CHAPTER 3

EFFICACY OF IRON-BIOFORTIFIED CROPS

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ABSTRACT

Biofortification aims to increase the content of micronutrients in staple crops without sacrificing agronomic yield, making the new varieties attractive to farmers. Food staples that provide a major energy supply in low- and middle-income populations are the primary focus. The low genetic variability of iron in the germplasm of most cereal grains is a major obstacle on the path towards nutritional impact with these crops, which is solvable only by turning to transgenic approaches. However, biofortified varieties of common beans and pearl millet have been developed successfully and made available with iron contents as high as 100 mg/kg and 80 mg/kg, respectively, two to five times greater than the levels in the regular varieties. This brief review summarizes the research to date on the bioavailability and efficacy of iron-biofortified crops, highlights their potential and limitations, and discusses the way forward with multiple biofortified crop approaches suitable for diverse cultures and socio-economic milieu. Like post-harvest iron fortification, these biofortified combinations might provide enough iron to meet the additional iron needs of many iron deficient women and children that are not covered at present by their traditional diets.

Key words: Biofortification, Iron, Beans, Pearl millet, Rice, Polyphenols, Phytic acid, Anemia, Efficacy, Nutrition-Agriculture linkages

BIOFORTIFICATION OF STAPLE CROPS WITH IRON

The staple crops which have shown the most potential to increase dietary iron intake through traditional or selective plant breeding are common beans, pearl millet, cowpea, chickpea, pigeon pea, and lentils. Plant breeders have successfully developed varieties of these common staples with iron content two to five times higher than typical commercial varieties. Even considering the low iron bioavailability from these staples due to their high phytic acid (PA) content, it has been estimated that the higher iron content in these biofortified pulses and pearl millet can provide an additional 20 to 30% of the estimated average iron requirement for non-pregnant, non-lactating women of reproductive age, and children three to six years of age who consume these as staples [1]. Careful testing of these varieties is underway so that they can ultimately be introduced into food systems with claims of nutritional superiority and agronomic competitiveness.

Before new biofortified varieties are released to farmers, their ability to improve iron status in at-risk populations must be demonstrated initially through well-designed and implemented, randomized controlled efficacy trials, and ideally also under market conditions *via* effectiveness studies. Until now, only three iron-biofortified staple foods, rice (in the Philippines), beans (in Rwanda), and pearl millet (in India), have been tested for nutritional efficacy, and two of these (biofortified beans and pearl millet) have been tested for iron bioavailability. Given the relatively short time that biofortification has been pursued as an intervention strategy, no effectiveness trials have yet been completed for iron-biofortified crops.

Bioavailability of Iron-biofortified Staple Foods: Evidence to Date

Beans: Beans are an important part of the diet for more than 300 million people and are particularly important in regions of Africa and Central and South America. Iron-biofortified beans contain approximately double the iron content of conventional beans, and iron bioavailability has been evaluated in a series of stable isotope absorption studies, mainly in Rwandese women with low iron status. The biofortified beans were provided as part of a composite meal using a multiple meal design, which has been shown to better reflect real-life iron bioavailability [2]. In the multiple meal studies, iron bioavailability from biofortified beans consumed with potatoes or rice was modest, ranging from 3.8% to 7.3% [3, 4, 5]. However, in these studies, the total amount of iron absorbed from biofortified beans in these studies per woman per day ranged from 234 to 431 µg, and represented up to 30% of the physiologic requirement for non-pregnant non-lactating women of reproductive age [6].

A major finding from these studies was that PA concentrations increased as the iron content of biofortified beans increased, and that the higher PA concentrations decreased fractional iron absorption so that the additional amount of iron absorbed from the biofortified beans was lower than expected. The overriding influence of PA can be seen by comparing iron absorption from biofortified beans to conventional beans over three different studies [3, 4, 5]. When the PA concentration of biofortified beans was 2.0 g/kg, 3.4 g/kg, or 5.4 g/kg higher than the control beans, the additional iron absorbed from the biofortified beans was 80% [5], 19% [4], and 0% [3] higher than in the control beans. Further evidence of the key role of PA in iron absorption from beans comes from the

