RESEARCH ARTICLE

*ScienceAsia* 29 (2003): 203-207

# Easy and Rapid Detection of Iron in Rice Grain

**Chanakan Prom-u-thai,[a,*] Bernie Dell,[b] Gordon Thomson[b] and Benjavan Rerkasem[a]**

[a] Department of Agronomy Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.
[b] School of Biological Sciences and Biotechnology, Murdoch University, Perth 6150, Australia.
*Corresponding author, E-mail: g4268101@cm.edu

*Received 2 Dec 2002*
*Accepted 21 Feb 2003*

**ABSTRACT:** In this study, we show how a preliminary determination of grain Fe in rice may be made with reaction to Perls' Prussian blue, a stain for Fe (III). Differential localization of Fe in grain parts was indicated by the intensity of reaction of tissue Fe to the dye. The blue colour reaction was most intense in the embryo, weak in the aleurone layer of the pericarp and invisible in the endosperm. The staining intensity also varied with the region of the embryo, generally being strongest in the scutellum, intermediate in the coleorhiza and weakest in the coleoptile. Variation in the reaction to Perls' Prussian blue was observed among eleven rice genotypes with varying grain Fe contents. The intensity of the blue colour reaction in the embryo of different rice genotypes was indicative of their grain Fe contents for both brown and white (polished) rice. Those with high grain Fe, >14 mg Fe $kg^{-1}$, were clearly distinguishable from those with <10 mg Fe $kg^{-1}$ with Perls' Prussian blue. We suggest that this simple staining procedure may be used to quickly screen for high Fe contents in large germplasms containing hundreds of rice entries, using reactions in genotypes with known grain contents as standards.

**KEYWORDS:** *Oryza sativa*, Rice, Seed, Iron localization, Perl's Prussian blue, Detection.

## INTRODUCTION

Increasing Fe content in the grain people consume is considered one way to increase Fe intake in those suffering from Fe-deficiency anemia.[1] Previous studies have shown that grain Fe content can vary widely among rice genotypes. Most of the commonly eaten rice genotypes in Asia contain only about 10 mg Fe $kg^{-1}$ in brown rice, but genotypes with 15 mg Fe $kg^{-1}$ or more have been found.[1,2] Selection and breeding for rice of higher grain Fe content is possible. However, in the past, Fe content of rice grain could only be measured by chemical analysis. This poses a problem when dealing with large numbers and limited amounts of samples as in screening of germplasms and evaluation for Fe content in progenies of crosses. Furthermore, even though the method for chemical analysis for Fe is well established, contamination is still a problem in many labs, which has resulted in unusually high grain Fe contents being reported. The application of the Prussian blue stain[3] has made possible rapid estimation of the amount of non-hemoglobin Fe in the marrow and in the blood of humans[4] and other vertebrates.[5] Biologically active Fe is normally very tightly complexed to protein, as in hemoglobin and myoglobin. Fortunately, there are various mechanisms within the tissue which allow the Fe to be detected, in the form of Fe (III) surrounded by hemosiderin. This Fe is reported to be easily stained with Perls' Prussian blue reaction.[6] We set out to see if Perls' Prussian blue may be used to detect the localization of grain Fe in rice, and whether seed with different grain Fe contents may be distinguished by the intensity of staining.

## MATERIALS AND METHODS

Seed of 11 genotypes of rice (*Oryza sativa*) were obtained from plants grown on San Sai soil under wetland conditions in the Department of Agronomy field plot at Chiang Mai University, Thailand. The genotypes included IR68144 (a high yielding genotype from IRRI), Basmati 370 (a traditional genotype from Pakistan), KDML 105, RD 6, Hom Klong Luang 1 (3 popular aromatic rice genotypes from Thailand), Neaw Ubon 2, Hom Pu Pan, Hom Nang Fa (3 newly released Thai genotypes), and CMU 122, CMU 123, CMU 124 (3 upland Thai genotypes). Seed Fe concentration was determined by wet-ashing and Inductive Couple Plasma Spectrophotometry (ICP)[7] in mature grain as whole grain brown rice

