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Improving iron and zinc value of rice for human nutrition

G. B. GREGORIO
D. SENADHIRA
T. HTUT
Plant Breeding, Genetics and Biochemistry Division, IRRI, MCPO Box 3127, 1271 Makati City, Philippines

R. D. GRAHAM
Department of Plant Science, University of Adelaide, Glen Osmond 5064, South Australia, Australia

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Introduction

High-yielding varieties of rice, wheat, maize, and other food staples are now grown widely in developing countries. Over the past three decades, cereal production has grown faster than demand and the resulting lower food prices and higher farm incomes have contributed to reduced protein-energy malnutrition (PEM) among the poor. Food staples are inexpensive sources of calories and protein, but are not rich sources of essential vitamins and minerals.

Nutritionists are now alarmed by malnutrition caused by poor dietary quality. Overall, mineral and vitamin deficiencies, now known as hidden hunger, affect a far greater number of people in the world than PEM.

Nutritional anemia, mostly from iron deficiency, is widespread among developing countries. Low dietary intake and bioavailability of iron, blood loss due to parasites, and unmet demand associated with rapid growth and pregnancy are the causes of iron deficiency.

Severe problem of anemia

WHO estimates that nearly 3.7 billion people are iron-deficient and that the problem is severe enough to cause anemia in 2 billion people. Of this figure, 40% are non-pregnant women and 50% are pregnant women. An estimated 58% of pregnant women in developing countries are anemic and their infants are more likely to be born with low birth weight and depleted iron stores. WHO also estimates that 31% of children under 5 years old are also anemic, most of this being iron deficiency anemia (WHO,1999).

In general, iron deficiency and anemia have profound negative effects on human health and development, including limited learning capacity in childhood, impaired immune function and reduced labor productivity. Evidence is accumulating that zinc deficiency leads to complications in pregnancy and childbirth, lower birth weight and poor growth in childhood, associated with diarrhea incidence, reduced immunocompetence, and morbidity.

Improving nutritional quality of crop plants

Producing enough food energy to maintain the world's population is not enough. Even if energy requirements are met, billions of malnourished, poor people will continue to live in poor health, with low productivity and an inferior quality of life. Nutritious foods that meet minimum daily nutritional requirements must be produced. Supplementation, fortification and education have been successful in reducing iodine deficiency and such intervention programs must be continued and extended to the Vitamin A problem. For other micronutrients, such programs are expensive, and unlikely to reach all of those at risk. Another strategy in alleviating iron-deficiency anemia is reducing aetiological factors such as parasites. Still another is dietary intake improvement by balancing cereal-based diets with vegetables and animal products. Results, however, are frustrating. Vegetables and animal products are expensive, seasonal, subject to spoilage, and difficult to store and transport. Moreover, their availability in some countries is not even one-fourth of what is required to meet the needs of the people. There is a need to develop low-cost long-term solutions to complement any existing interventions. This appears to be improvement of nutritional quality of food staples.

The nutritional quality of crop plants can be improved by breeding. In fact, it is not a new approach. The philosophy of breeding for nutritional improvement has been developed and well perceived. However, an important requirement is that the improved varieties with nutritional characteristics must meet farmers' agronomic criteria. In case of increasing micronutrients like iron and zinc in the grain, improvement of both nutritional characters and agronomic criteria should be achieved at the same time. High micronutrient content in the seed will certainly possess a significant advantage like rapid crop establishment especially in nutrient deficient soil. It is reported that seed is the main mineral nutrient source for the seedlings and that the seed iron content is high in plants adapted to soils, which are low in available iron.

Rice and micronutrient

Rice is the staple food for 2.4 billion people in the developing world. Over the past three decades, the number of rice consumers had increased by 70%. However, at the same time, rice production had nearly doubled, contributing to substantial increases in the consumption of rice and caloric intake per capita, particularly in Asia. Half of the world's rice production is consumed where it is grown —in resource-poor farm households.

Among the important cereals, rice has the highest food yield and food energy yield. Rice provides 35-59% of energy consumed for nearly 3 billion people in Asia. Rice also contributes a substantial portion of protein intake. The contribution of rice to protein in the diet is about 69% in South Asia and about 51% in South East Asia. The rice grain has no provitamin A but has small amounts of iron and zinc. However, these small amounts seem to contribute substantially because of the large amount consumed. A nutritional survey conducted in the Philippines suggested that about 50% of the iron intake, even among high-income households, come from the cereals, rice and corn (Figure 1). The problem of iron deficiency was also revealed in the survey (Figure 2).

