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Easy and Rapid Detection of Grain Iron Content in Fingermillet [*Eleusine Coracana* (L.) Gaertn] Germplasm

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In this study, a preliminary evaluation of grain iron content in fingermillet using Perls' Prussian blue reagent, a stain for Fe $^{3+}$was established. Prussian blue solution of two per cent concentration was used in identifying grain iron content of genotypes based on the development of blue colour intensity. The rank correlation between measured grain Fe content and the colour intensity score was highly significant and positive (r = 0.62; P<0.01), indicating that higher the Fe content in the grain, more will be the intensity of blue colour developed. Pearls' Prussian blue method could be effectively used as an initial method of screening and genotypes can be scored for high grain Fe content. The grain Fe content of the same genotypes was quantified using Atomic Absorption Spectrophotometer method. Wide variation was observed in fingermillet genotypes for grain Fe content. It ranged from 3.46 (TNEc 0921) to 8.72 (TNEc 0601) mg per 100g of grain. The accessions namely TNEc 0308, TNEc 0407, TNEc 0601, TNEc 0788 and TNEc 0910 were rich in grain Fe content coupled with high grain yield per plant. Therefore, these accessions could be employed in the genetic improvement of fingermillet through hybridization or selection. This simple staining procedure could be used to screen genotypes with high Fe content in large number of germplasm accessions.

Key words: Fingermillet, Pearls' Prussian blue, Grain iron content, Germplasam, Genetic improvement.

Fingermillet [*Eleusine coracana* (L.) Gaertn] 2n = 4x = 36, belongs to the tribe Chloridae of the family Poaceae. Fingermillet is third in importance among millets in the country in area and production after sorghum and pearlmillet. Fingermillet is the most important small millets in the tropics (12% of global millet area) and is cultivated in more than 25 countries in Africa (eastern and southern) and Asia (from Near East to Far East), predominantly as a staple food grain. Fingermillet is an important cereal because of its excellent storage properties and the nutritive value of the grains. It is also a good source of micronutrients like Calcium, Iron, Phosphorus, Zinc and Potassium. Fingermillet, being a promising source of micronutrients and protein (Malleshi and Klopfenstein, 1998) besides energy, can make a contribution to alleviate micronutrient and protein malnutrition, also called 'hidden-hunger', affecting more than half of the world's population, especially women and preschool children in most countries of Africa and South-East Asia (Underwood, 2000).

Increasing grain iron content is one of the effective way to increase iron intake and reducing the incidence of Fe-deficiency anemia (Welch and Graham *et al.,* 2004). Intake of diet, poor in iron (Fe), zinc (Zn) and protein is the major cause for micronutrient and protein malnutrition. Iron deficiency leads to anemia; about 79 per cent of the preschool children between 6 and 35 months of age and 56 per cent of women between 15 and 49 years of age are anemic in India (Krishnaswamy, 2009). The most cost effective approach for mitigating micronutrient and protein malnutrition is to introduce fingermillet varieties selected and/or bred for increased Fe, Zn and protein contents through plant breeding. Attempts to breed fingermillet for enhanced grain micronutrient and yield are still in its infancy. Exploitation of existing variability among germplasm accessions is the first step and short term strategy for developing fingermillet cultivars to address the micronutrient malnutrition in the target population (Upadhyaya *et al.,* 2011). Previous studies have shown that grain Fe content can vary widely among fingermillet genotypes. Most of the commonly cultivated fingermillet genotypes contain only about 3.9 mg of Fe per 100g of grain, but genotypes with 8 mg or more have also been found in germplasm collections. Hence, Selection and breeding for fingermillet with high grain Fe content is possible. However, in the past, Fe content of fingermillet grain could only be measured by chemical analysis. This poses a problem in screening when dealing with large numbers of germplasm and limited quantity of seed samples available in progenies of crosses. A procedure based on Prussian blue stain proposed for rapid screening of grain Fe content in rice (Prom-u-thai *et al.,* 2003; Krishnan *et al.,* 2003) and pearlmillet (Velu *et al.,* 2006), which involves scoring

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of colour intensity development is for the first time used in this study to screen the fingermillet genotypes for high grain Fe content. The main objective of this study was to simplify the estimation and effective screening for grain Fe content in fingermillet and its variability.

Materials and Methods

Twenty five fingermillet genotypes with two check varieties were evaluated at Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore during *rabi,* 2011-2012. The field experiment was laid out in randomized complete block design with three replications. Each genotype was grown in single row of 3 metre length with a spacing of 30 cm x 10 cm.