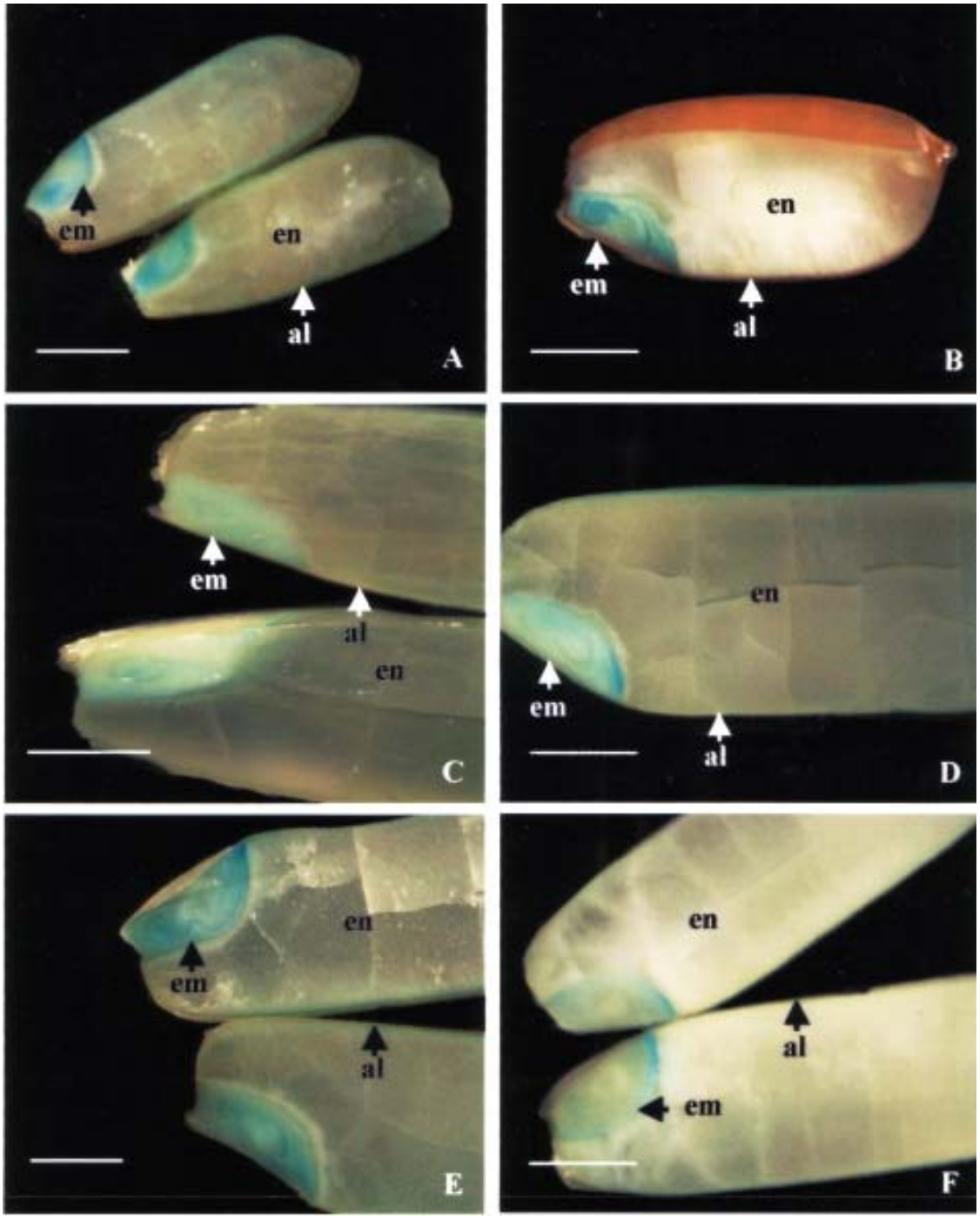


**Fig 1.** Stereo-micrographs of brown rice grains cut longitudinally in half, showing distribution and intensity of staining with Perl's Prussian blue. Most of the Fe (III) reaction is located in embryo part of the grain. Em, embryo; en, endosperm; al, aleurone layer. Genotypes: A) IR68144; B) CMU122; C) Neaw Ubon 2; D) KDML 105; E) Basmati 370; F) Hom Pu Pan. Scale bar A, B, F = 1.5 mm, C, D, E = 1 mm.

