Progress in Breeding for Trace Minerals in Staple Crops

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ABSTRACT  Staples are not considered an important source of minerals in the diet. However, because of high staple consumption, any increase in mineral concentration might well have a significant effect on human nutrition and health. The nutritional quality of staple crops (rice, cassava, wheat, maize, and beans) can be improved by breeding. Studies have shown the potential to exploit the genetic variation in seed concentration of iron and other minerals without the general negative effect on yield of adding new traits. The relationship between yield and mineral concentration may be positive, particularly in mineral-deficient soil. Initial evaluations have shown that some crop varieties have high Fe, Zn, and carotene in their edible portions. The next step for conventional breeding will be to study the genetics of trace mineral inheritance to determine the best selection technique. Initial investigations of the genetics for high iron in rice have indicated a complex mode of inheritance, demonstrating additive and dominant gene and environmental effects. Breeding strategies have been developed based on these genetic findings. The use of biotechnological tools, such as molecular marker-assisted selection, will significantly increase the pace and prospects of success for breeding to improve the nutritional value of staple food crops. J. Nutr. 132: 500S–502S, 2002.

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Micronutrient variability in staple food crops

During the past several years, scientists at several Consultative Group on International Agricultural Research centers [the International Rice Research Institute (IRRI), the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), the Centro Internacional de Agricultura Tropical (CIAT) and the International Institute of Tropical Agriculture] have been collecting data (1–6) on the potential for breeding to significantly increase the levels of bioavailable Fe, Zn and provitamin A carotenoids in edible portions of rice, wheat, maize, beans and cassava. The discussion below summarizes these findings.

Bean. Researchers at CIAT have been studying the degree of genetic variability that exists in Fe and Zn concentrations in seeds of common beans (Phaseolus vulgaris L.) (3). Researchers at CIAT (7) evaluated a core collection of 1,000 accessions of common beans and found a range in Fe concentrations from 34 to 89 μg/g (average = 55 μg/g Fe) (3). Zinc concentrations in these same accessions ranged from 21 to 54 μg/g (average = 35 μg/g Zn). Some bean accessions from Peru were recently found to contain especially high levels of Fe, averaging >100 μg/g Fe. The range in seed/Zn concentrations in the core collection was narrower than seed/Fe concentrations, ranging from 21 to 54 μg/g Zn. Wild types tended to have lower Zn concentrations than did common cultivated types. Some seeds from genotypes originating in Guatemala were highest in Zn levels. The data collected suggest that there is sufficient genetic variability to significantly increase Fe concentrations (by ~80%) and Zn (by ~50%) in common beans. The results also indicate that the traits responsible for genetic improvement in Fe and Zn concentrations are stable across various environments. For both Fe and Zn seed concentrations, there were significant location and location × genotype effects, demonstrating (as expected) that environments can influence the concentrations of Fe and Zn in bean seeds. However, high-Fe and high-Zn genotypes will accumulate more of these nutrients than will low-Fe and low-Zn genotypes grown at the same location during the same growing season.

Interestingly, CIAT researchers also found a very highly significant positive correlation of 0.52 between the concentrations of Fe and Zn across different genotypes. Thus, genetic factors for increasing Fe are cosegregating with genetic factors for increasing Zn. Therefore, selecting for a higher Fe level in bean seeds will also tend to select for increased Zn levels in the seeds.

Wheat. A wide range of wheat germplasm is being studied at CIMMYT (8) to determine the range of concentrations of
Fe and Zn in the whole grain and environmental interactions with these concentrations. In one study, the ranges in Fe and Zn concentrations (dry weight basis) in wheat grain from plants grown in El Batán, Mexico, in 1994 were 28.8–56.5 μg/g (mean = 37.2 μg/g; SD = 4.10 μg/g; n = 132) for Fe and 25.2–53.3 μg/g for Zn (mean = 35.0 μg/g; SD = 4.99 μg/g; n = 132) (3). Clearly, enough genetic variation exits within the wheat germplasm to substantially increase Fe and Zn concentrations in wheat grain. Among all wheat germplasm studied, the species *Triticum dicoccum* had the highest concentrations of Fe and Zn, which warrants additional study.

There was a high correlation between grain-Fe and grain-Zn concentrations in the wheat lines studied. Although significant genotype × environment interactions were obtained for Fe and Zn grain concentrations, there was still a strong genetic component for Fe and Zn accumulation in the grain. This finding indicates that it should be possible to improve Fe and Zn levels in wheat grain simultaneously through plant breeding. Additional research has also shown that there is no negative linkage between grain yield and Fe and Zn density in the grain.

**Maize.** The Fe and Zn concentration in the grain for 1,814 maize core germplasm and breeding populations of CIMMYT were evaluated in 13 trials in Zimbabwe and Mexico from 1994 to 1999 (9). They reported a range in the grain-Fe concentration between 9.6 and 63.2 μg/g (mean of 23.76 μg/g), and grain-Zn concentration between 12.9 and 57.6 μg/g (Mean of 33.27 μg/g). These variations in Fe and Zn in the kernel were attributed to both genetic differences and environmental conditions, which the germplasm was grown.