Prussian blue staining method

Prussian blue solution of 2 per cent concentration was used in identifying high grain iron genotypes (Prom-u-thai *et al.,* 2003). A quantity of 10.0 g of potassium ferrocyanide was mixed with distilled water, and the volume was made up to 500 ml. A volume of 10.0 ml concentrated hydrochloric acid (HCl) was mixed with distilled water to make the volume to 500 ml. This solution was prepared by mixing equal volumes of 2 per cent HCl and 2 per cent ferrocyanide solutions. Dry fingermillet grain samples were ground to flour with a pestle and mortar and 0.5 g of flour sample was placed in Borosilicate glass test tubes or Petri-dish. The Prussian blue solution (10 ml) was poured onto the flour in each test tube or Petri-dish. Colour development was recorded after 10 minutes and the color intensity was visually scored on a 1-4 scale, where score 1 represents formation of no colour; 2 for less intense blue colour; 3 for medium blue colour and 4 for more intense blue colour. Frequency distribution was studied for grain Fe content with colour intensity scores.

Atomic Absorption Spectrophotometer method

Biochemical analysis for grain Fe content was carried out on triplicate ground samples of seeds from individual plant by digestion with 9:4 diacid mixture (HNO_3: $HCLO_4$) followed by atomic absorption spectrometry (AAS) method using ECIL AAS (Perkin Elmer) (Zarcinas *et. al.,* 1987 and Singh *et. al.,* 2005).

Simple correlation coefficients between the grain Fe content, 1000 grain weight and grain yield per plant were estimated to examine association among them (Snedecor and Cochran, 1994).

Results and Discussion

Perls' Prussian blue staining has been first reported by Baker (1958) in animal tissue for locating Fe^{3+}because it is fast, reproducible and the reagent penetrates bulky tissue to give a distinctive blue reaction product. In plants, this technique had been first report by Krishnan *et. al.* (2001) in rice. In fingermillet, this technique was used for the first time to score the germplasm accessions based on the grain Fe content. These qualitative scoring based on the intensity of the colour development was validated by quantification of grain Fe content using Atomic Absorption Spectrophotometer (AAS) method (Table 1)

Table 1. Grain iron (Fe) content in fingermillet germplasm accessions

Genotypes	Colour intensity	Nutrient level	Score	Fe content (mg/100g)
TNEc 0089	Medium blue	Medium	3	5.17
TNEc 0152	No colour	Low	1	4.04
TNEc 0231	More intense blue	High	4	5.67
TNEc 0308	Medium blue	Medium	3	4.78
TNEc 0407	More intense blue	High	4	5.80
TNEc 0416	More intense blue	High	4	7.40
TNEc 0601	More intense blue	High	4	8.72
TNEc 0629	More intense blue	High	4	7.52
TNEc 0707	Medium blue	Medium	3	4.64
TNEc 0775	Medium blue	Medium	3	4.77
TNEc 0776	More intense blue	High	4	8.17
TNEc 0786	Less intense blue	Medium	2	4.35
TNEc 0787	More intense blue	High	4	8.42
TNEc 0788	More intense blue	High	4	5.65
TNEc 0907	Medium blue	Medium	3	4.92
TNEc 0910	More intense blue	High	4	8.22
TNEc 0921	No colour	Low	1	3.46
TNEc 0936	Medium blue	Medium	3	4.85
TNEc 1865	Medium blue	Medium	3	4.81
TNEc 1902	Less intense blue	Medium	2	4.54
TNEc 1977	Less intense blue	Medium	2	4.52
TNEc 1980	More intense blue	High	4	6.64
TNEc 2161	More intense blue	High	4	8.06
TNEc 2179	No colour	Low	1	3.80
TNEc 2187	Medium blue	Medium	3	4.68
CO 9	More intense blue	High	4	5.62
CO(Ra) 14	More intense blue	High	4	5.99

In this study, fingermillet grain Fe content ranged from 3.46 (TNEc 0921) to 8.72 (TNEc 0601) mg per 100g of grain. The blue colour was more intense in the genotypes having a high Fe content of 5.67 (TNEc 0231) to 8.72 (TNEc 0601), mg per 100g of grain. Eleven accessions namely TNEc 0601, TNEc 0787, TNEc 0910, TNEc 0776, TNEc 2161, TNEc 0629, TNEc 0416, TNEc 1980, TNEc 0407, TNEc 0231 and TNEc 0788 showed more intense blue colour development which was validated by quantification method. These high Fe content genotypes can be employed in the genetic improvement of fingermillet.

