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Ferritin isolation from different genotype of rice and its biochemical characterization

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Abstract
Ferritin content was analyzed by Bradford's method and biochemical confirmation was concluded by reductive release of ferritin iron using Ferrozine. BJ-21 and ARB-6 contained highest ferritin among the selected genotypes. Though ferritin was reported to store 92% of the total Fe indicating positive correlation between Ferritin and Fe content in grain, some deviations were observed among genotypes like AM-72, AM-1, BJ-23, IRJS-11 which had comparatively lower ferritin content than others but had higher Fe content, suggesting that it is not always that high ferritin leads total increase of Fe content in rice grain.

Keywords: ferritin, paddy and brown rice, stress tolerance, SDS PAGE

Introduction
Rice a member of the poaceae family belonging to the genus *Oryza*. *Oryza* includes twenty undomesticated and two domesticated species (cultigens). Feral species of rice are widely dispersed in the humid tropics and subtropics of Africa, Asia, Central and South America, and Australia (Chang, 1985) [1]. The data are evident to prove that rice is the basic food for most of the area around the world. Rice is an important source of carbohydrate but it is lacking in fat, protein and micronutrient. The content of carbohydrates is measured roughly around 80g, fat with 0.66g and protein with 7.13g per 100g of rice. The remaining vitamins and minerals are found in traces. One of the important mineral nutrients are Fe with approximately 0.80 mg per 100g of rice which is not sufficient enough for proper growth and development of human who depend upon rice as their staple food (Ibrahim *et al.*, 2013) [4].
Ferritin is ubiquitous and highly conserved iron binding protein in all organisms. Research focusing on the analysis of the iron demand by the plant is abundant. The plant Ferritins are similar to animal Ferritins. Plant Ferritin is generally observed in cells which are inactive in photosynthesis such as in roots or root nodules, seeds and young etiolated leaves or hypocotyls, reproductive cells and senescing cells. Plant Ferritins are nuclear encoded and located primarily in plastids (Shanker and Venkateswarlu, 2011) [7]. In waterlogged soils, however, Fe availability increases and can reach toxic concentrations. Rice is an important staple crop worldwide and faces iron deficiency or excess, depending on the growth conditions (Silveira *et al*., 2007) [8] so Ferritin importance increases in aerobic condition to chelate Fe. Most of the iron in rice grains accumulates in the outer aleurone layer and embryo, which are removed during milling, and the edible endosperm contains lesser amounts of iron. The development of iron-biofortified food is preferable to the use of iron supplementation and increasing the Ferritin content. The structure of Ferritins determined by Theil *et al*. (1987) [10] states that they are spherical, cage-like proteins with nanocavities formed by multiple polypeptide subunits (four-helix bundles) that manage iron/oxygen chemistry. Catalytic coupling yields diferric oxo/hydroxo complexes at Ferroxidase sites in maxi-Ferritin subunits (24 subunits, 480 kDa; plants, animals, microorganisms). Oxidation occurs at the cavity surface of mini-Ferritins/*Dps* proteins (12 subunits, 240 kDa; bacteria). Oxidation products are concentrated as minerals in the nanocavity for iron−protein cofactor synthesis (maxi-Ferritins) or DNA protection (mini-Ferritins). The protein cage and nanocavity characterize all Ferritins, although amino acid sequences diverge, especially in bacteria. Catalytic oxidation/di-iron coupling in the protein cage (maxi-Ferritins, 480 kDa; plants, bacteria and animal cell specific isoforms) iron the cavity surface (mini-Ferritins/*Dps* proteins, 280 kDa; bacteria) initiates mineralization. Gated pores (eight or four), symmetrically arranged, control iron flow. The multiple Ferritin functions combine pore, channels and catalytic functions in compact protein structures required for life and disease response. With the help of electron microscopic studies,

Ferritin was discovered in plants by Hyde *et al.* (1963) [3]. Embryo extract containing iron metal Protein complex known as phytoferritin was discovered by using microscopy, centrifugation and spectrophometery by Extracting phytoferritin protein which is 90% pure from embryonic axes, cotyledons, and young bean leaves has been developed. The techniques in this process include the use of differential centrifugation and DEAE-cellulose column chromatography. The electron microscopic image, ultraviolet absorption spectrum, iron:protein ratio, and sedimentation behavior of phytoferritin closely bear a resemblance to those of animal Ferritin. Electron microscopic studies reveal that PhytoFerritin is located abundantly in the proplastids of pea epicotyls and root meristems and in plastids of young light or dark-grown bean seedlings. Phytoferritin has been identified in the plastids of the peripheral layers of pea cotyledons.