**Cassava.** Variation in β-carotene concentration in cassava roots from a CIAT core collection (630 genotypes) from the global cassava germplasm collection (~5,500 genotypes) was reported by Iglesias et al. (10). Additionally, the relationship between root color and heritability as well as the stability of root β-carotene for different root-processing techniques were studied. Chavez et al. (11) reported a range in β-carotene concentrations in fresh roots from 0.1 to 2.4 mg/100 g⁻¹.

The inheritance of β-carotene root-concentration seems to be determined by two genes (one controlling transcript of shoot precursors to roots and one responsible for the biochemical processes affecting the accumulation of β-carotene in the roots). Furthermore, visual screening by using intensity of orange color seemed feasible. However, Chavez et al. (11) also stated that there was a need to rely on quantitative screening techniques to increase the efficiency of the screening program. It is possible that other provitamin-A carotenoids could also be responsible for the deep yellow color observed in accessions that have intermediate β-carotene concentrations.

Iglesias et al. (10) concluded that there is enough genetic variability within the available cassava germplasm that would make it possible to produce cassava-roots that contain enough β-carotene to meet the daily requirements of adults (i.e., 6 mg/d β-carotene) if the β-carotene in cassava roots is highly bioavailable. The genotypes containing the highest levels of B-carotene were collected from the Amazon region of Brazil and Colombia, where yellow root lines are preferred by the indigenous farmers. Processing techniques were shown to have a large effect on the final β-carotene content in the food prepared from cassava roots, with some genotypes being more stable to various forms of processing than others. This factor must also be included in any breeding program to increase β-carotene in cassava roots.

**Micronutrient variability in rice**

Since 1992, researchers at IRRI [Gregorio et al. (12)] have been evaluating the genetic variability of Fe concentration in rice grain. In 1995 the research was expanded to include Zn [Graham et al. (3)]. The range in Fe and Zn concentrations in brown rice within the eight sets of genotypes (n = 1,138) tested in one study was 6.3–24.4 μg/g for Fe and 13.5–58.4 μg/g for Zn. Thus, within those genotypes tested, there was approximately a fourfold difference in Fe and Zn concentrations, suggesting some genetic potential to increase the concentrations of these micronutrients in rice grain.

The highest grain-Fe concentrations (i.e., ranging from ~18–22 μg/g) were found in several aromatic rice varieties, such as Jalmagna, Zuchem and Xia Bue Nuo. These same aromatic lines also contained the highest grain/Zn concentrations (ranging from ~24–35 μg/g). Additional research using F2-derived populations demonstrated that the aromatic trait was not pleiotropic for grain-Fe or grain-Zn concentrations, and, therefore, this trait may be used to screen for high Fe and Zn levels in rice grain, but the linkage is broken at a low frequency.

Several studies were carried out at IRRI to examine the effect of soil and climate on grain-Fe and grain-Zn concentrations among genotypes. Factors studied included wet vs. dry season, normal vs. saline soils, acid vs. neutral soils, and N supply. The data from these various studies demonstrated that high-Fe and high-Zn grain traits are expressed in all rice environments tested, although there is some evidence of significant genotype × environment interactions that can ultimately affect Fe and Zn concentrations in extreme environments (3).

These IRRI results indicate that there is significant genetic diversity in the rice genome to increase Fe and Zn concentrations substantially in rice grain. Currently, 7,000 samples have been analyzed together with supplementary sets from China and Bangladesh. These data make up a valuable database on genetic variation for Fe and Zn variability in rice grain. However, the effects of rice grain processing on Fe and Zn levels in the edible product (i.e., polished and parboiled rice grain) as well as the bioavailability of the Fe and Zn in the grain for humans still await final results from continuing evaluations of these factors.

A high-iron trait can be combined with high yielding traits. This was demonstrated in the serendipitous discovery of an aromatic variety already in the IRRI testing program—a cross of a high yielding variety (IR72) and a tall, traditional variety (Zawa Bonday) from India, from which IRRI identified an improved line (IR68144-3B-2-2-3) with a high concentration of grain iron, ~21 ppm in brown rice. This elite line has good tolerance to rice tungro virus and has excellent grain qualities. Yields are ~10% below IR72, but, in partial compensation, maturity is earlier. This variety has good tolerance to mineral-deficient soils, such as P, Zn and Fe. It has no seed dormancy and excellent seedling vigor, suggesting that it could be a good direct-seeded rice. Among the high-iron varieties were several aromatic rice genotypes, an observation which led to the discovery of IR68144-3B-2-2-3. Aromatic rice was consistently higher in grain iron concentration and often also in zinc than its nonaromatic counterparts.

