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The usefulness of iron bioavailability as a target trait for breeding maize (*Zea mays* L.) with enhanced nutritional value

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ABSTRACT

Iron (Fe) deficiency is the most widespread nutritional problem, affecting as many as half of the world's population. Only a small fraction (2–15%) of Fe from plant sources is typically bioavailable, that is, available for absorption and nutritionally useful for humans. This study evaluated Fe concentration and bioavailability for three diverse sets of 12, 14 and 16 maize hybrids grown in two- or three-location trials to assess the feasibility of selecting for Fe bioavailability in breeding programs. Bioavailability of Fe, assessed using the in vitro digestion/Caco-2 cell model, varied significantly among hybrids in two of the three trials. Location effects were larger than location by genotype interaction effects, additive but not non-additive gene action was significant, and heritability estimates were mostly between 0.55 and 0.65 for Fe bioavailability estimators. Bioavailability of Fe was not associated with Fe concentration in grain or with grain yield. Negative correlation of Fe bioavailability with zinc concentration in grain for one of the three hybrid trials, and positive correlation with provitamin A concentrations in one trial were indicative of inhibitor and enhancer effects on Fe bioavailability, respectively. Although use of the Caco-2 cell model is promising, particularly because it integrates the whole meal, or food matrix effect on Fe bioavailability, the complex nature of the assay and moderate heritability of bioavailability estimators make it most suitable as an intermediate selection tool, following high throughput selection for molecular markers of Fe bioavailability, currently in development by other researchers, and preceding validation and efficacy trials with animal and human models.

1. Introduction

Iron (Fe) deficiency is the most widespread form of malnutrition and afflicts an estimated two billion people, resulting in increased perinatal mortality, impaired cognitive capacity and development, and decreased productivity (Horton et al., 2009; Zimmermann and Hurrell, 2007). Fortification of foods, typically wheat flour, and diet diversification can alleviate the problem for consumers with adequate and consistent access to these foods, but young children or rural poor households may not be well served by these strategies. Biofortification, or the development and popularization of staple crop varieties with enhanced micronutrient (Fe, in this case) content has been proposed as an effective and sustainable strategy to alleviate malnutrition, particularly of rural families with limited access to markets and healthcare facilities (Bouis and Welch, 2010; Mayer et al., 2008; Ortiz-Monasterio et al., 2007). Successful biofortified varieties offer improved yield stability and potential in addition to nutritional quality, thereby enhancing household income, food security, and overall livelihoods.

Several studies have reported genetic variation for Fe concentration in maize grain, but there is disagreement whether the extent of variation is adequate to support successful non-transgenic breeding programs. An evaluation of more than 1800 improved and landrace maize genotypes, including CIMMYT's white-grained germplasm bank core accessions, all of CIMMYT's white and yellow maize germplasm pools and populations, and numerous elite and advanced southern African breeding lines, found Fe concentration values ranging from 9.6 to 63.2 mg kg^{-1}, suggesting that significant

Abbreviations: Ferr, ferritin (ng ferritin/mg cell protein); FerPC, ferritin as percent of ferritin of the control hybrid; FerFe, ferritin produced per available iron in each maize sample; Feraa, ferritin (ng ferritin/mg cell protein) in the Caco-2 assay with added ascorbic acid; FeraaPC, ferritin in the Caco-2 assay with added ascorbic acid, as percent of ferritin of the control hybrid; FeraaFe, ferritin produced per available iron in each maize sample, in the assay with added ascorbic acid; ProA, total provitamin A concentration; BCx, beta-cryptoxanthin concentration; BC, beta-carotene concentration.

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variation exists (Bänziger and Long, 2000). Most reports, however, involve far fewer genotypes and report small, but significant genotypic variation for Fe content in maize, for example, Oikeh et al. (2003a) reported 16.8–24.4 mg kg^{-1} Fe in 20 tropical late maturing open-pollinated maize cultivars grown at one location in Nigeria. Environmental, and genotype by environment interaction effects have generally been reported as highly significant for Fe concentration in maize grain (Long et al., 2004; Oikeh et al., 2003a, 2004b; Šimić et al., 2009). In addition to large environment and genotype by environment interaction effects, an important concern for breeders improving Fe concentration of maize is that because Fe is located mainly in the aleurone layer of grain, smaller kernels typically have larger Fe concentration than large kernels, and this unfavorable correlation must be carefully addressed during selection.

Bioavailability, or the efficiency with which Fe in grain can be absorbed for use by human consumers, is an important nutritional consideration (for a comprehensive review of factors influencing Fe bioavailability, see Hurrell and Egli, 2010). It is generally agreed that Fe from vegetable sources, including maize, is absorbed (bioavailable) with 5–15% efficiency, but recent studies using maize porridge reported only 1.8% (Hurrell et al., 2003) and 2.1% (Beiseigel et al., 2007) Fe absorption. The bioavailability of Fe in maize is strongly inhibited by phytic acid, or phytate, which is the principal store of phosphate, but binds minerals in seed. Drakakaki et al. (2005) demonstrated that Fe bioavailability from maize is enhanced proportionately to phytase expression in genotypes transformed with the phytase gene from *Aspergillus niger*. However, phytate is associated with desirable agronomic (e.g. early seedling vigor) and human health benefits (e.g. as an antioxidant when consumed in diets) (Shi et al., 2003). Therefore, despite the obvious nutritional desirability of enhancing Fe bioavailability in maize and other seeds, there is considerable controversy regarding the desirability of reducing phytate in seeds, and use of available low phytate mutant stocks has been limited.

