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Research Note

Simple and Rapid Method of Estimation of Iron in milled rice of Early Segregating Generations

R.Kalaimaghal and S.Geetha
Anbil Dharmalingam Agricultural College and Research Institute,
Tamil Nadu Agricultural University, Trichy 620 009, Tamil Nadu
Email: geethagovind1@gmail.com

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Abstract
Prussian Blue staining method was compared with conventional method of quantitative estimation through Atomic Absorption Spectroscopy method. The results confirmed the reliability and cost effectiveness of this staining method. It can be used as a rapid qualitative assessment of Fe content in milled rice grains of early segregating generation

Keywords: Rice, Fe, Estimation, Rapid method

Malnutrition arising from critically important mineral micronutrients such as iron (Fe) is a serious health problem affecting nearly half of the world's population (WHO 2002). The nutritional disorder, anemia, due to iron deficiency, is widespread in rice consuming countries. South East Asia shows the highest prevalence of anemia in women and especially the pregnant women are worst affected (Mason *et al*, 2001). The disorders due to Fe deficiency could be rectified by appropriate Fe rich diet or Fe supplementing tablets or tonics or postharvest - fortification of food grains with iron. However, breeding crop varieties with enhanced levels of Fe would be a sustainable economic way (WHO, 2002) to alleviate the problems due to Fe deficiency. Bouis (2003) has also indicated the possibilities of breeding high yielding cultivars coupled with nutritional level enhancement through crop improvement strategies.

Since rice is the dominant cereal crop in most Asian countries and is the staple food for more than half of the world's population even a small increase in micronutrient content in rice would have a significant impact on human health. Iron content vary widely among rice genotypes and considerable natural genetic variability for Fe content exists among rice genotypes (Anandan et al. 2011 and Brar et al. (2011) and hence through planned breeding programme it would be possible to enhance the Fe content of grains in rice genotypes.

The breeding programme aimed at biofortification of rice with iron involves screening of a large number of germplasm, commercial varieties and early generations of segregating materials and is conventionally done by Atomic Absorption Spectroscopy (AAS) which is quite expensive (atleast Rs.40 per sample) . A simple, cheaper, relatively reliable and rapid method of screening would highly be useful to plant breeders to facilitate the screening process. Prom – u – thai *et al* (2003) proposed Prussian Blue Stain Method for rapid screening of grain Fe content of rice by scoring the color intensity in the embryo of cut and treated seeds through a stereo microscope. Later, Velu *et al* (2006) simplified this method and used for screening Fe content in Pearl Millet. Purusothaman (2010) studied milled rice samples of seventy eight rice genotypes varying in Fe content with different concentrations and concluded that 5% concentration of Prussian Blue solution for 30 minutes was optimum for differentiating the rice genotypes for Fe content based on colour intensity.

Under Indian conditions 90 per cent of rice is consumed after milling (polishing) only and hence while breeding for high Fe content , assessment of Fe content in polished rice would be of more value than in unpolished rice. However, only a very few publications (Prom-u-thai et al. (2006) and Martinez et al.(2010)) are available with regard to Fe content in milled rice. Considering the importance of breeding for high Fe in polished rice and the need for an easy method for the qualitative estimation of Fe in rice, the present study was undertaken with the objectives of assessment of Fe content in milled rice of F3 population of rice population and to validate the rapid Prussian Blue staining method .

The parent IR 68114 – 3B – 2 – 2-3 (7.50 ppm) was identified to be the best donor for high Fe content in milled rice after screening 78 genotypes during 2010 (Purusothaman 2010). Prom – u – thai *et al* (2004) have also reported that IR 68114 – 3B – 2 – 2 – 3 was a high Fe containing genotypes. Hence this parent was used as a male (donor) parent and hybridized with a short duration high

yielding popular rice variety ADT 37 (1.5ppm) with low Fe content, during 2009 – 2010 and subsequently the F_3 generation of this cross was developed.

Preparation of rice flour samples of milled rice: During wet season of 2010, F_3 family rows of the cross combinations of rice ADT 37 / IR 68144 – 3B – 2 – 2 – 3 were raised. The grains of 50 randomly selected families, were carefully harvested from five randomly selected plants from each of 50 F_3 families. The rice grain samples were air dried to 12 – 14 per cent moisture content and dehulled using rubber Palm dehusker. The dehulled brown rice was further milled using sand paper (No. 100). The milled rice grains were powdered and used for analysis. At all stages of rice flour preparation, utmost care was taken not to use any of Fe or other metal containing vessels / tools.

Atomic Absorption Spectro Photometer Method**:** Half a gram of powdered rice grains sample with 12 ml of triple acid mixture (9:2:1 Nitric : Sulphuric : Pachloric acid) was kept for overnight digestion. The digested samples were kept on a hot plate till solution turned colourless. The extract was diluted to 50 ml and fed to the Atomic Absorption Spectrophotometer. The concentration was expressed in ppm.