Material and Methods

The experiment was carried out during *Kharif* season 2014–15 at aerobic rice/biotechnology rice research laboratory Department of Plant Biotechnology, University of Agricultural Sciences, GKVK campus, Bangalore, India located at 120 58‘North; longitude 770 35‘East and altitude of 930 meters above sea level (MSL).Ten genotypes with high grain Zn and Fe were selected based on earlier studies of Bekele and Sumantha 2013-14. Table 1 shows the list. The experiment was carried out in Randomized Complete Block Design (RCBD) with three replications. Selected genotypes were grown in the field condition. Each genotype was grown in three replications with 12 plants in each replication. A spacing of 25 cm between rows and 15 cm between plants was given. The field management practices were followed with the recommended package of UAS, Bangalore to raise healthy crop. Fertilizers were put with 5 t ha-1 of FYM and NPK in the ratio of 100:50:50 kg ha-1. N was provided in the form of urea at basal, 30 and 60 DAS at 50%, 25% and 25% respectively. P was provided as single super phosphate (16% P_2O_5) and K as Murite of potash (60% K_2O). Irrigation was done at a regular basis. Crop was protected by all kinds of infestation by insect and disease by proper management practises. The soil at the experimental site contained 0.74 mg kg^{-1} for Zn (estimated using DTPA, diethylenetriamine penta acetic acid extractable method), a pH of 6.1 and 7.4 mg kg^{-1} of organic carbon.

Table 1: List of super elite accessions used in experiment

Different genotypes of rice	Parentage
AM-1	Azucena x Moromutant
AM-65	Azucena x Moromutant
AM-72	Azucena x Moromutant
BJ-21	Budda x jeerigesanna
BJ-23	Budda x jeerigesanna
BI-33	Budda x IR64
IRJS-11	IR64xjeerigesanna
IRJS-107	IR64xjeerigesanna
IRJS-197	IR64x jeerigesanna
Sebati	Improved Variety

Isolation of Ferritin From Aerobic Rice (Protocol)

The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes. The Bradford is recommended for general use, especially for determining protein content of cell fractions and assessing protein concentrations for gel electrophoresis. The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change. The assay is useful since the extent of coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range.

Warm up the spectrophotometer before use. Dilute unknowns if necessary to obtain between 5 and 100 µg protein in at least one assay tube containing 100 µl samples. If desired, add an equal volume of 1 M NaOH to each sample and vortex. Add NaOH to standards as well if this option is used. Prepare standards containing a range of 5 to 100 micrograms protein (albumin or gamma globulin are recommended) in 100 µl volume. Add 5 ml dye reagent and incubate 5 min. Measure the absorbance at 595 nm and recorded the reading.

The SDS page was conducted to separate Ferritin protein based on its molecular weight. The SDS PAGE findings resulted in 24kDa band, which indicated the presence of Ferritin protein. Reductive release of Ferritin iron: a kinetic assay was performed to ensure the presence of Ferritin protein isolated from rice samples. Ferritin iron release, a process of considerable interest in biology and medicine, occurs most readily in the presence of reducing agents. The kinetic assay for measuring the rate of Ferritin iron removal promoted by various reductants also confirms the existence of Ferritin. The new procedure uses Ferrozine as a chromophoric, high-affinity chelator for the product, Fe (II).

Results and Discussions

Estimation of Ferritins was done by employing Bradford assay and the arithmetic mean was calculated for both Ferritin content in paddy & brown rice by using paired for the means. As the fig. 1 and fig. 2 suggests that BJ21 was having highest content followed by BI-33 in paddy and brown rice and AM-72 was found to have least in both cases.

