Biofortified pearl millet cultivars to fight iron and zinc deficiencies in India

Inadequate intake of energy-providing organic macronutrients (largely carbohydrate, followed by protein and fat, in that order), leads to under nutrition, with a consequent feeling of hunger. The result of chronic hunger, reflected in such apparent physical manifestations as underweight (being too thin for one's age), wasting (being too thin for one's height) and stunting (being too short for one's age), has long been debated as a food security issue in various global fora. Unlike the macronutrients mentioned above, which are consumed in larger quantities for proper growth and development, there are several nutrients, called micronutrients, which are needed in trace amounts, but they play vital roles in various physiological functions. The deficiencies of these micronutrients, do not lead to obvious hunger effects, nor do they lead to common physical manifestations as those arising from the deficiencies of macronutrients. Thus, these micronutrient deficiencies are also termed as hidden hunger. Deficiencies of some micronutrients, of course, are more widespread, and their adverse health consequences more severe. For instance, iron (Fe) and zinc (Zn) deficiencies have been reported to be most widespread, affecting more than two billion people worldwide, mostly in the low- and middle-income countries. Despite its impressive economic growth, India ranks poorly in this respect with alarmingly high levels of deficiencies of these two micronutrients, especially in the rural populations and urban poor. About 80% of the pregnant women, 52% of the non-pregnant women, and 74% of the children in the 6-35 months age group in India suffer from iron deficiency-induced anaemia. About 52% of the children below 5 years are zinc deficient. The Fe deficiency causes varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, and pregnancy complications (e.g., babies with low birth weight and poor learning capacity). Iron deficiency-induced severe anaemia is a direct cause of maternal and child mortality. Zinc deficiency in children makes them vulnerable to diarrhoea, pneumonia, mortality, and causes stunting. Such adverse health effects of the deficiencies of these micronutrients leads to huge economic losses. A recent study showed that actions to solve iron and zinc deficiencies in China would cost less than 0.3% of the GDP, but failure to do so could result in a loss of 2-3% of the GDP. No such cost-benefit analysis has been done for India, but an economic loss due to combined deficiencies of iron, zinc, iodine and vitamin A has been shown to be of the order 2.5% of the GDP. Pharmaceutical approach of supplementation, industrial approach of food fortification, and agricultural approaches of dietary diversification and biofortification have been advocated as some of the strategies to address micronutrient deficiencies. Crop biofortification, which refers to the breeding of cultivars with higher levels of micronutrients, is increasingly being recognized as a cost-effective and sustainable approach. In case of iron and zinc, unlike Vitamin A, it has another advantage of unhindered and ready consumer acceptance as grains of biofortified cultivars with higher levels of iron and zinc are similar, in terms of appearance and taste, to those normally consumed.

Pearl millet, variously known as bajra, bajri, sajja and cumbu in different states of India, is a highly nutritious cereal grain. Grown on 8-9 million ha, it ranks third after rice and wheat, and is a major source of dietary energy and nutritional security. It has high levels of protein with better amino acid balance than other major cereals such as rice, wheat and maize. It also has high levels of fat content, dietary fibre, and several minerals, including iron and zinc. Studies at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, conducted in alliance with the HarvestPlus Biofortification Program of the Consultative Group on International Agricultural Research, and in partnerships with several public and private sector pearl millet research programs, have shown large variability in released and commercial cultivars, both for iron and zinc content. For instance, a multi-location trial of 18 open-pollinated varieties and 122 hybrids, jointly conducted by ICRISAT and All India Coordinated Pearl Millet Improvement Project, showed iron content varying from 42 to 67 mg/kg in varieties and from 31 to 61 mg/kg in hybrids. The zinc content varied from 37 to 52 mg/kg in varieties and from 32 to 52 mg/kg in hybrids. Clearly, all of these pearl millet cultivars had much higher iron content than the best rice varieties (less than 20 mg/kg). Many, but not all, had markedly higher iron content than the best wheat varieties (less than 45 mg/kg). Also, many had markedly higher zinc content than the best rice varieties (less than 30mg/kg), but only few had higher zinc content than the best wheat varieties (less than 45 mg/kg). Thus, when talking of pearl millet grains as a rich source of iron and zinc, as commonly assumed, there will be a need to talk in terms of specific cultivars. It is in this context that pearl millet biofortification program has been undertaken to breed biofortified cultivars with higher levels of iron and zinc content.