Perl's Prussian Blue Staining Method**:** The Prussian Blue Solution was prepared by mixing five percent potassium ferrocyanide and five percent hydrochloric acid as standardized by Purusothaman (2010). Half a gram of dried rice grain flour of each sample was placed on petridishes and 10 ml of Prussian Blue Solution (5%) was added to the flour samples and kept for 30 minutes for the development of blue colour and based on colour intensity the scores were given as detailed in Table 1. The ferrice Fe is released from any attachments to protein by treatment with dilute hydrochloric acid and then reacts with a dilute solution of Potassium ferro cyamide to produce an insoluble compound, ferric ferro cyanide (Prussian Blue). Scoring Procedure based on colour intensity is as 1- No colour; 2-Light blue; 3-Medium Blue and 4- Dark Blue.

Simple phenotypic correlation coefficients (Gomez and Gomez, 1984) was worked out between the scores given based on colour intensity and AAS readings to assess the reliability of staining procedure.

The Fe content ranged from 2.1 ppm to 9.43 ppm in milled rice (Table 2). The average value was 4.42 ppm. Martinez et al (2010) evaluated 11,400 rice genotypes and reported that the Fe content in polished rice ranged from 2-3 ppm and in brown rice it was from 10-11 ppm. Various range of intensities of blue colour was produced in the samples. The colour intensity varied from 'no color' to 'dark blue'. The genotypes with less than 2.5 ppm did not develop any colour may be due to the fact that the Fe available in the sample is far below the limit of visible staining of Prussian Blue. As suggested by Velu *et al* (2006), higher concentrations of Prussian Blue Solutions may be tried for rice samples with very low level of Fe. In general, genotypes with relatively high Fe content developed medium to dark blue color intensity and those with low level of Fe developed light blue or no colour. The genotypes with more than 8 ppm of Fe got the score of "4 "and those with 4-6 ppm were given a score of "3" and other genotypes with less than 4 ppm obtained a score of "2 "or "1" . Using this technique the gentoypes were categorized into very low(score"1") , low (score"2") medium (score"3")and high (score"4")Fe containing genotypes.

However, as an exceptional case, gentoypes with medium (4.64 ppm) and high (8.99 ppm) showed similar type of colour intensity *i.e.*, medium blue. Another sample with 3.91 ppm, developed, no color which is otherwise expected to develop 'light blue' color intensity. The correlation between Prussian Blue staining method and AAS method was highly significant and positive with the correlation coefficient values of 0.81 revealing the reliability of this staining method. Using this method 100 number of rice flour samples could be analysed per day and this method consumes less labour and time as already reported by Velu *et al* (2006).

The results of parent – progeny regression studies in F_2 and F_3 generation of this cross combination revealed the high genetic potentiality of this trait ie., Fe content in rice (Kalaimaghal, 2011). Further, she has reported about the positive and significant correlation between grain Fe and Zn content in rice in the same cross combination indicating the possibility of indirect selection for Zn through the selection for Fe through the selection for Fe. Transgressive segregants for high Fe content were also witnessed in the present study which could effectively be exploited in further breeding programme.

It is concluded that in general, Prussian Blue Staining Method is a cost effective rapid method and could effectively be used for assessing the level of Fe content in rice grains especially in large sized germplasm and large segregating population and hence the gentoypes with very low levels of Fe could be eliminated at early stages of segregation. James Stangoulis (2010) has also reported Prussian Blue Staining method could be used for high thorough put screening of Fe content in rice breeding programme.

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Table 1. Fe content using AAS method and staining method in milled rice grains from F_3 families

Family number	Fe content by AAS method (ppm)	Rapid method (score)	Family number	Fe content by AAS method (ppm)	Rapid method (score)
1	9.83	4	27	9.83	4
2	5.21	3	28	5.21	3
3	8.99	3	29	8.99	4
4	4.81	3	30	2.81	1
5	3.91	2	31	3.91	2
6	3.29	2	32	2.29	1
7	2.95	1	33	2.95	2
8	2.60	1	34	3.60	2
9	2.64	1	35	3.64	2
10	3.56	2	36	3.56	1
11	2.96	2	37	2.96	1
12	3.73	2	38	3.73	1
13	3.28	2	39	3.28	2
14	3.01	2	40	3.01	1
15	2.98	2	41	2.98	1
16	2.21	1	42	2.21	1
17	8.99	4	43	8.99	4
18	4.81	2	44	2.81	1
19	3.91	1	45	3.91	1
20	2.29	1	46	3.29	2
21	2.95	1	47	2.95	1
22	2.60	1	48	2.60	1
23	4.64	3	49	4.64	3
24	3.56	2	50	3.56	2
25	2.96	1	ADT 37	1.50	1
26	3.73	2	IR 68114 – 3B – 2 – 2-3	7.50	3