Plant Genetic Resources: Characterization and Utilization (2011) **9**(2); 327–329
doi:10.1017/S1479262111000116
ISSN 1479-2621

Iron biofortification of maize grain

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Abstract

Mineral nutrient deficiencies are a worldwide problem that is directly correlated with poverty and food insecurity. The most common of these is iron deficiency; more than one-third of the world's population suffer from iron deficiency-induced anaemia, 80% of which are in developing countries. The consequences of iron deficiency include increased mortality and morbidity rates, diminished cognitive abilities in children and reduced labour productivity, which in turn stagnates national development. The developed world has made tremendous success in alleviating nutrient deficiencies through dietary diversification, food product fortification, improved public health care and supplementation. In developing countries, these strategies are often expensive and difficult to sustain, especially in rural areas. The rural poor typically consume what they grow and are dependent upon a small number of staple crops for the vast majority of their nutrition. Therefore, genetic improvement of staple crops (biofortification) is the most cost-effective and sustainable solution to this global health problem. In this study, we describe a strategy to enhance iron nutritional quality in maize using a human cell culture (Caco-2)-based bioassay as a phenotyping tool to guide genetic analysis of the trait. We also report validation of this approach using an animal feeding study.

Keywords: biofortification; Caco-2 bioassay; iron nutrition; maize; quantitative trait loci

Introduction

Iron (Fe) deficiency is a worldwide problem that is directly correlated with poverty and food insecurity. Approximately, one-third of the world's population suffer from Fe deficiency-induced anaemia, 80% of which are in developing countries (Boccio and Iyengar, 2003). The consequences of Fe deficiency include increased mortality and morbidity rates, diminished cognitive abilities of children and reduced labour productivity that in turn stagnates national development (Caballero, 2002). About 75% of the world's poor households live in rural areas, and the majority are small-scale farmers (Pinstrup-Andersen, 2002). The resource-poor typically consume what they grow and are dependent upon a small number of staple crops for the vast majority of their nutrition (Bouis, 2000; Welch and Graham, 2000). This limits the feasibility of processed food fortification as a micronutrient deficiency-alleviating tool for this group and emphasizes the importance of plant-based solutions for human nutrition problems.

Fe is less available for absorption into the human body from vegetarian as opposed to non-vegetarian diets (Fig. 1, adapted from Hunt (2003)). The influence of biochemical factors on Fe availability depends on the form of Fe. Fe in plants exists primarily as non-haeme Fe. One factor influencing non-haeme Fe bioavailability is solubility; hence an increase in Fe concentration alone is not a sufficient answer to dietary Fe deficiency problems (Lucca *et al.*, 2001). Thus, evaluating the bioavailability of Fe is a necessity in order to improve the Fe nutritional quality in staple food crops. Given the high cost of quantifying Fe bioavailability via human and animal studies, *in vitro* screening of food samples represents the most feasible system for phenotyping large number of samples to identify factors and interactions

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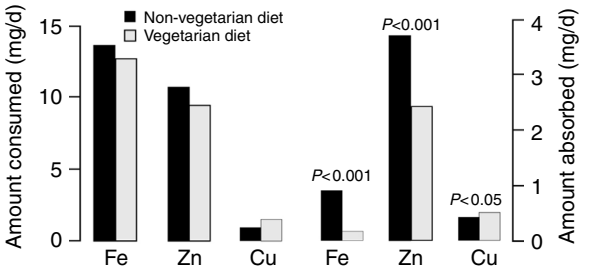

Fig. 1. Bioavailability is a key component of nutritional quality. While vegetarian and non-vegetarian diets may have similar quantities of nutrients such as Fe, the difference in solubility, absorption and ultimate utility ('bioavailability') is significantly different and should be considered in discussions of nutritional quality and breeding goals (Adapted from Hunt (2003)). Zn, zinc; Cu, copper.

that affect Fe bioavailability (Wienk *et al.*, 1999). The current state of the art for *in vitro* screening is a two-stage assay of a simulated gastric and intestinal digestion of food together with feeding human intestinal epithelial cells, specifically the Caco-2 cell line (Glahn *et al.*, 1998). We utilized the Caco-2 bioassay to evaluate Fe bioavailability in maize with the intermated B73 × Mo17 recombinant inbred (IBM RI) population (Lee *et al.*, 2002). The data collected from the bioassay allowed the identification of quantitative trait loci (QTLs) and have also been useful to guide selection for enhanced (and diminished) Fe nutritional quality in novel germplasm.