study in Rwandese women, in which iron biofortified (88 mg Fe/kg; 13.20 g PA/kg) and control (54 mg Fe/kg; 9.8 g PA/kg) bean meals were provided to women with low iron status, in a multiple meal design [4]. The biofortified bean meals and the control bean meals contained either their native PA concentrations or were almost completely dephytinized. At normal PA concentrations, fractional iron absorption from the biofortified beans (7.1%) was lower than for the control beans (9.2%), and the total amount of iron absorbed from the iron-biofortified beans was only slightly higher (19%) compared to the control beans. However, dephytinization increased fractional iron absorption and, after 95% dephytinization, iron absorption from both beans increased to about 13% and the total amount of iron absorbed from the biofortified bean was 51% higher than from control beans.

A potential explanation for the results from Petry *et al*. [4] is that some of the additional iron bred into the beans is stored as a non-bioavailable form of iron bound to PA. This is consistent with findings from Hoppler *et al.* in their study examining iron speciation in beans [7]. The authors reported that the amount of iron stored as ferritin in beans was relatively constant (13-35%) and increased only slightly with increasing iron concentrations. However, as iron concentrations increased, so did the PA concentrations, indicating that the extra iron might be less bioavailable and bound to PA. It can thus be hypothesized that increasing iron content in beans without simultaneously increasing PA would improve iron bioavailability from biofortified beans. Based on these findings, low PA beans (with 90% less PA) were developed. The usefulness of the low PA varieties, however, is still uncertain: although iron absorption from low PA biofortified beans was 50 to 60% higher compared to the parent beans [8], a recent multiple meal study reported that low PA beans do not provide more bioavailable iron than biofortified beans. Further, the low PA beans administered in the study had poor cooking quality and hemagglutinin residues (PHA-L) in the beans caused gastrointestinal problems in 95% of the participants [5]. Moreover, further improvements in cooking characteristics and digestibility of iron-biofortified low PA beans are needed before they can be evaluated in efficacy feeding trials and effectiveness studies.

Some polyphenol compounds, present in considerable amounts in the hulls of colored bean varieties, are also potential inhibitors of iron absorption [9, 10, 11, 3]. However, although bean polyphenols have been shown to be inhibitory in the absence of PA, their inhibitory effects are less evident in the presence of PA. When bean polyphenols from bean hulls were added to a bread meal (zero PA) provided to Swiss female university students, 50 mg and 200 mg of bean polyphenols decreased iron absorption by 14% and 45%, respectively [12]. In a subsequent double meal study in Rwandese university students iron absorption from a high polyphenol bean was compared to iron absorption from a low polyphenol bean, with similar PA concentration (~400mg). Although the high polyphenol bean meal contained 200 mg more polyphenols, iron absorption was only 27% lower than from the low polyphenol bean meal and this difference was no longer observed when rice and potatoes were provided with the beans in a multiple meal design [4]. A limited-to-negligible influence of bean polyphenols even at low PA levels on iron absorption was also indicated in the aforementioned low PA bean study [8]. In this study, the colored low PA bean (high polyphenols, low PA) had a higher iron absorption than the white low PA bean (low polyphenols, low PA), indicating that other bean compounds

can overrule the inhibiting effect of bean polyphenols It is not clear, therefore, whether high iron, white, low PA beans would be the best option for the iron biofortification of beans or whether beans should be bred primarily for low phytic acid, with less emphasis placed on their color. The low PA bean study mentioned above [8] indicates the latter option as the highest absorption occurred with a colored low PA bean high in polyphenols. Nevertheless, it should be noted that there is a large heterogeneity within polyphenol compounds [13], some bean polyphenols may decrease iron absorption more than others, different colored beans have different polyphenol profiles, and color plays an important role in consumer preference in low and middle income countries.

