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Localization of Iron in Grains of Arobic Rice (*Oryza sativa* L.) by Histochemical Method

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A B S T R A C T

Micronutrient malnutrition has reached to alarming situation with billions of people suffering from Iron, Zinc and Vitamin-A deficiencies. Micronutrient supplementation, food fortification, and biofortification are the three basic approaches used to alleviate micronutrient deficiencies. In the present study ten genotypes rich in iron were used. The crop was grown under aerobic condition and harvested rice grains used for the study. Localization of iron in rice grain was done using Perl's Prussian blue staining of aerobic rice grain sections showed distribution of iron accumulation (blue compound of ferric ferrocyanide) throughout the alureone and subaleurone layers.

Introduction

Aerobic rice is responsive to high inputs (water, nutrients) to reach high yields under non-flooded conditions. In China, the Upland Rice Laboratory of the Chinese Agriculture University (CAU) is breeding the aerobic rice varieties, called "Han Dao" by Bouman *et al.*, (2002) (1). In India, University of Agricultural Sciences (UAS), GKVK, Bengaluru developed aerobic rice variety called "BI-33" released in 2009 (Gowda *et al.*, (2011) (2); www.aerobicrice.in), MAS 956-1 and MAS 26 released in 2008 (www.aerobicrice.org).

Histochemistry is a powerful technique for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as protein, lipid, starch, phytin, and minerals such as calcium, potassium and iron in rice grains (Krishnan *et al.*, (2001) (3); Krishnan and Dayanandan (2003) (4)). Iron is found in haemoglobin, which transports oxygen in the blood of vertebrates. When iron levels are low, the amount of available oxygen declines,

causing a common symptom of iron deficiency called anaemia. Iron deficiency causes a range of health problems in humans, including poor pregnancy-related complications, brain damage in infants, chronic hypoxia and reduced work performance by Goto and Yoshihara (2001) (5). Dietary iron deficiency owing to an insufficient and inappropriate diet is a severe nutritional problem by Goto *et al.,* (2001) (6) that affect 30 % of the world's population by WHO (1992) (7).

Plant-based foods are potential sources of all essential minerals and organic nutrients that are directly or indirectly required by humans. Rice is the most important staple food crop in the world; nearly half of the world's population depends on rice as the source of their calories. Hence make an effort to know the distribution of iron within the rice grains.

Materials and Methods

Perl's Prussian blue technique was employed for localization of iron by Pearse, (1972) (8). This is an extremely reliable and sensitive technique that can even detect small quantities of iron microscopically. Rice caryopses were soaked in distilled water for about 2–3 hours. Thin transverse sections of aerobic rice grains were treated with a mixture of freshly prepared 2% potassium ferrocyanide and 2% hydrochloric acid for 20–30 min. Prussian blue stain. The treatment with acid was done to release ferric ions from the tissue, which immediately reacts with the cation of potassium ferrocyanide to produce a blue insoluble compound, ferric ferrocyanide. Observations and photographs were taken using a microscope Krishnan *et al.,* (2003) (9).

Results and Discussion

In the present study, ten elite lines of aerobic brown rice grain of transverse sections showed bright blue color indicates the presence of iron in the sections of aerobic rice grains are presented in the figures 1–10. The highest bright blue color was observed in Jeerigesanna fallowed by Jaya, BJ-21, AM-1, AM-72, Chittimuthyalu, AM-65, ARB-6, Black rice and Azucena. It clearly indicates that distribution of iron and accumulation (blue compound of ferric ferrocyanide) was high in the alureone and sub-aleurone layers. Similar results reported by Pearse (1972) (8), 1988(10); Krishnamurthy, (1998) (11).

Localization of iron in aerobic rice grains

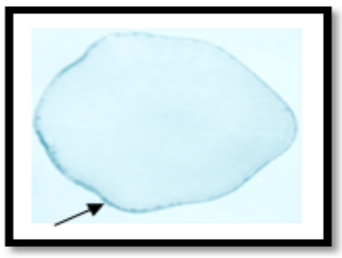

Fig 1: AM-72

Fig 2: ARB- 6

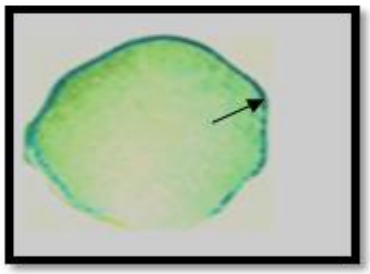

Fig 3: Jeerigesanna

Fig 4: AM-1

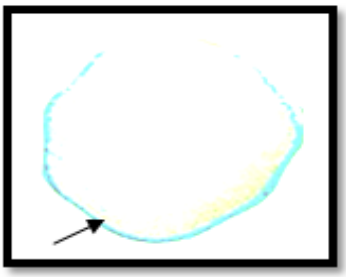

Fig 5: BJ-21

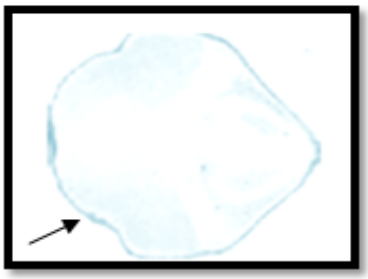

Fig 6: Black rice

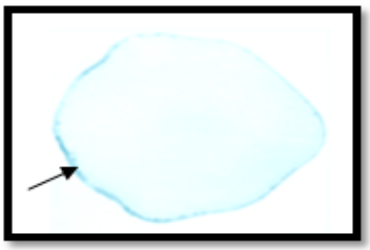

Fig 7: AM-65

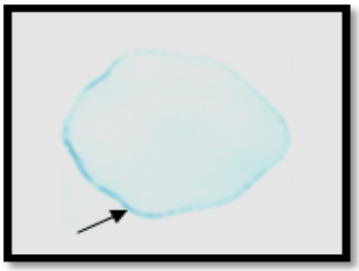

Fig 8: Chittimuthyalu

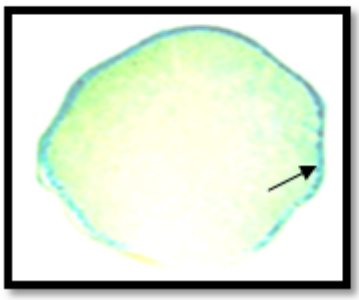

Fig 9: Jaya

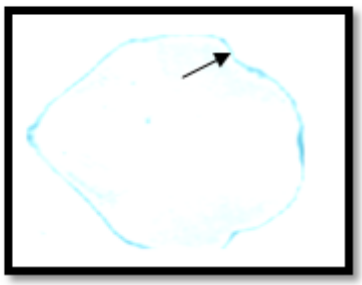

Fig 10: Azucena

It was shown that histochemical analysis is a powerful tool and can be used for preliminary screening of biofortified aerobic rice lines with high iron content (qualitatively) to select the desired line for further analysis. This technique does not require antibody or radiolabelled probe. Previously, iron content of grains was measured only by chemical

analysis. But this method is beset by technical problems when dealing with a large germplasm screening and limited amounts of sample materials. It has also been reported that iron content could vary widely among rice genotypes by Prom-u-thai *et al.,* (2003) (12). The histochemical technique can easily be used in large germplasm screenings to select the right background material for genetic engineering or for breeding programmes.

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