In countries where the staple food is rice, per capita consumption is so high, ranging from 87 to 214 kg/year, that even slightly more nutritious rice could mean healthier people. However, rice is considered a starchy staple and, as a result, attention to its nutritional aspects is still minimal. Other than the efforts of IRRI in 1960s and 1970s to improve its protein content, there had been little or no work on improving the nutritive value of rice. Research priorities for improving grain quality that were discussed and recommended at international rice research conferences held in 1985 and 1990 did not focus on nutritive values but emphasized milling, cooking, and eating qualities as high priority research items. This was possibly because IRRI's experience with breeding for protein content was not successful. However, rice is the cereal lowest in iron, often containing only 5 or 6 mg/kg after milling, and there appears from other studies to be potential to exploit genetic variation in seed content of iron and other minerals without the generally negative impact on yield commonly seen with protein in many crops; indeed, the relationship to yield may be positive for some minerals when their availability is low in the soil. Although rice is not considered a major mineral supplier, any increase in its concentration could significantly help reduce the iron and zinc deficiency problem.

Variability in iron and zinc content in rice grain

In 1992, IRRI began to examine the effect of certain soil characteristics on the Fe content in the grain. This research was influenced by the efforts of the Philippine Government to eliminate the iron malnutrition problem in the country by artificially enriching consumption rice with iron. Varieties were tested under normal and Fe toxic soil conditions. In spite of the many problems associated with sample preparation for analysis, wide differences among varieties were observed for the Fe content in grain. The problems encountered in sampling were rectified later and in 1995 the work was expanded to include Zn. Collaboration with the University of Adelaide was established for mineral analysis according to international standards. Up to now nearly 7,000 samples have been analyzed and together with supplementary sets from China and Bangladesh, these data is a valuable database on genetic variation for Fe and Zn variability in rice grain.

Since the effect of the environment (soil and climate) on the grain mineral content was not known, the initial test strains were planted in IRRI farm under uniform soil and crop management conditions. From harvesting to analysis, care was taken to prevent any contamination, with soil in particular. There were several plantings, in different locations and seasons. Brown rice samples were analyzed for minerals by Inductively Coupled Plasma Atomic Emission Spectometry (ICP-AES) at the Department of Plant Science, University of Adelaide, Australia. After several screening, some with exceptionally high mineral content were observed and were reevaluated in later tests. Data obtained from each screening set is summarized in Table 1.

Among the 1138 samples analyzed, Fe concentration ranged from 6.3 to

24.4 mg/kg with a mean value of 12.2 mg/kg. for Zn, the range was 15.3 to 58.4 mg/kg with a mean of 25.4 mg/kg. A comparison of high Fe and Zn varieties isolated from the tests with IR36 and IR64, the two most popular varieties in Asia, is shown in Table 2. Traditional variety Jalmagna, had almost double the amount of Fe compared with the popular varieties. Its Zn concentration was also high and nearly 40% more than IR64. Jalmagna is a floating rice grown in some parts of Eastern India. The variety Madhukar showed slightly high Fe density and very high Zn. Madhukar is a popular variety in some rainfed and deepwater areas of Eastern India. Soils of this region are slightly alkaline and Zn deficient, and Madhukar is well known as a highly Zn efficient rice variety. However, other known Zn efficient rice varieties such as Kuatik Putih, Bille Kagga and Getu did not show high grain Zn concentration. Zuchem, a traditional japonica type rice variety grown in very high altitudes (> 2000 m above sea level) of Bhutan, expressed both high Fe and Zn in grain. Its Fe content was not as high as in Jalmagna. Xua Bue Nuo, a traditional variety from China, also showed high Fe. This variety was included in tests because its name has some relation to Fe. None of the improved varieties showed exceptionally high Fe or Zn content.

Among the high iron varieties were a number of aromatic rices. Therefore, comparisons of aromatic and non-aromatic varieties were made (Table 3). Aromatic rice was consistently higher in grain iron concentration and often also in zinc, than the non-aromatic comparisons. It follows that people eating aromatic rices have better intakes of iron than those eating non-aromatic types, up to twice as much. Calculations suggest that because of the high consumption of rice, the extra iron if it is, as bioavailable as in normal rice, would have a meaningful impact on the health of anemic women and children.

Effect of soil on grain mineral content

Although there has not been any in-depth Genotype x Environment studies on iron-dense grain trait in rice, evidences from preliminary experiments indicates that environment has significant impact on iron concentration in the rice grain. The significant portion of environmental effect may be expected to come from the rice rhizosphere environment with respect to plant iron nutritional aspect. Studies from IRRI revealed that soil properties have considerable effect on rice grain mineral content. An experiment conducted in both normal and saline soils, in a coastal area in Pili, Iloilo, Philippines showed that grain mineral content of four rice varieties varied across soil types (table 4.)