Pearl millet: Pearl millet is widely consumed in certain regions of India, particularly in Gujarat, Rajasthan, Maharashtra, and Karnataka, and also in populations living in the arid and semi-arid regions of western and central Africa [14, 15]. Iron-biofortified pearl millet has been bred to contain approximately three times higher iron than conventional pearl millet. Iron in biofortified pearl millet has reached almost 80 mg/kg, and the additional iron has been shown to provide important amounts of bioavailable iron in two stable isotope absorption studies. The first of these studies was conducted in iron-deficient children aged two years from Karnataka, India [16]. In this study, three different test meals were fed to children on two consecutive days. The meals were made from regular or biofortified pearl millet and provided approximately 60 g of pearl millet flour in the form of a sweetened porridge, savory porridge, or flatbread. In the control group (n=18), the three test meals were made from conventional pearl millet and provided 4.1 mg of dietary iron per day. In the test group (n=19), the test meals were made from iron-biofortified pearl millet and provided 7.7 mg of dietary iron per day. While mean fractional iron absorption (6-9%) did not differ between the two groups, the iron-biofortified intervention group had significantly higher total iron absorbed per day compared to the control group (0.7 mg vs. 0.2 mg). These results indicate that, in contrast to conventional pearl millet, the amount of iron absorbed from iron-biofortified pearl millet, when consumed in quantities reported in this study, can meet the estimated physiological iron requirements for children in this age group.

The second study was conducted in 20 Beninese women with marginal iron status (plasma ferritin <25.0 µg/L) [17]. This study used a cross-over design in which each woman acted as her own control and consumed three different test meals: regular commercial pearl millet, iron-biofortified pearl millet, or regular commercial pearl millet fortified with iron post-harvest. The composite test meals consisted of a traditionally prepared Beninese pearl millet paste (60 g flour/meal) accompanied by a leafy vegetable sauce or an okra sauce, and each test meal was administered for five consecutive days (two meals/day). Mean fractional iron absorption (7.5%) was identical for meals containing the regular commercial or iron-biofortified pearl millets. The amount of total iron absorbed per day from iron-biofortified pearl millet meals was two-fold higher than the regular commercial pearl millet meals (1.13 mg vs. 0.53 mg). These results indicate that women of reproductive age from Northern Benin with a daily per capita consumption of approximately 160 g pearl millet [18] could meet more than 70% of their daily physiological iron requirements (1.46 mg/day) [19] by consuming the iron-biofortified pearl millet variety. The equivalent amount of the regular commercial pearl millet used in the study would only provide approximately 20% of their iron requirements. In this

study, mean fractional iron absorption from the post-harvest iron fortified regular pearl millet (10.4%) was significantly higher than that from the iron biofortified pearl millet, resulting in greater total iron absorption from the post-harvest iron-fortified millet meals due to a lower PA to iron molar ratio in the post-harvest fortified millet. This suggests that breeding for an iron-biofortified pearl millet variety with lower PA concentrations would further increase the levels of bioavailable iron in iron-biofortified pearl millet. Findings from the studies in India and Benin suggest that biofortification of pearl millet could be a very promising approach for increasing the bioavailable iron in the diet of remote millet-consuming populations with limited access to conventional post-harvest iron-fortified foods.

Rice: No iron bioavailability studies have been made with iron biofortified rice varieties developed through plant breeding programs; however, a serendipitously discovered variety of rice with modestly high iron concentration (9.8mg/kg) was examined in an efficacy feeding trial in the Philippines [20]. An estimated iron absorption value from the rice provided in the Philippine trial can be obtained from stable isotope studies reported by Thankachan *et al.* [21] which were performed with rice-based meals in India using non-biofortified rice with a naturally high similar iron concentration (6.7 mg/kg). In this study, a single rice meal was fed to two groups of Indian women who had normal iron status or who had iron-deficiency anemia (IDA). The rice was not biofortified. The composite meal was comprised of rice (60g), tomato purée, oil, turmeric and chili powder, and 3mg iron was added as labeled ferrous sulfate. Mean iron absorption was 6.3% in women with normal iron status and 18.9% in women with IDA. The International Nutritional Anemia Consultative Group (INACG) reviewed iron absorption from rice meals as measured in human subjects using radioisotopes [22]. All meals contained polished rice, vegetables and spices. Mean iron absorption from five studies containing 221 participants was 6.5% after adjusting to 40% reference dose absorption. This mean fractional iron absorption value from rice meals is very close to the estimated absorption value calculated by Beard *et al.* [23] in a review of six algorithms applied to the diets from the Philippine efficacy study of high iron rice.