In pearl millet, there are two types of cultivars grown by farmers. These are open-pollinated varieties and hybrids, though the latter dominate the scene in India on account of their grain yield superiority and greater uniformity for plant and grain traits. In the variety trial, an ICRISAT-bred variety ICTP 8203, released in 1988 and under cultivation since then, had the highest iron content of 67 mg/kg, followed by another ICRISAT-bred variety ICMV 221 that, released in 1993 and under cultivation since 1995, had 61 mg/kg iron. While ICTP 8203 had also the highest level of zinc content of 52 mg/kg, and ICMV 221 ranked second with 45 mg/kg zinc content. In the hybrid trial, four hybrids viz., Ajeet 38, Proagro XL 51, PAC 903 and 86M86 had the highest iron content of 55-56 mg/kg and zinc content of 39-41 mg/kg.

Since iron deficiency is a more widespread and serious problem than zinc deficiency, and much larger variability has been observed for iron than for zinc content, research at ICRISAT has focused on genetic improvement of iron content, with zinc being improved as an associated trait, considering that both traits are highly significantly and positively correlated. Utilizing the large genetic variability for iron content observed within variety ICTP 8023, an improved version of it, selected for higher iron content, was developed. In multi-locational trials, jointly conducted by ICRISAT, Mahatma Phule Krishi Vidyapeeth and All India Coordinated Pearl Millet Improvement Project, the improved version had a mean iron content of 71 mg/kg (9% more than ICTP 8203), and 2.2 t/ha^{-1} of grain yield (11% more than ICTP 8203), with no changes in zinc content (40 mg/kg), seed size, flowering time and other traits. This new variety was released in 2014 as *Dhanashakti* for cultivation, especially in Peninsular India. Since *Dhanashakti* is an improved version of ICTP 8203 (a commercial variety already under cultivation), it was rapidly adopted by farmers, reaching 65,000 households in 2014, and is expected to completely replace ICTP 8203 on more than 200, 000 ha by 2017 or 2018. *Dhanashakti* and the four hybrids (Ajeet 38, Proagro XL 51, PAC 903 and 86M86) identified for high iron content were included in the Nutri-Farm Pilot Program of the Government of India, launched in 2014. Large genetic variability for iron and zinc content was also observed in another ICRISAT-bred variety ICMV 221, which was released in 1993. It is mainly cultivated in Tamil Nadu and parts of Maharashtra. Utilizing this variability, a higher version of it, designated as ICMV 221Fe11-2, has been developed, which has been found having 81 mg/kg iron (14% higher than ICMV 221), and 51 mg/kg zinc (11% higher than ICMV 221) in three years and two seasons of trials conducted at ICRISAT. This improved version also had 3.92 t/ha of grain yield, which, though marginally higher by 5%, was similar to that of ICMV 221. This improved version of ICMV 221 with higher iron and zinc content is yet to be widely tested in All India trials to obtain data on validated superiority margins of their iron and zinc content. In the meantime, its seed is available for producing truthfully labelled seed that can be marketed to farmers, as it had been done in case of *Dhanashakti*.

Hybrids are produced by crossing male-sterile lines (A-lines) as female parents with restorer lines (R-lines) as male parents. A-lines are maintained by crossing with their counterpart male fertile maintainers lines (B-lines) that are genetically identical to A-lines except for male sterility. ICRISAT analyzed a large number of B-lines and R-lines in its stock and identified those with high levels of iron and zinc for producing hybrids with high levels of these micronutrients. Two hybrids (ICMH 1201 and ICMH 1301), developed using different parental lines, with high iron an zinc content have now been tested in a large number of multi-location trials, while many others are at different stages of testing. Based on the performance over 48 field trials, ICMH 1201 had 75 mg/kg iron content (similar to the high-iron variety ICTP 8203) but had 3.6 t/ha grain yields (38% higher than ICTP 8203). ICMH 1201 flowered only 3 days later than ICTP 8203. ICMH 1301 tested in 32 trials had 77 mg/kg iron content (similar to ICTP 8203) and 3.3 t/ha grain yield (33% higher than ICTP 8203). It is significant to note that almost all the high-iron cultivars (both varieties and hybrids) had more than 40 mg kg of zinc content.