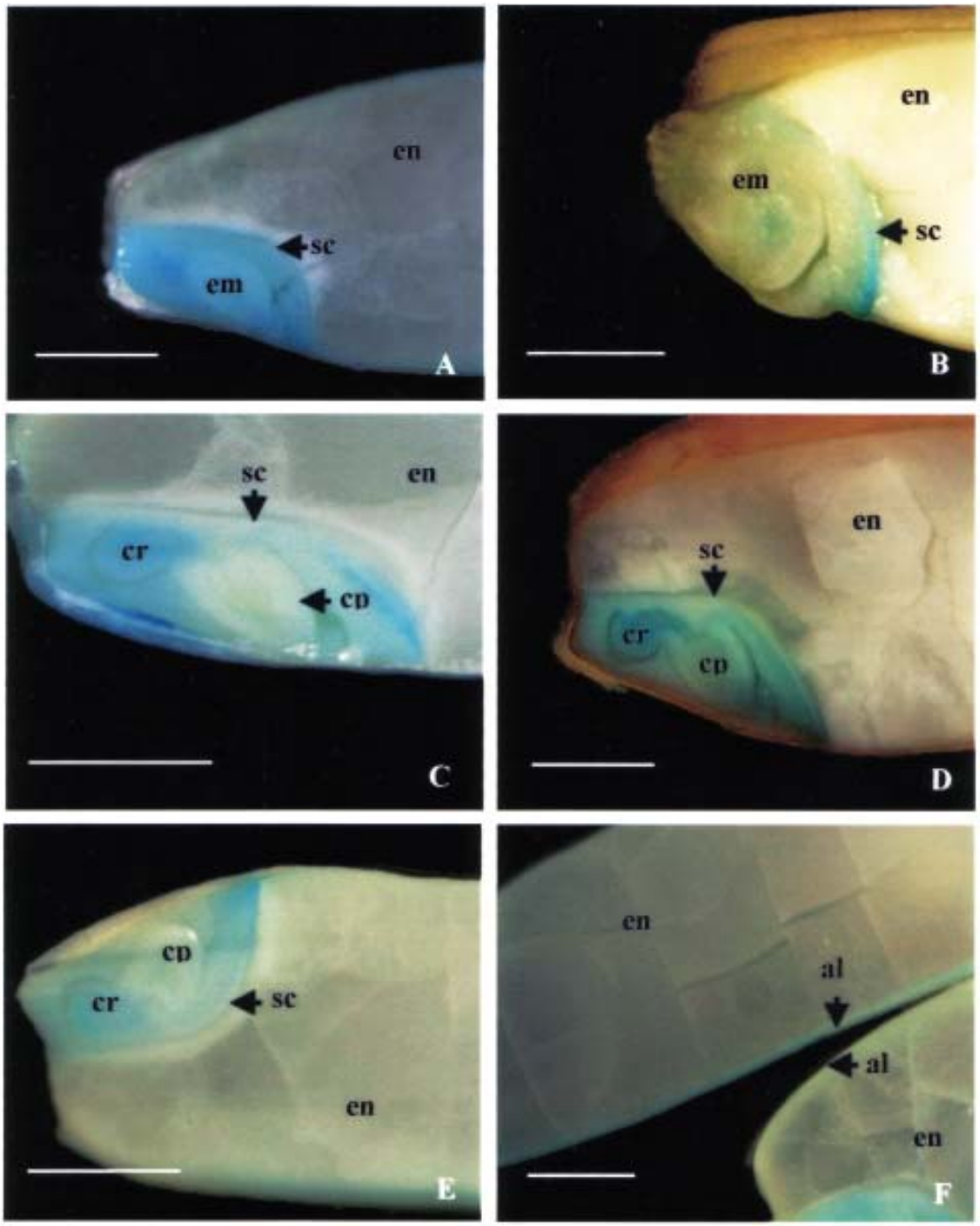


**Fig 2.** Stereo micrographs of brown rice grain cut longitudinally in half, showing the distribution and density of staining with Perls' Prussian blue. A-E showing the different reaction in different part of embryo: em, en: endosperm, sc: scutellum, cr: coleorhiza, cp: coleoptile. F showing the reaction of Perls' Prussian blue in aleurone layer of KDML 105. Genotypes; A) IR68144; B) Hom Klong Luang1; C) CMU 124; D) CMU 122, E) Basmati 370; F) KDML 105. Scale bar = 1 mm.