Efficacy of Iron-Biofortified Staple Foods: Evidence to Date
An essential step to demonstrating the efficacy of iron-biofortification to improve nutritional status is the randomized, controlled feeding trial. These feeding trials are conducted under carefully controlled experimental conditions in iron-deficient human participants. They generally evaluate the effects of consuming iron-biofortified versus control foods on iron status biomarkers over four to six months of monitored daily food intakes.

Beans: In an efficacy feeding trial of iron-biofortified beans, 195 iron-depleted (serum ferritin <20.0 µg/L) Rwandese university women were randomized to consume iron-biofortified beans or control beans daily for 18 weeks [24]. At baseline, 37% were anemic (Hb<120 g/L), 86% were iron deficient (serum ferritin <15.0 µg/L), and 55% had negative body iron stores. The iron-biofortified bean group consumed 12.9 mg of iron per day from beans, compared to 7.6 mg per day from control beans. This accounted for 63% and 48% of total daily iron ingested, respectively. Women in the iron-biofortified group had significantly greater increases in hemoglobin, log serum ferritin, and total

body iron compared to controls after consuming approximately 150 g of beans (dry wt) daily for 18 weeks (Table 3.2). The plausibility of these findings was supported by an analysis that showed that there was a significant 0.7 g/L increase in hemoglobin and a 0.06 mg/kg increase in total body iron for every 100 mg of iron consumed from beans over the 18 weeks of the feeding trial. Physical performance and physical activity were evaluated with the same methods used in the pearl millet trial, to examine functional effects of the iron-biofortification intervention [25]. VO_2max decreased in both groups over the 18 weeks of intervention; however, women in the iron-biofortified group had a significantly slower rate of decline, suggesting that the additional iron from biofortified beans attenuated the decline in physical fitness that occurred during the academic semester. This decline in physical fitness was consistent with the significant increase in time spent in sedentary activities during weekends when students had choices of how to spend their free time. However, women in the iron-biofortified group spent 50% less time in sedentary activity (102 vs. 201 minutes, p = 0.04). Together, the results of the Rwanda bean and India pearl millet studies provide the strongest evidence to date for the efficacy of iron-biofortification, and support the continuation of research to examine the effectiveness of programs to promote the planting, marketing, and consumption of iron-biofortified crops to populations where these crops are major staple foods.

Pearl millet: Efficacy of iron-biofortified pearl millet was evaluated in secondary school children from western Maharashtra, India (Table 3.1) [26]. This randomized, controlled feeding trial was conducted among 246 boys and girls (12-16 years) attending a boarding school for children from low-income rural families. At baseline, 28% were anemic (Hb<120 g/L), 43% were iron deficient (serum ferritin <15.0 µg/L), and 21% had negative total body iron stores. Iron-biofortified or conventional pearl millet in the form of a flatbread (*bhakri*) was provided to the children twice daily for six months with assessment of iron status at baseline and after four and six months of feeding. During the first four months, the iron-biofortified group consumed 19.6 mg of iron per day, while the control group consumed 5.2 mg per day. After four months, the change in serum ferritin (median change: 5.7 µg/L vs. 1.2 µg/L) and total body iron (median change: 0.8 mg/kg vs. 0.0 mg/kg) concentrations was significantly higher in the children consuming iron-biofortified pearl millet flatbread, compared to conventional pearl millet. Also, significant from a public health perspective, among children who were iron-deficient at baseline (serum ferritin <15.0 µg/L), those who consumed biofortified pearl millet were 64% more likely to resolve their iron deficiency by six months compared to the control group (RR: 1.64, 95% CI: 1.07-2.49, p = 0.02). In order to evaluate the potential benefits of iron-biofortification beyond changes in iron status, an analysis of the effects of the intervention on physical performance and physical activity was conducted. Accelerometers were used to monitor physical activity over one week at baseline and at end line; preliminary analyses demonstrated that individuals in the iron-biofortified group had significantly decreased time spent in sedentary behaviors and increased light and moderate-to-vigorous activity, compared to the control pearl millet group. There was also a significant positive relationship between change in hemoglobin and change in physical fitness measured as VO_2max over the six months of the intervention in both boys and girls. This study demonstrated that iron-biofortified pearl millet improves iron status in children and has the potential to improve functional measures related to iron status. Further research is needed to determine the effectiveness of iron-biofortified pearl

millet when introduced to the general population in areas where it is grown and consumed as a staple food.