**Effect of milling on rice grain iron content**

A comparison of Fe content at different polishing times for high-iron traditional varieties (red pericarp) with IR64 and IR68144-3B-2-2-3 (white pericarp) was undertaken, which demonstrated a strong interaction between genotype and time of milling (12). Grain color seemed to be associated with the amount of iron content. The grain appearance of red pericarp
varieties like Jalmagna, Tong Lang Mo Mi and Xua Bue Nuo became fairer as polishing time increased. Drastic changes of color were observed in Jalmagna and Tong Lang Mo Mi from 15 to 45 min polishing time, which corresponded to large declines in Fe content. For Xua Bue Nuo, a very slight change in color was observed, whereas Fe content declined relatively little.

For IR64, a popular commercial variety with the lowest Fe in brown rice, the Fe content dropped by more than one-third with 15 min polishing (the time equivalent to that of commercial polishing); after 15 min, Fe content remained almost unchanged. A loss in Fe content of approximately one-third at 15 min milling was observed for two high-iron traditional rices, Jalmagna and Tong Lang Mo Mi, but their iron concentrations continued to decrease substantially as polishing time increased. These observations suggest that much of the iron is in the outer layers of the grain. However, Xua Bue Nuo, a traditional variety from China, and high-iron IR68144-3B-2-2-3 were less affected by polishing time. At 15 min polishing, IR68144-3B-2-2-3 had ~80% more iron than IR64.

**Genetic analysis of the high fe trait and breeding strategy for rice**

Genetic component analysis was undertaken of the high-Fe trait in grain using four traditional high-Fe rice varieties (Azucena, Basmati 370, Xua Bue Nuo and Tong Lang Mo Mi), three advanced lines (IR61608, PP2462-11 and AT5-15), and three IRRI released varieties (IR36, IR64 and IR72) (12).

Results show highly significant differences among the crosses and among parentals and F1 progenies. This clearly indicates a genetic effect on grain Fe concentration, suggesting that selection among F1 progenies is possible. The genetic analysis of variance revealed the presence of additive gene action (fixable genes) in addition to a significant nonadditive genetic variance (nonfixable genes or unpredictable genes). Environmental effects are present but smaller than the genetic effects. Narrow-sense heritability of the trait was found to be moderately low (43%). The large difference between estimates of narrow-sense and broad-sense heritability (88%) further confirmed the importance of a nonadditive-type gene action.

Analysis of combining ability indicated a high and significant general combining ability. This suggests that some parents may be combined with a range of varieties to produce a high-Fe trait in progenies. Specific combining ability is also present, indicating that specific combinations between the parents (e.g., Azucena × Basmati 370) would produce increasing Fe concentration in progenies. The presence of reciprocal effects suggests the importance of the choice parent. For example, a female parent of Tong Lang Mo Mi would always produce higher Fe progenies than a male parent.

Based on the inheritance study, selection during breeding should be undertaken in a later generation (such as F5), where the dominance effect (unfixable genes) is not present. A bulk breeding method is suggested in early generations during which selection for other agronomic characteristics should be undertaken—without selection yet for the high-Fe trait. An alternative method that might work well is single-seed descent using the F5 generation. Because of the influence of environment and cultural practices in determining Fe concentration, selection should be done in an optimum environment such as application of N and P to maximize genetic variability.

Three groups of genes were found to be associated with the high-Fe trait. These groups of genes were located on chromosomes 7, 8 and 9 and explained 19–30% of the variation in Fe content. Three groups of genes also were identified for aroma. They were located on chromosomes 5, 7 and 8 and explained 16–38% of the variation. Thus, two genes associated with high-Fe and aroma have two chromosomes in common (7 and 8), although these genes are located in different locations on the chromosome. This indicates a slight linkage between aroma and the high-Fe trait.

Permanent mapping populations of F2 recombinant inbred lines were developed to map high-Fe and -Zn traits. Mapping of genes is an important step in developing marker-assisted selection for any trait of interest. This selection technique is rapid, highly reliable, and relatively inexpensive. Marker-assisted selection will be used to select two to three traits at a time.

**DISCUSSION**

Results so far obtained under the Consultative Group on International Agricultural Research Micronutrient Project indicate that the breeding parameters are not difficult and are highly likely to be low cost. The following points are seminal:

- Adequate genetic variation in concentrations of B-carotene, other functional carotenoids, iron, zinc and other minerals exists in the major germplasm banks to justify selection.
- Micronutrient/density traits are stable across environments.
- In all crops studied, it is possible to combine the high-micronutrient/density trait with high yield, unlike protein content and yield that are negatively correlated.
- Genetic control is simple enough to make breeding economic.
- It will be possible to improve the content of several limiting micronutrients together, thus pushing populations toward nutritional balance.
- Bioavailability of the extra nutrient in elite breeding lines is high for rats, and, where the density is high enough for the test, also for human colon cell lines. Tests on human populations are now a high priority.

**LITERATURE CITED**