**Table 1.** Differential staining with Perls' Prussian blue in parts of the grain of 11 rice genotypes.

| Genotype | Intensity of staining, part of grain * | | |
|---|---|---|---|
| | Embryo | Aleurone layer | Endosperm |
| IR68144 | + + + § | + | 0 |
| KDML 105 | + + | + | 0 |
| BASMATI 370 | + + + | + | 0 |
| Hom Klong Luang 1 | + + | + | 0 |
| Hom Nang Fa | + + | 0 | 0 |
| Hom Pu Pan | + + | 0 | 0 |
| Neaw Ubon 2 | + | 0 | 0 |
| RD 6 | + | + | 0 |
| CMU 122 | + + + | + + | 0 |
| CMU 123 | + + + | 0 | 0 |
| CMU 124 | + + + | + + | 0 |

* Without palea and lemma, unpolished. For each genotype and part of the grain 3 seeds were stained, and all three had the same rating. § 0 = no stain; + weak staining; + + moderate staining; + + + intense staining.

**Table 2.** Differential staining with Perls' Prussian blue in different parts of the embryo and grain Fe concentration in 11 rice genotypes.

| Genotype | Whole | Part of embryo | | | mg Fe $kg^{-1}$ † | |
|---|---|---|---|---|---|---|
| | | Scutellum | Coleoptile | Coleorhiza | Brown rice | White rice |
| | Intensity of staining | | | | | |
| IR68144 | + + + ‡ | + + + | + + + | + + + | 19.7 ± 0.3 | 15.6 ± 1.6 |
| KDML105 | + + | + + | + | + + | 7.8 ± 0.2 | 7.2 ± 1.3 |
| BASMATI 370 | + + + | + + + | + | + + + | 14.3 ± 0.2 | 8.0 ± 0.6 |
| Hom Klong Luang 1 | + + | + + | + + | + | 7.9 ± 0.5 | 6.5 ± 0.5 |
| Hom Nang Fa | + + | + + | 0 | + | 8.0 ± 0.2 | * |
| Hom Pu Pan | + + | + + | 0 | + | 8.4 ± 0.5 | * |
| Neaw Ubon 2 | + | + | 0 | + | 8.1 ± 0.6 | 7.8 ± 1.9 |
| RD 6 | + | + | + | + | 8.6 ± 0.2 | 7.5 ± 1.4 |
| CMU 122 | + + + | + + + | + + | + + + | 16.7 ± 0.7 | 13.8 ± 0.9 |
| CMU 123 | + + + | + + + | + + + | + + + | 15.8 ± 0.8 | 11.4 ± 1.2 |
| CMU 124 | + + + | + + + | + + | + + + | 13.2 ± 0.8 | 10.1 ± 1.5 |

‡ 0 = no colour; + weak staining; ++ moderate staining; +++ intense staining.

† Brown rice was whole grain with embryo, without palea and lemma; white rice had palea and lemma removed by milling, and pericarp removed by polishing, embryo largely abscent. Each value was mean of three analyses ± SE. For each genotype and part of the grain 3 seeds were stained, and all three had the same rating.

* not determined.

with a teflon knife (Advanced Personna Brand) in a Petri dish. The specimens were submerged in freshly prepared Perls' Prussian blue[3] solution (2% hydrocloric acid mixed with 2% potassium ferrocyanide) for 10 minutes. The seed were then gently washed continuously in distilled water for 2 minutes. The ferric Fe is released from any attachments to protein by treatment with dilute hydrochloric acid and then reacts with a dilute solution of potassium ferrocyanide to produce an insoluble compound, ferric ferro cyanide (Prussian blue).[5] The intensity of staining was rated from 0 (no staining), + (weak staining) to +++ (most intense) under a stereo microscope.