Rice: The first efficacy study demonstrated a "proof-of-concept" when consumption of a rice variety with modestly higher iron content than the regular commercial rice for nine months increased serum ferritin concentrations and total body iron in non-anemic Filipina religious sisters (Table 3.1) [20]. Hemoglobin concentrations did not change in this study, presumably due in part to the multiple causes of anemia. However, in women whose hemoglobin was normal but who had reduced body iron stores at baseline, there was a significant increase in serum ferritin concentrations and total body iron. Also, as a test of the plausibility of these findings, there was a significant positive association between the amount of iron consumed from biofortified rice and the increase in total body iron over the nine months of feeding. These findings suggest that even at low doses of iron, as seen in iron-biofortified versus control rice, one can observe improvements in iron status in deficient individuals if they consume rice in sufficiently high quantities for a long period of time.

The narrow genetic variability of iron in rice, coupled with the large amount of iron removed during the polishing process rules out selective rice breeding as a cost-effective approach to develop high iron rice varieties with potential to improve population iron status. Genetic engineering offers more viable alternatives for biofortification of cereals, including rice [27]. Transgenic modification has increased iron and zinc content of the rice grains to levels that achieve dietary nutrient targets without penalizing the crop's yield [28].

Variation in Iron Concentrations of Staple Foods Tested
The genetic potential for biofortifying staple foods with iron varies considerably, with cereals in general having a lower potential than pulses. The genetic variation in iron content of brown rice varieties has been reported as 7 to 23 mg/kg compared to 22 to 56 mg/kg in whole wheat [29], although much of the iron is in the grain coat and is removed by polishing or milling. The native iron concentrations in polished rice as consumed are, therefore, much lower than in beans or pearl millet as consumed. In the aforementioned efficacy studies, there was thus a natural variance in iron concentrations: iron concentrations in the consumed portion of the high iron staple relative to the control staple was much lower in rice (10 vs. 2 mg/kg) compared to beans (86 vs. 51 mg/kg) or pearl millet (86 vs. 22 mg/kg). The amount of additional iron supplied per kg of pearl millet (64 mg) was seven times higher than that supplied by rice (8 mg/100 g), although daily consumption of rice was twice as high. The daily intake of relatively large quantities of the staple food with high iron content - Rwandan beans (150 g/d dry wt) and Indian pearl millet (250 g/d as flour), respectively - contributed more than 60% of the median absorbed iron requirement for non-pregnant, non-lactating women and more than 100% of the median requirement for school-age children [26].

CONTINUED RESEARCH AND CHALLENGES

Further analysis will focus on the effects of improving iron status on physical and cognitive performance in order to determine costs versus benefits of iron biofortification. Also, HarvestPlus and its partners plan to examine the ability of iron-biofortified beans and pearl millet to reduce the prevalence of iron deficiency in at-risk populations through effectiveness studies during the next five years. Challenges remain to breed for additional crops and varieties of the current crops with higher iron content and bioavailability that approach the levels observed in the Rwanda and India studies. For example, recent biofortified varieties of lentils have nearly twice the iron content (114 mg/kg) compared to the average of commercial varieties (65 mg/kg) [30, 31]. Lentils are widely consumed globally, particularly in South Asia, where they are a major source of protein in a predominantly vegetarian diet. India is one of the largest consumers of pulses, with approximately 13% of per capita total protein intake from beans and lentils. Forty percent (40%) of the global area for lentil production is in India out of a total of 3.6 million hectares [32]. Bioavailability studies and efficacy trials with lentils and other similar crops are the logical next steps.

Additionally, research focusing on biologically vulnerable population groups will be undertaken on the efficacy of interventions that combine several crops, such as biofortified cereals and pulses. Together, the biofortified staple crops have the potential to provide all of the iron that is lacking in the diet and, like post-harvest fortification, should be able to prevent iron deficiency in at-risk groups.