Compared to the most popular and highest-yielding hybrid 86 M 86, which also had the highest iron among the commercial hybrids, both biofortified hybrids ICMH 1201 and ICMH 1301 had 33-34% higher iron content. The biofortified hybrids had 18-20% less grain yield compared to 86 M 86, but these flowered 2-6 days earlier than 86M 86. Production of Truthfully labelled seed (TLS) of ICMH1201 was undertaken by Shaktivardhak Seed Company in 2014 for commercialization under its brand name Shakti-1201, and it was adopted by 35,000 farmers in 2015, mostly in Maharashtra and Rajasthan. Seed production of hybrid ICMH 1301 is yet to start. Several breeding lines and germplasm accessions with 90-100 mg/kg iron content and 70-80 mg/kg zinc content have been identified at ICRISAT. Much greater progress in breeding high-yielding biofortified hybrids is expected in the near future by utilizing parental lines that are being developed, using the high iron and high zinc genetic resources, through targeted breeding for these micronutrients.

Farmers adopting biofortified cultivars generally ask three questions. First, whether iron and zinc content of any cultivar will be same regardless of the environments where it has been grown. As in case of grain yield, iron and zinc content of any cultivar will vary from one environment to the other. ICTP 8203 has been most widely tested since it has been included as a test and check entry in multi-location trials over several years, producing 261 data points. Its average iron content of this variety in these trials was 69 mg/kg, and it varied from 40 to more than 100 mg/kg; and average zinc content was 44 mg/kg, which varied from 20 to 90 mg/kg. However, in 80% of the cases, iron content varied from 51 to 80 mg/kg and zinc content varied from 26 to 55 mg/kg, which is what most of the time farmers are likely to find. Second, whether iron and zinc content of any cultivar depends on the iron and zinc levels in the soils. Research shows that iron and zinc content in pearl millet grains does not depend on the levels of these micronutrients in the soil, so long the soils are not deficient for them. Third, whether grains produced in the environments with high grain yields will have any less iron and zinc content than those produced in the environments with low grain yields. Analysis of a large number of trials has shown that the iron and zinc contents in pearl millet grains are not associated with the grain source, whether produced in low or high-yielding environments. Nutritionists as well as consumers ask another question: whether the bioavailability of iron and zinc in grains produced from cultivars with high levels of these micronutrients will be any less than those produced from cultivars with low levels of these micronutrients. Three feeding trials including children below three years of age from Benin (Africa) and Karnataka (India) and 12–16 years age from Maharashtra in India, and non-pregnant non-lactating (NPNL) women in 17-35 year age group in Benin (Africa), have shown that the bioavailability of iron in pearl millet is 7.0-7.5%, regardless of their levels in grains. Thus, the consumption of whole grain products made from, say 240 g/day of variety *Dhanashakti*, and assuming even 7% bioavailability, would provide much more iron than daily requirement in men (0.84 mg), and meet 70% of the daily requirement in NPNL women (1.65 mg) and 42% of the daily requirement in pregnant women (2.8 mg). Above consumption rate will also provide 80% of the recommended daily allowance of 12 mg/day of zinc. In a similar way, biofortified variety ICMV 221 Fe 11-2, and biofortified hybrids ICMH 1201 and ICMH 1301 will also help fight iron and zinc deficiencies. Finally, in the context of prevailing reservations and controversies on GMO products, another question generally asked by the policy makers, and by some nutritionists and farmers alike, is whether biofortified pearl millet cultivars are GMO products. These are not. The biofortified pearl millet cultivars mentioned above and those under development are based on the utilization of natural genetic variability in pearl millet germplasm, the same way as those bred for grain yield and other traits.