Materials and methods

The IBM RI set was received from the Maize Genetics Cooperation Stock Center (Urbana, IL, USA) and grown at the Musgrave Research Farm of Cornell University (Poplar Ridge, NY, USA). The IBM RI set was planted in 2001, 2003 and 2005. Grain samples from each year were analyzed by an inductively coupled argon plasma atomic emission spectrophotometer to measure Fe concentration. Fe bioavailability was measured in the IBM RI from only the 2003 season using the Caco-2 *in vitro* digestion method as described by Glahn *et al.* (1998). Briefly, 50 g lots of clean grain were cooked, freeze dried and ground to fine powder. A subsample of 1 g was then resuspended in water, treated with pepsin (pH 2.0), neutralized, treated with pancreatin/bile salts (pH 7.0) and then layered over a dialysis membrane (15 kDa cut-off). Soluble Fe from the cooked and digested food matrix passed through the dialysis membrane and was available to Caco-2 cells for uptake (2 h feeding, 22 h recovery). Cells were harvested and washed, and total protein was then isolated. Bioavailable Fe levels were estimated by measuring the Fe storage protein ferritin by a commercial immunoradiometric assay; ferritin is produced in linear response to Fe uptake by the Caco-2 cells. Grain Fe concentration and bioavailable Fe values were used to map QTLs using QTL Cartographer (Wang *et al.*, 2006). BC_2S_2 families were derived from select IBM RI in both parental backgrounds and used to isolate QTL, using a combination of SSR markers and phenotypic selection. BC_2S_3 sister lines with contrasting Fe nutritional values were used to derive new related inbreds and to create related hybrids, to facilitate grain production for animal feeding studies. Hybrids with predicted high and low Fe nutritional values were grown in 2008 and fed to newly hatched chickens to validate the results of the Caco-2 assay (Tako *et al.*, 2010).

Results and discussion

Given that the majority of Fe from plant-based foods is inaccessible to those who consume them, enhancing Fe bioavailability has greater potential to improve nutritional quality than merely increasing the amount of Fe present. To that end, the Caco-2 bioassay was used as a phenotyping tool to evaluate Fe nutritional quality in a maize RI population. Once QTLs were identified, backcross derivatives from RI lines were isolated to combine the superior (or inferior) alleles for the three largest QTLs. As backcross derivatives were constructed in both parental backgrounds, using both the superior and the inferior QTL configurations, hybrids were easily constructed to be heterozygous essentially everywhere except the three QTL regions under selection. Hand-pollinated hybrids produced similar amounts of grain (not significant, $P < 0.05$), while the potentially superior hybrids had more Fe in the grain (21.2 ± 0.2 *vs.* 18.8 ± 0.4 μg Fe/g dry weight, mean ± standard error (SE)) and had more bioavailable Fe according to the Caco-2 bioassay (44.4 ± 4.2 *vs.* 26.5 ± 1.6 μg ferritin/mg total protein, mean ± SE). The improvement in grain Fe concentration (12%) was far smaller than the improvement in grain Fe bioavailability (67%), indicating that the majority of improvement in nutritional quality must have arisen from changes in grain chemistry that enhance Fe bioavailability.

While the results of the Caco-2 bioassay have been consistent over several seasons, the impact of these results is tempered by the artificial conditions of the bioassay. To validate the use of the Caco-2 bioassay as selection tool, grain from the contrasting hybrids was fed to newly hatched chickens for 28 d. Bird health and Fe bioavailability were estimated from weekly blood tests that measured haemoglobin (Fig. 2). In addition to the

https://doi.org/10.1017/S1479262111000116 Published online by Cambridge University Press

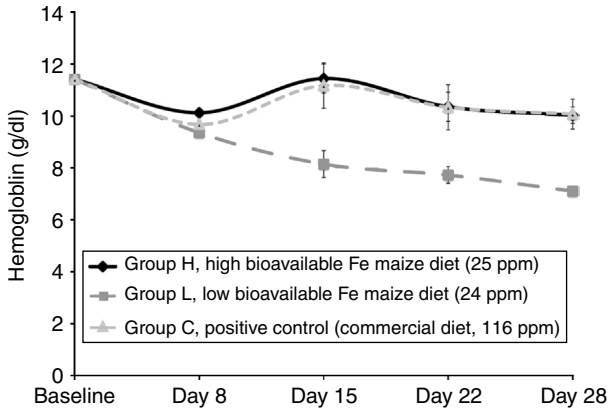

Fig. 2. Poultry experiment validates predictions from bioassay-based selection. Marker and phenotypic selection were used to develop hybrids with potentially superior and inferior Fe nutritional quality. These efforts were evaluated with a 28 d feeding study in newly hatched chickens, where haemoglobin levels were used to estimate Fe levels and overall animal health ($n = 6$ birds, haemoglobin levels are mean ± SE).

experimental diets where the contrasting hybrids provided nearly all of the dietary Fe, a control diet was fed to a third group of birds and contained supplementary Fe according to veterinary protocols. While the control and superior hybrid diets differed more than fourfold for Fe concentration, birds fed both diets produced identical amounts of haemoglobin in response and were Fe replete. In contrast, the superior and inferior hybrid diets contained similar Fe concentrations but produced highly significant differences in the birds for haemoglobin production and Fe repletion (Fig. 2). These results indicate that the Caco-2 bioassay was a useful phenotyping tool for evaluating Fe bioavailability, which could be useful for the improvement of any staple crop. In addition, we have produced novel maize varieties with altered Fe nutritional quality, which may serve as donors for breeding programmes for biofortification of maize. As several BC_2S_2 families were used to create superior and inferior derivatives, these stocks will also be highly useful for genetic, agronomic and genomic-based studies into the bases of Fe nutritional quality in maize. We should be able to identify the underlying genes and compounds that improve Fe bioavailability and transfer this knowledge to improve maize germplasm adapted to particular environments. This knowledge can also be used to enhance other staple crops using conventional breeding or biotechnology.

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