In that experiment, salt sensitive variety, IR29, increased the Fe content under saline soil condition and in saline tolerant varieties, IR74, IR9884, and Pokkali, it slightly decreased. Independent of the tolerance for salinity, Zn content decreased with salinity in the soil.

An experiment testing eight rice varieties along with iron dense traditional varieties, Jalmagna and Madhukar, in an acid soil site in San Dionisio, Iloilo, Philippines, showed that grain iron content of varieties sensitive to iron toxicity increased in comparison with the same experiment conducted in normal soil (Table 5). However, tolerant varieties decreased grain iron content slightly. The conclusion was that tolerant varieties may possess exclusion mechanism for excess toxic iron in the soil. Therefore, root-soil interaction with respect to iron nutrition and other plant nutritional characteristics must have modified iron-dense grain character.

Effect of milling on grain iron content

A comparison at different polishing times of high iron traditional varieties (red pericarp), with IR64 and high-iron rice IR68144-3B-2-2-3 (white pericarp) is shown in Figure 3. The graph demonstrates a strong correlation between iron content and time of milling.

In the popular variety, IR64 that contained the lowest iron in brown rice, the Fe content dropped by more than 33% with 15 minutes polishing. This remained almost unchanged as polishing time increased. A loss of about 33% after 15 minutes milling was also observed for high iron traditional rices – Jalmagna and Tong Lan Mo Mi, but their iron concentrations decreased drastically as polishing time increased. These observations confirm reports that in rice grain, iron and other micronutrients are deposited in high concentration in the outer layer of brown rice. However, iron content in the rice grain can vary upon rice genotype and cultural condition as exampliﬁed by the traditional variety from China, Xua Bue Nuo, and high-iron IR68144-3B-2-2-3, which were less affected by polishing time. Data showed that with polishing time equivalent to that of commercial polishing (15 minutes), IR68144-4B-2-2-3 had about 79% more iron than IR64. In red pericarp varieties grain color appeared to be associated with the amount of iron content in the grain (Figure 4).

The grain appearance of red pericarp varieties like Jalmagna, Tong lan Mo Mi, and Xua Bue Nuo become fairer as polishing time increases. However, drastic changes in color were observed in Jalmagna and Tong Lan Mo Mi from 15 to 45 minutes polishing. This trend is congruent to the decreasing Fe content. For Xua Bue Nuo, very slight change in color was observed and its Fe content was also less affected (Figure 4).

Improved rice with enhanced iron and zinc in the grain

To meet the farmer's agronomic criteria, a high-iron trait was combined with high yielding traits. This was observed and its Fe content was also less affected (Figure 3) as demonstrated in the cross of a high yielding variety IR72 and tall traditional variety Zawa Bonday of India, from which IRRI identified an improved line IR68144-3B-2-2-3 with high concentration of grain iron about 21 mg/kg in brown rice. This elite line has good tolerance to rice tungro virus, has excellent grain qualities and is aromatic. The yields were about 10% below IR72 but in partial compensation, the maturity is earlier and has good tolerance to mineral deficient soils like phosphorus, Zn, Fe. It has no seed dormancy but has excellent seedling vigor, which could be good direct seeded rice.

Mapping genes for high iron and zinc traits in the grain

Rapid and cheaper means of selecting for efficiently and loading traits of micronutrients is an urgent need. Breeding programs for complex traits were usually delayed primarily due to slow detection segregants in breeding populations. The advent of the molecular marker technique provides greatest prospect in doing precise breeding for such complex traits. Previously genotyped rice population was used to tag the genes/QTLs for the high Fe trait in the grain. A Total of 175 polymorphic markers linkage map of double haploid derived lines from the cross IR64 and Azucena was analyzed to map the genes/QTLs for high Fe traits and aroma in the grain. The population was phenotyped for Fe concentration in the grain and analyzed together with the molecular markers available. Three important Quantitative Trait Loci (QTLs) were detected for high Fe trait and located on chromosomes 7, 8, and 9, explaining 30.3%, 21.3% and 19.0% respectively of the phenotypic variation. Three QTLs were identified for aroma and they were located on chromosomes 5, 7 and 8, showing 16.4 to 38.3% of the variation. Two QTLs in high Fe trait and aroma have common in two chromosomes (7 and 8) but in different loci with 24.8 and 36.4 cM between QTLs respectively. This indicates a slight linkage between aroma and high Fe trait.