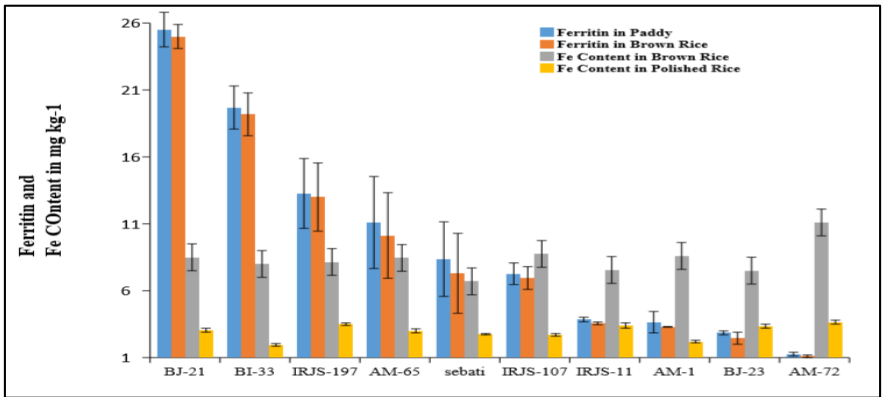

Fig 1: Ferritin and Iron content in paddy and brown rice

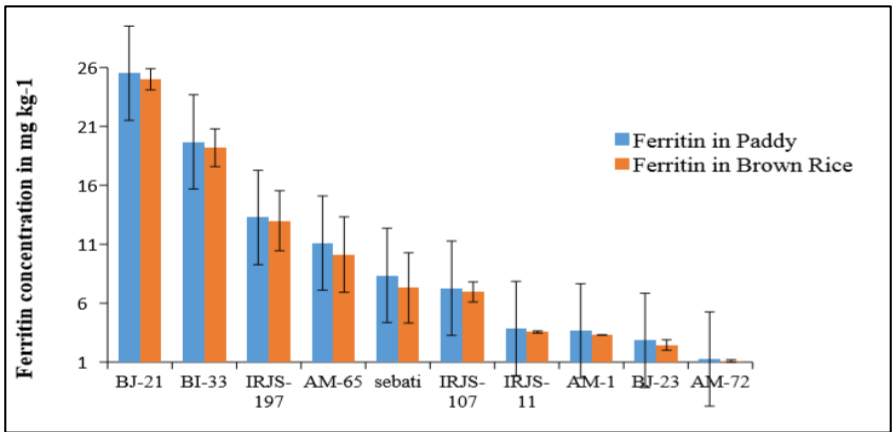

Fig 2: Ferritin content in paddy and brown rice using Bradford method

The Ferritin content was dissimilar in different varieties tested the possible reason could be varietal variation among the rice crop grown or may due to presence of different gene coding for Ferritin protein because gens responsible for Ferritin protein synthesis has very large family which differs crop to crop and variety to variety and in rice till now two different genes (*OsFER1* And *OsFER2*) were identified by Stein *et al.* (2009) [9] which synthesizes Ferritin protein so the possible reason could be one gene has more expression level then another moreover probably the oxidative stress resistance variety will show more Ferritin content (Deak *et al.*,1999) [2]. BI -33 showed significantly high level of Ferritin after BJ-21 which proves that it is drought tolerant as mentioned in work of Patil *et al.* (2012) [5].

The purpose of SDS-PAGE is used to separate proteins according to their size, and no other physical Feature. The SDS page was conducted to separate Ferritin protein based on its molecular weight. The SDS PAGE findings resulted in 24kDa band as shown in fig.3 which indicated the presence of Ferritin protein.

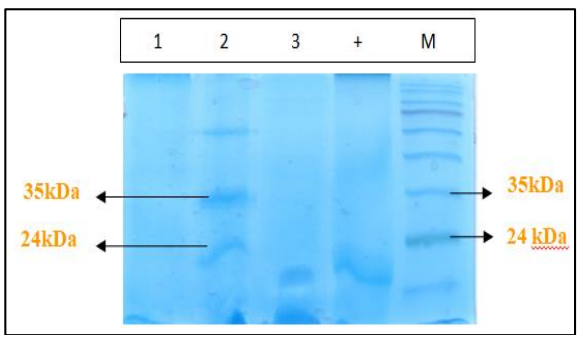

Fig 3: The SDS PAGE findings resulted in 24kDa band.

M: Marker
(+): Positive control
Lane 1: Sebati
Lane 2: AM72
Lane 3: BI33

The research also shows that Iron accumulation does not parallel the high expression level of Ferritin and vice versa and the accumulation of Fe in seeds does not alone depend on Ferritin but efficacy of iron transporter genes. The result present strongly suggests that is Ferritin plays a vital role in Fe homeostasis and is useful for drought resistance varieties.

The possible reason for loss of Ferritin from whole grain to brown rice may be perhaps due to removal of husk and the loss of bran results in loss of nutrient and relating proteins (Puri *et al.*, 2013) [6]. Ferritin content in BJ-21 and BI-33 was found to be more which suggest that these varieties are drought and oxidative stress tolerant and it was found that it was not always that high Ferritin leads total increase of Fe content in rice grain. Ferritin plays an important role in iron homeostasis and is responsible factor in suppressing the formation of reactive oxygen species that is [OH^-] ion which causes the death of cell during drought condition so it plays an important role in prevent the cell from dying in drought condition and make it tolerant towards stress.

Knowledge on these preferential elemental constitutions of the different grain tissues and their remobilization patterns during germination makes the possibility of designing target products with nutritionally optimal constitution more Feasible. The result may also be useful in order to optimize various germination procedures (both processes and management strategies) of edible grains, in order to enhance the nutritional quality of food mixtures based on coarse cereals.

Conclusions

Estimation of Ferritins was done by employing Bradford assay and the arithmetic mean was calculated for both Ferritin content in paddy & brown rice and loss of Ferritin from paddy to brown rice was observed significant by using paired for the means. As the fig. 2 suggests that BJ21 was having highest content followed by BI-33 in paddy and brown rice and AM-72 was found to have least in both cases.

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