## Results and Discussion

Perls' Prussian blue staining has been recommended as a method for locating Fe(III) in animal tissue because it is fast, reproducible and the reagent penetrates bulky tissue to give a distinctive blue reaction product.[8] The technique has recently been used to report the presence of iron in the aleurone layer of rice grain.[9] However, that study only indicated the location of Fe in the aleurone layer. There was no information on Fe in other parts of the grain, especially the embryo. Furthermore, the presence of Fe found was not related to the grain Fe content nor was there any comparison across genotypes. In this paper, we show for the first time a pattern of staining with Perls' Prussian blue within the rice grain and across genotypes that clearly indicates differential localization of Fe (Fig 1). The staining was most intense in the embryo, weak in the aleurone layer of the pericarp, and not detectable in the endosperm (Table 1). Differences were also seen in different parts of the embryo (Fig 2, Table 2). The staining was weakest in the coleoptile, intermediate in the coleorhiza and strongest in the scutellum, which was similar to the embryo as a whole. The most intense blue colour (+++) in the embryo (and the scutellum) was associated with a grain Fe content of >13 mg Fe $kg^{-1}$ for brown rice and >10 mg Fe $kg^{-1}$ for polished rice (Table 2). The intensity of staining was less well correlated with grain Fe at lower concentrations as genotypes with embryo staining rated as + and ++ were not distinguishable by their grain Fe contents. No reaction with Perls' Prussian blue was observed in the endosperm, even at 15.6 mg Fe $kg^{-1}$ as found in white, polished IR68144. The Fe present in the endosperm may not be in the form that reacts with the dye, or possibly the concentration of Fe in the endosperm may be below the limit of visible staining by Perls' Prussian blue. By contrast, staining of the embryo of Neaw Ubon 2 (19.9 ± 1.8 mg Fe $kg^{-1}$), although faint (+) was still clearly visible. The reaction to Perls' Prussian blue in the aleurone layer of the pericarp also followed roughly the Fe contents of whole grain in individual rice genotyes, but the distinction is less clear between genotypes with high and low grain Fe.

## Acknowledgements

The authors wish to acknowledge financial support from the Thailand Research Fund and the McKnight foundation. The first author is a recipient of the Royal Golden Jubilee Ph.D. scholarship. Thanks to Thailand Rice Research Institute for rice seed germplasm, W. Boonma for CMU 122, CMU 123, CMU 124 and IRRI for IR68144.

## References

1. Senahira D, Gregorio G and Graham RD (1998). Breeding iron and zinc – dense rice. Paper presented at the International Workshop on Micronutrient Enhancement of Rice for Developing Countries, September 3, 1998. At Rice Research and Extension Center, Stuttgard, Arkansas.
2. Prom-u-thai C and Rerkasem B (2001). Grain iron concentration in Thai rice germplasm. *Dev Plant Soil Sci*. **92**, 350-1.
3. Perls M (1867) Nachweis von Eisenoxyd in gewissen Pigmentation. Virchows Archive für pathologische Anatomie and Physiologie and für Klinisch Medizine. **39**, 42-7.
4. Dorothy R and Harrette B (1955) The application of the Prussian blue stain to previously stained films of blood and bone morrow. *Blood J* **10**, 160-6.
5. Doucet MY and Viel (2002) Alveolar macrophage graded hemosiderin score from bronchoalveolar lavage in horses with exercise-induced pulmonary hemorrhage and controls. *J Vet Int Med* **16**, 281-6.
6. Stevens A (1977) Pigments and minerals. In: Theory and practice of histological techniques, Bancroftt JD and Stevens A. (eds), pp 186-208, Churchill Livingstone, Edinburgh.
7. Zarcinas BA, Cartwright B and Spouncer LR (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun Soil Sci Plant Anal* **18**, 131-46.
8. Baker JR (1958) Principles of biological microtechnique. A study of fixation and dyeing. London. pp 304.
9. Krishnan S, Ebenezer GAI and Dayanandan P (2001) Histochemical localization of storage components in caryopsis of rice (*Oryza sativa* L.). *Current Science* **80**, 567-71.