Seven accessions namely, TNEc 0089, TNEc 0907, TNEc 0936, TNEc 1865, TNEc 0775, TNEc 2187 and TNEc 0707 exhibited medium blue colour development whereas, the accessions namely, TNEc 1902, TNEc 1977 and TNEc 0786 developed lesser blue colour. The following low Fe content genotypes grouped *viz.,* TNEc 0152, TNEc 2179 and TNEc 0921 no colour development. These results suggest that Perls' Prussian blue staining will be effective in differentiating genotypes with high, medium and low Fe content.

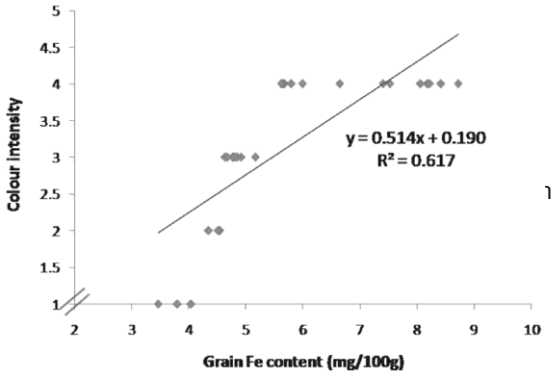


Fig 2. Correlation between grain Fe content and colour intensity

genotypes recorded a score 3. Each of the less intense blue colour (score 2) and no colour (score 1) was show in 11.11 per cent genotypes.

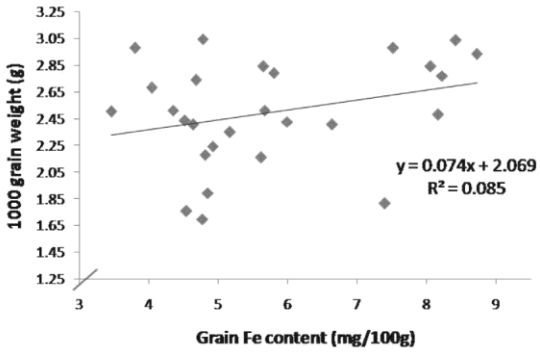
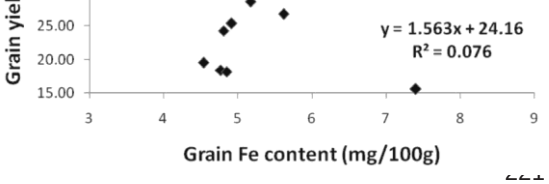


Fig 3. Correlation between grain Fe content and 1000 grain weight

The rank correlation between measured grain Fe content using AAS method and the color intensity score using Perls' Prussian blue method was highly significant and positive, indicating that higher the Fe content in the grain, the more will be the intensity of blue colour developed. In general, the intensity of



blue color served as a reliable qualitative selection criterion for grain Fe in fingermillet (Fig. 2). This method is efficient in classifying the genotypes with high grain Fe content. When a large number of germplasm accessions or progenies or breeding lines are to be screened for Fe content, this method will be highly efficient in discarding accessions with low Fe content or *vice versa* (Velu *et al.,* 2006; Prom-u-thai *et al.,* 2003; Krishnan *et al.,* 2003).

Fig 4. Correlation between grain Fe content and grain yield per plant

The simple correlation between measured grain Fe content and the 1000 grain weight were non significant, indicating that the grain Fe content and 1000 grain weight are not associated (Fig. 3).

Table 2. Performance of high yielding varieties with grain iron (Fe) content in fingermillet

Genotypes	Fe content (mg/100g)	1000 grain weight (g)	Grain yield per plant (g)
TNEc 0089	5.17	2.35	28.55
TNEc 0152	4.04	2.68	38.40
TNEc 0231	5.67	2.51	32.40
TNEc 0308	4.78	3.04	41.69
TNEc 0407	5.80	2.79	44.68
TNEc 0416	7.40	1.81	15.60
TNEc 0601	8.72	2.93	41.67
TNEc 0629	7.52	2.98	40.38
TNEc 0707	4.64	2.40	30.56
TNEc 0775	4.77	1.70	18.43
TNEc 0776	8.17	2.48	32.42
TNEc 0786	4.35	2.51	33.68
TNEc 0787	8.42	3.03	41.67
TNEc 0788	5.65	2.84	43.62
TNEc 0907	4.92	2.24	25.38
TNEc 0910	8.22	2.77	42.60
TNEc 0921	3.46	2.50	35.68
TNEc 0936	4.85	1.89	18.20
TNEc 1865	4.81	2.18	24.28
TNEc 1902	4.54	1.76	19.48
TNEc 1977	4.52	2.43	31.56
TNEc 1980	6.64	2.40	30.15
TNEc 2161	8.06	2.84	45.39
TNEc 2179	3.80	2.98	40.94
TNEc 2187	4.68	2.74	39.62
CO 9	5.62	2.16	26.72
CO(Ra) 14	5.99	2.42	31.56
Mean	5.75	2.49	33.16
SE	0.30	0.07	1.70
Minimum	3.46	1.70	15.6
Maximum	8.72	3.04	45.39

However, four accessions namely, TNEc 0601, TNEc 0629, TNEc 0787 and TNEc 2161 are having high 1000 grain weight with high grain Fe content. These genotypes can be further used for genetic improvement programme.