Significant genotypic variation for Fe bioavailability from maize has been reported (Mwaniki et al., 2006; Oikeh et al., 2003a, 2004a; Šimić et al., 2009), indicating the possibility of breeding for enhanced Fe bioavailability instead of or along with breeding for increased Fe concentration as a strategy to improve the Fe nutritional value of maize. The feasibility of breeding for enhanced Fe bioavailability, however, needs to be examined considering the complexity, cost and effectiveness of available assays for use in applied breeding programs. Currently, the in vitro digestion/Caco-2 cell model is the leading in vitro assay for Fe bioavailability, and it is used in several laboratories for research purposes. The Caco-2 cell model has been applied to staple food crops including maize, wheat, beans, lentils and rice, and its results have agreed closely with results from Fe absorption studies in humans (Beiseigel et al., 2007; Yun et al., 2004). This model is a relatively high throughput system, capable of processing several hundred samples in a few months, for relatively low cost.

More recently, a poultry model of Fe bioavailability (Tako et al., 2010) was utilized to confirm in vitro observations of high and low bioavailable Fe maize (Lung'aho et al., 2010). In these studies, maize with high or low Fe bioavailability was developed via quantitative trait loci (QTL) mapping guided by the in vitro digestion/Caco-2 cell model. Poultry diets consisting of 75% maize were formulated and fed for a 4-week period. Birds consuming the high bioavailable Fe diets maintained a significantly higher Fe status than those consuming the low bioavailable Fe maize. This combination of in vitro selection followed by validation using in vivo animal models can be a productive approach to identifying factors and developing varieties that influence Fe bioavailability in staple food crops.

Biofortification of maize with more than one nutrient, for example, provitamin A (ProA) and Fe, may also be a desirable strategy because these nutrients interact in synergistic ways to enhance each others' nutritional effectiveness. Tanumihardjo et al. (2010) have described the synergistic roles of zinc (Zn) and proA carotenoids in human nutrition. Several authors have reported that vitamin A is an enhancer of Fe bioavailability (for example, Singh et al., 2006; Garcia-Casal et al., 1998), and existence of other traits affecting Fe bioavailability in maize kernels is likely.

The objective of this study was to consider the potential use of Fe bioavailability, measured by the in vitro digestion/Caco-2 cell model, as a target trait for breeding to enhance nutritional value of maize. The specific objectives were to examine the extent of genetic, environmental and genotype by environment effects for Fe concentration and bioavailability, and to investigate whether proA or Zn concentrations in grain are associated with Fe bioavailability for three diverse sets of maize hybrids grown in multiple environments.

2. Materials and methods

2.1. Germplasm

Three sets of single-cross (crosses of two inbred lines) hybrids were formed and evaluated in replicated field experiments at two or three locations during 2006 and/or 2007 (Table 1). The Highland Yellow hybrids were combinations among CIMMYT's most agronomically superior (e.g. good combining ability for grain yield, disease resistant, etc.), highland-adapted (suited to areas between 1800 and 2500 m above mean sea level, masl) lines, possessing above average concentrations of proA carotenoids. The Highland Zinc trials contained hybrids among advanced or elite lines from CIMMYT's highland maize breeding program, previously identified to have above average Zn concentration in grain. Finally, the Testcross Hybrid trial used subtropical or mid-altitude (1000–1800 masl) experimental lines from CIMMYT's proA maize biofortification project. These germplasm materials were chosen: (1) to sample a wide range of environments (subtropical and highland), (2) because Zn and Fe concentrations have sometimes been reported as positively correlated (Ortiz-Monasterio et al., 2007), and (3) because proA carotenoids have been reported to enhance the bioavailability of Fe (Garcia-Casal et al., 1998; Singh et al., 2006).

The experimental environments were summer (rainfed) plantings at CIMMYT experiment stations at El Batan (19°N, 99°03′W, 2250 masl) and Tlaltizapan (18°40′N Lat, 99°7′W, 950 masl), Mexico, and EIAR (Ethiopian Institute of Agricultural Research) experiment station at Ambo, Ethiopia (8°57′N, 38°07′E, 2225 masl). Additional, managed-stress environments, were a low nitrogen (LoN) field at El Batan during summer 2006, and drought stress (DR) at Tlaltizapan during winter 2006–2007. The LoN field has been grown with continuous maize and received chemical fertilizer as recommended, except zero nitrogen, for more than 5 consecutive years. The DR evaluation was conducted as per CIMMYT protocols (Bänziger et al., 2000), which maximize drought stress at flowering time and provide one relief irrigation during grain fill. To enable effective selection in both drought and low nitrogen evaluations, the target is to impose stress that results in average yield reduction to one-third of the yield under optimal or well managed conditions.

Each experiment was grown using an alpha-lattice design (Patterson et al., 1978) with two replications of one-row plots, which were generally 5 m long and spaced 75 cm between and 25 cm within rows (53,300 plants per hectare). Grain samples for laboratory analyses of proA carotenoids, Zn, Fe, and Fe bioavailability were obtained by bulking grain from controlled, hand-pollinations made in two or three plants of each field plot. All ears were harvested with their husk and taken to a clean laboratory area for de-husking and shelling without use of metal implements and with care to avoid Fe contamination.