Permanent mapping populations of F8 recombinant inbred lines (RIL) were developed to map high Fe and Zn trait (Table 6). These populations were also used to map other abiotic stresses tolerance like Al toxicity, Zn deficiency and excess water.

The marker assisted selection technique is rapid, highly reliable and less expensive. Rapid because small portion of a leaf from a young plant could be use to detect if the high Fe trait in the grain is present and results could be available within few days. It is highly reliable because molecular markers are not affected by environment. Since grain analysis using Inductively Coupled Plasma Atomic Emission Spectometry is very expensive, by the use of marker assisted selection it will be less expensive and this could be use to select two to three traits at a time. This new technique will significantly increase the prospect of efficiently breeding in improving the nutritional value of rice.

Genetic analysis of high iron trait and breeding strategy

A 10 x 10 complete diallel involving four traditional high Fe rice varieties (Azucena, Basmati 370, Xua Bue Nou and Tong lang Mo Mi), three advanced lines (IR61608, PP2462-11, and AT5-15), and three IRRI released varieties (IR36, IR64, and IR72) were used in studying the genetics of high Fe trait in the grain. Evaluation of the 90 F1s and 10 parental genotypes were done at IRRI experimental farm with three replications. Dehulled brown rice were analyzed for Fe density. Results shows highly significant difference among the crosses and between parentals and F1s, this clearly indicate the genetic effect on grain Fe concentration and selection among F1 could be done. Genetic analysis showed the complexity of the mode of inheritance of the high Fe trait. Both additive and dominance gene actions contributed significantly, and the influence of the environment,smaller, in the expression of the trait. Thus for breeding for high Fe concentration in the rice grain, selection should not be applied in the early segregating generation since dominance effect (unpredictable/unfixable genes) are still present. Selection should be delayed in the later generation like F5 were the dominance effect (unfixable genes) is not pronounced. Bulk breeding method is suggested in early generation were selection for other agronomic character could be done and no selection for high Fe trait. Single seed descent (SSD) could be another method upto F5 generation. The significance of the reciprocal effects suggests that the choice of male and female parent is also critical. Some donor parents are better to be used as male than female or the other way around, depending on the choice of male or female the progeny's phenotype is greatly affected. There is great potential of exploiting heterosis by developing hybrid rice with enhanced Fe in the grain because of the pronounced dominance gene action of the trait. Selection under very controlled condition with bigger population size is recommended because of the influence of the environment or cultural practices in the expression of the high Fe trait. To increase the genetic variability of the trait, plants should be raised in an optimum environmental condition.

The future

For the poor, micronutrient-dense rice is the affordable source of Fe and Zn. The high consumption rates makes developing rice that load high amounts of Fe and Zn into their seed a wise breeding strategy. But these opportunities need better understanding of the genetics and inheritance of the trait and further studies on the genotype x environment interaction of these varieties. Although part of this work is currently underway, the rate of progress can be enhanced through collaboration with NARS and the use of biotechnology tools.

Initial evaluations have shown that there are rices with high Fe and Zn in grain but more evaluation will be valuable. The next step will be to study the genetics, to determine the best selection technique for use in breeding and to test bioavailability of Fe and Zn in rice to humans.

The key issues involved in breeding are: (1) the agronomic advantage of rices with mineral-dense seeds, (2) the bioavailability of additional nutrients contained in the seed and (3) the nutritional implications of milling, parboiling, preparation and cooking, and the genotype interactions in these processes. Some advanced lines with good agronomic characteristics and high grain Fe content have been identified at IRRI. Although preliminary, these findings are good reasons to be optimistic about the breeding strategy for micronutrient dense rices.

Résumé...Abstract...Resumen

G. B. GREGORIO, D. SENADHIRA, T. HTUT, R. D. GRAHAM — **Amélioration de la teneur en fer et en zinc du riz pour l'alimentation humaine.**