Similarly, there is no significant difference between grain Fe content and the grain yield per plant (Fig. 4). In general, grain Fe content did not significantly influence either the 1000 grain weight or the grain yield per plant. Hence, it can be inferred that genetic enhancement of grain iron content, 1000 grain weight and grain yield per plant are independent and does not influence each other.

Conclusion

The success of genetic improvement in any character depends on the nature of variability present for that character. Hence, an insight into the magnitude of variability present in the gene pool of a crop is of utmost important to a plant breeder for starting judicious plant breeding program. Although breeding for high yield is the primary objective of breeders, the improvement of the nutritional quality of these cereal crops should also be an important consideration to be given due to prevalence of malnutrition world-wide. Pearls' Prussian blue method can be effectively used as an initial method of screening, and genotypes could be identified for high grain Fe content. This saves the cost, time and labour involved in quantitative estimation of grain Fe content. Highly significant and positive correlation between grain Fe and colour intensity was observed in fingermillet genotypes. Entries having high grain Fe content combined with grain yield per plant can be used in crop improvement programmes.

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References

Baker, J.R. 1958. Principles of biological microtechnique. A study of fixation and dyeing. London. pp 304.

Krishnaswamy, K. 2009. The problem of double burden of disease and its consequences—a brief overview. In: Programme and abstracts, Symposium on Nutritional Security for India—Issues and Way Forward, Indian National Science Academy, New Delhi, 3–4 August, pp. 5–6.

Krishnan, S., Datta, K., Baisakh, N., Vasconcelos, M. and Datta, S.K. 2003. Tissue-specific localization of P-carotene and iron in transgenic rice *(Oryza sativa* L.). *Current Science*, **84**:1232-1234.

Krishnan, S., Ebenezer, G.A.I. and Dayanandan, P. 2001. Histochemical localization of storage components in caryopsis of rice (*Oryza sativa* L.). *Current Science,* **80**: 567-71.

Malleshi, N.G. and Klopfenstein, C.F. 1998. Nutrient composition, amino acid and vitamin contents of malted sorghum, pearl millet, finger millet and their rootlets. *Int. J. Food Sci. Nutr.* **49**: 415–422.

Prom-u-thai, C., Bernie, D., Thomson, G. and Benjavan, R. 2003. Easy and rapid detection of iron in rice grain. *Science Asia* **29**: 203-207.

Singh, D., Chonkar, P.K. and Dwivedi, B. S. 2005. Manual on soil, plant and water analysis. Westville Publishers, New Delhi.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods, eighth ed. Iowa State University Press, Ames, Iowa, USA.

Underwood, R.A. 2000. Overcoming micronutrient deficiencies in developing countries: is there a role for agriculture? *Food Nutr. Bull*. **21**: 356–360.

Upadhyaya, H.D., Ramesh, S., Shivali Sharma, Singh, S.K., Varshney, S.K., Sarma, N.D.R.K., Ravishankar, C.R., Narasimhudu, Y., Reddy, V.G., Sahrawat, K.L., Dhanalakshmi, T.N., Mgonja, M.A., Parzies, H.K., Gowda, C.L.L. and Sube Singh. 2011. Genetic diversity for grain nutrients contents in a core collection of finger millet (Eleusine coracana (L.) Gaertn.) germplasm. *Field Crops Res.,* **121**: 42–52.

Velu, G., Kulkarni, V.N., Muralidharan, V., Rai, K.N., Longvah, T., Sahrawat, K.L. and Raveendran, T.S. 2006. A Rapid Screening Method for Grain Iron Content in Pearl Millet. An open access journal in ICRISAT.

Welch, R.M. and Graham, R.D. 2004. Breeding crops for enhanced micronutrient content. *Plant and Soil,* **245:** 205-214.

Zarcinas, B.A., Cartwright, B. and Spouncer, L.R. 1987. Nitric acid digestion and multi element analysis of plant material by inductively coupled plasma spectrometry. Comm. *Soil Sci. Pl. Analysis,* **18:** 36-40.

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