.0

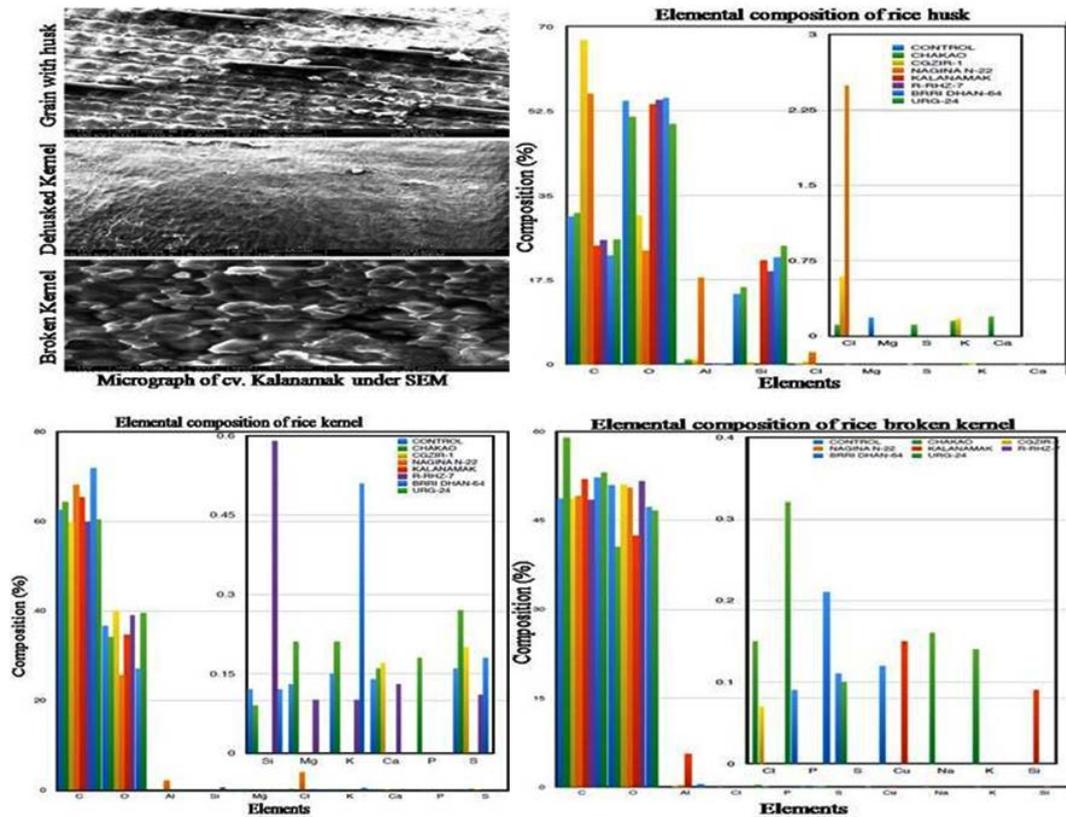


Fig. 1. Elemental composition of rice grains assessed under Environmental Scanning Electron Microscope(ESEM)

in variety of elements. Graphical analysis of the data with enlarged insert for trace elements clearly substantiates the above findings. Although, ESEM cannot detect the elements present in the rice grains up to a high accuracy level, but it gives a quick and a rough estimation which is very useful for the study of morphology or surface properties of rice grains¹⁰.

Majority of Fe is lost during polishing as pericarp of brown rice contains higher proportion iron (55%), but polished rice still retains substantial amount of Zn as endosperm and embryo contain 57% and 9% Zn respectively. Therefore, brown rice and wild rice are considered better in terms of nutritional perspective¹¹. However, present status of Zn (<12–14 mg/kg in polished rice) in commonly grown rice varieties is not sufficient to meet the daily dietary requirement of Zn. With even 20% Zn absorption, and retention of 90% in the blood serum, a daily ration of 422gm Zn biofortified rice (having Zn conc. @ 28mg Zn/kg kernel) can supplement at least 25% of daily requirement. Zn is the constituent (co-factor) of more than 300 enzymes involved in the cellular metabolism related to carbohydrates, lipids, proteins, and nucleic acids. Hence, it seems to have potential role in maintaining normal health in plants and animals.

In general, *Oryza ruffipogon* and *Oryza nivara* among wild species of rice are rich in Zn and Fe; while, basmati type and deep water rice varieties harbour marginally higher content of these micronutrients. Seven wild accessions of rice are reported to harbour higher Zn content¹². Milled rice contains 2-3 ppm Fe and 16-17 ppm Zn, while brown rice is reported to harbour 10-11 ppm Fe and 20-25 ppm Zn. in a panel of 11,400 rice genotypes during 2006-2009¹³. In contrast, the grain iron content in the present investigation was shown to be higher than Zn content in all test genotypes (Table 2). This is ascribed to the fact that the crop was grown in iron toxic soil (450ppm Fe) and higher concentration of iron (Fe⁺²) in the rhizosphere seem to have antagonistic effect on uptake of many nutrients including Zinc¹⁴. Nagina 22- an upland rice variety revealed higher content of Zn (28.1mg/kg) followed by R-RHZ-7 and URG 24 (24.0-24.7mg/kg) in brown rice using ICP-OES (Table 2). A panel of sixty rice accessions including local genotypes and improved lines showed variation in grain Zn content from 14.32ppm to 42.49ppm with

an average of 15.44 ppm in brown rice¹⁵. Similarly, a set of diverse collection of IRRI rice germplasm was reported to have significant variation of grain Zn content¹⁶. Recently, the base line for zinc content is fixed at 22.0mg/kg for Zn biofortification breeding. Hence, the above rice varieties can serve as donors in recombination breeding. Currently, biofortification programme is being carried out at OUAT using the above donors. R-RHZ-7 was shown to be a Fe-rich (67.5mg/kg) rice variety as compared to the mega variety 'Swarna' (36.8mg/kg), but the benefit of high Fe-content is likely to be less harnessed in milled rice due to its much loss during polishing. However, genomic strategies combined with traditional breeding are expected to create new horizon to achieve towards nutritional security¹⁷.

CONCLUSION

Since, rice is the major crop and the principal source of food in our daily dietary ration, knowledge of its elemental architecture is very important for nutritional and industrial purpose. As per ESEM study, the elemental composition in rice grain is not uniform, and importantly carbon decreases in the interior of the kernel than its surface layer. But, reverse is true for oxygen in most of the rice grains. The black rice land race, 'Chak Hao' harbours many elements (Al, Si, Mg, Cl, K, Ca, P and S) except Cu. Copper(Cu) is specific to the interior (endosperm) of Swarna and Kalanamak; and Na is unique to Chak Hao endosperm. However, ICP-OES result indicated Nagina 22 and R-RHZ 7 as excellent source of Zn and Fe respectively compared to other test genotypes. Hence, the above nutrient rich rice varieties can serve as useful materials for biofortification programme in rice.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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