Certainly, the impact of biofortified crops on population health will depend on the size of the market share that these crops occupy, determined to a large extent by the adoption by farmers and acceptance by consumers in general. In this respect, iron-biofortified staple crops have no visible traits that differentiate them from their conventional counterparts, their agronomic yields are superior or comparable to conventional varieties, and the culinary characteristics should be indistinguishable from those of the varieties they are intended to replace. Therefore, during the scaling up of these crops, monitoring systems should be in place to document the adoption and consumption of the biofortified varieties, as well as the persistence of the high-iron trait over time.

CONCLUSIONS

The common bean is a good vehicle for iron biofortification. When regularly consumed, biofortified beans can improve iron status and physical performance. Iron bioavailability and biological impact in beans could be increased by lowering phytic acid concentrations through conventional plant breeding without sacrificing agronomic traits.

Iron-biofortified pearl millet is also an excellent source of bioavailable iron, and can significantly improve iron status and reduce the prevalence of iron deficiency in high-risk groups, such as women of reproductive age, young children, and school-aged children. Efforts to integrate this crop into public feeding programs for populations living in arid and semi-arid regions of South Asia and West Africa should be implemented.

Introduction of multiple iron-biofortified crops into the same population through a combination of cereals (for example, wheat, rice, or maize) and pulses that have been successfully biofortified (for example, common bean, cowpea, chickpea, lentils, or pigeon pea) could successfully provide all of the iron that is lacking in the diet and improve the iron status and health of populations. One trial assessing the efficacy of multiple biofortified crops is currently being planned for implementation in India, and similar approaches globally could potentially provide sustainable agriculture-based solutions for both macro- and micro-nutrient malnutrition, particularly in vulnerable populations.

Table 3.1: Comparison of Results from Three Iron Biofortification Efficacy Studies

Crop (Location)	Rice (Philippines)		Beans (Rwanda)		Pearl millet (India)	
Participants	*Adult females [a]*		*Adult females [b]*		*Youth [c]*	
Experimental group	**High iron**	**Control**	**High iron**	**Control**	**High iron**	**Control**
N	**69**	**69**	**94**	**101**	**98**	**95**
Hemoglobin (g/L)	1.1	0.9	2.8 [d]	-1.0	1.3	0.9
Ferritin (µg/L)	1.1 [d]	-4.27	5.50 [d]	3.60	5.7 [d]	1.2
Transferrin receptor (mg/L)	0.35	-0.15	-0.10	-0.20	0.21	0.33
Body iron (mg/kg)	0.63 [d]	-0.25	1.40 [d]	0.90	0.83 [d]	0.02
Sample description	*Non-anemic (Hb≥120 g/L) at baseline*		*Low ferritin (<20.0 µg/L) at baseline*		*Low ferritin (<15.0 µg/L) at baseline*	

Values are change in iron status indicator from baseline to end line

[a] Mean values from Haas *et al.* [20]

[b] Mean values from Haas *et al.* [24]

[c] Males and females, median values from Finkelstein *et al.* [26]

[d] Significant difference between iron-biofortified and control groups, $p<0.05$

Table 3.2: Iron Intake from Iron-Biofortified Staple Food

Crop (Location)	Rice (Philippines)		Beans (Rwanda)		Pearl millet (India)	
Experimental group	**High iron**	**Control**	**High iron**	**Control**	**High iron**	**Control**
Iron content						
Iron concentration (mg/kg-dry)	10	2	86	51	87	30
Iron intake from staple (mg/d)	1.8	0.4	13.5	8.0	17.6	5.7
Percent of total dietary iron	18	5	64	46	90	81
Iron intake relative to requirements						
Percent iron absorption [a]	7.3	7.3	7.1	9.2	7.4	7.5
Absorbable iron (µg/d)	134	30	959	737	1300	428
Median Iron requirement[b] (µg/d)	1460		1460		1170[c]	
% daily median requirement from staple	9	2	66	51	111	36.5

[a] Iron absorption estimates: Philippines rice based on calculations by Beard *et al.* [23] using algorithm by Hallberg & Hulthen (2000); Rwanda beans based on Petry *et al.* [8]; Pearl millet from Cercamondi *et al.* [17]

[b] Median total absolute requirements of absorbed iron (µg/day) from FAO/WHO, Human Vitamin and Mineral Requirements [19]

[c] Median requirement for 11-14 year old males (from FAO/WHO) [19]

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