La carence en oligoéléments touche plus de 3,7 milliards de personnes dans le monde, essentiellement des femmes et des enfants en raison de leurs besoins physiologiques. Les carences en fer et en zinc sont à l'origine de déficiences immunitaires, de complications au niveau de la grossesse et de l'accouchement, d'un développement difficile chez l'enfant, de capacités d'apprentissage et d'une productivité moindres. En 1992, l'Irri a commencé des recherches sur l'influence de certaines caractéristiques du sol sur la teneur en fer des céréales, recherches qui ont été étendues au zinc en 1995 en collaboration avec l'université d'Adélaïde, en Australie, concernant l'analyse des minéraux. Le criblage du germoplasme a révélé une importante variabilité génétique concernant le fer et le zinc du riz complet. Les teneurs en fer et en zinc des variétés couramment cultivées sont de l'ordre de 12 milligrammes par kilogramme et 25 milligrammes par kilogramme, respectivement ; certaines variétés traditionnelles présentent des valeurs doubles de celles-ci. L'étude génétique du caractère de teneur élevée en fer a montré l'importance des effets de dominance et d'additivité et l'incidence moindre de l'environnement. Par ailleurs, trois QTL de teneur élevée en fer ont été marqués sur des chromosomes de riz. Les teneurs élevées en fer et en zinc peuvent être combinées avec des caractères agronomiques améliorés. L'Irri a identifié du riz amélioré associant une bonne aptitude au rendement et des grains à concentration élevée en fer et en zinc dans les grains. Compte tenu de l'importance de la consommation de riz dans les pays en développement, des variétés à teneurs élevées en fer et en zinc pourraient avoir un impact significatif sur la nutrition et la santé des populations concernées.

Mots-clés : riz, génétique, sélection, carence en oligoéléments, anémie, fer, zinc.

G. B. GREGORIO, D. SENADHIRA, T. HTUT, R. D. GRAHAM — **Improving iron and zinc value of rice for human nutrition.**

Micronutrient deficiency 'hidden hunger' affects more than 3.7 billion people worldwide, predominantly women and children because of their physiological needs. Iron and zinc deficiencies cause impaired immune function, complications in pregnancy and childbirth, poor child growth and learning ability, and reduce labor productivity. In 1992, IRRI began to examine the effect of certain soil characteristics on the iron content in the grain and was expanded in 1995 to include zinc and collaboration with university of Adelaide, Australia for mineral analysis. Germplasm screening showed large genetic variation for Fe and Zn in brown rice. Common cultivars contain about $12mg\ kg^{-1}$ of iron and $25mg\ kg^{-1}$ Zn. Some traditional varieties have doubled these amounts. Genetics of high Fe trait showed the importance of additive and dominance gene action but less affected by environment. Moreover three QTLS for high Fe trait were tagged in rice chromosomes. High iron and zinc traits can be combined with improved agronomic traits. IRRI identified improved rice with good yielding ability and high concentration of iron and zinc in the grain. Because of the high consumption of rice in developing countries, the extra iron and zinc would have meaningful impact on human nutrition and health.

Key words : rice, genetics, breeding, micronutrient deficiency, anemia, iron, zinc.

G. B. GREGORIO, D. SENADHIRA, T. HTUT, R. D. GRAHAM — **Mejoramiento del contenido en hierro y zinc del arroz para la alimentación humana.**

La carencia en oligoelementos afecta a más de 3 700 millones de personas en el mundo, principalmente mujeres y niños debido a sus necesidades fisiológicas. Las carencias en hierro y zinc originan deficiencias inmunitarias, complicaciones en el embarazo y parto, desarrollo difícil de los niños, capacidad de aprendizaje y productividad inferiores. En 1992, el IRRI comenzó a investigar sobre la influencia de ciertas características del suelo en el contenido de hierro de los cereales, investigación que se amplió al zinc en 1995 en colaboración con la universidad de Adelaida (Australia) en lo concerniente al análisis de minerales. El cribado de germoplasma reveló una importante variabilidad genética del hierro y el zinc del arroz completo. Los contenidos en hierro y zinc de las variedades más cultivadas son de unos 12 miligramos por kilogramo y 25 miligramos por kilogramo, respectivamente; algunas variedades tradicionales tienen valores dos veces más altos que éstos. El estudio genético del carácter de alto contenido en hierro mostró la importancia de los efectos de dominancia y aditividad y la menor influencia del medio ambiente. Por otro lado, tres loci de expresión cuantitativa (QTL) con un contenido alto en hierro fueron marcados en cromosomas de arroz. Los contenidos altos en hierro y zinc pueden combinarse con caracteres agronómicos mejorados. El IRRI ha identificado un arroz mejorado asociando en unas semillas una buena aptitud para el rendimiento y semillas con una alta concentración de hierro y zinc. Teniendo en cuenta la importancia del consumo del arroz en los países en desarrollo, las variedades con alto contenido en hierro y zinc podrían tener un impacto significativo en la nutrición y la salud de las poblaciones afectadas.

Palabras clave: arroz, genética, selección, carencia en oligoelementos, anemia, hierro, zinc.