

Review Article

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## A Review on Biofortification - To Improve Nutritional Quality of Cereals

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### ABSTRACT

Biofortification is a probable and cost-effective means of providing micronutrients to populations that may have limited access to diverse diets and other micronutrient intercession. Micronutrient deficiency, also known as ‘hidden hunger’ is one of the most important provocations facing humanity today. According to world health organization (WHO) report about 3 billion people worldwide suffer from micronutrients deficiency. In India, malnutrition problems like anaemia about more than 52% of women suffer, 44% of children below 5 years are underweight and 38% are stunted. Therefore, to overcome the malnutrition in human beings, improvement of crop quality by bio-fortification process for important crop plants through biotechnological applications that is sustainable solution. Target crops for increase micronutrient status like rice and maize for Fe, Zn, Pro-vitamin A and wheat, pearl millet, sorghum, small millets for Zn and Fe. Two approaches for bio-fortification *i.e.*, agronomic and genetic used to increase nutrients level in stable food crops. Bio-fortified crop varieties are developed by plant breeding using selective breeding and genetic modification. In wheat recreated synthetics, wild relatives and landraces are being used as progenitor for high Zn/Fe source and approaches are backcross, bulk scheme and wide-cross uses for development of fortified varieties. Pearl Millet, as a species, has higher levels of Fe and Zn densities than other major cereal crops many varieties developed in pearl millet. The development of the ‘golden rice’ proved that biofortified crop developed through genetic engineering. In maize QPM is a product of conventional plant breeding (*i.e.*, it is not genetically modified) it produces 70-100% more of lysine and tryptophan than the most modern varieties. Given the severity of mineral malnutrition in humans worldwide, biofortification of micronutrients, especially Fe and Zn, in cereals must be encouraged.

#### Keywords

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### Introduction

Biofortification is a Greek word “bios” means life and Latin word “fortificare” means make strong. Biofortification refers to increasing genetically the bioavailable mineral content of food crops (Brinch-Pederson *et al.*, 2007).

Anaemia is the most common human malnutrition, resulting from iron (Fe) deficiency and affecting 32.9% people worldwide meanwhile, zinc (Zn) deficiency affects 17% of the world’s population (Wessells *et al.*, 2012; Kassebaum *et al.*, 2014). In the twenty-first century, there is

strong trouble worldwide concerning the ability to produce nutritionally rich food because cereals are inheritably poor in essential micronutrients. Moreover, owing to grow a rapidly human population and industrialization; this situation may be further being composed of by the production of cereals in areas with low mineral phytoavailability (White and Broadley, 2009). Hence, malnutrition and poor health affect these people, who may suffer from blindness or stunting, and sometimes even face death. To overcome this “hidden hunger” through medical supplements and fortification have been pursued (Underwood, 2000). In fact, food fortification has a long history of use in industrialized countries and perceive on the addition of micronutrients to processed foods. However, food fortification tends to have a rapid but less sustainable impact, because various safety, technological and cost considerations may place restriction on such interventions (Allen *et al.*, 2006). By increasing the micronutrient content of energy-rich crops, micronutrient intakes among the poor can be increased, thereby leading to decreases in the prevalence of micronutrient deficiencies. It differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. Traditionally, vitamins and minerals have been provided to the masses through nutrient supplementation programs, but it falls short of the goals set by the international health organizations as the supplementation programs rely on external funding that is not guaranteed to be available from year to year. Other limitations are purchasing power of poor people, their access to markets and health-care systems, and lack of awareness regarding the long-term health benefits of these nutrient supplements. Hence, biofortification of different crop varieties offers a sustainable and long-term solution in

providing micronutrients-rich crops to people. Furthermore, biofortified crops with increased bio available concentrations of essential micronutrients are deployed to consumers through traditional practices used by agriculture and food trade which therefore provides a feasible way of reaching undernourished and low income group families with limited access to diverse diets, supplements and fortified foods.

### **Malnutrition data**

Malnutrition form an important global health issue, affecting key development outcomes including poor physical and mental development in children, vulnerability of disease, mental retardation, blindness and general losses in productivity and potential. The World Health Organization (WHO) estimate 3 billion people worldwide suffer micronutrients deficiency, hunger and undernourishment, which is defined as dietary energy intakes below the minimum levels necessary to achieve and maintain a healthy weight, over nourishment, which is defined as dietary energy intake which exceeds requirements for maintenance of a healthy bodyweight, micronutrient deficiencies, which is defined as a lack of essential vitamins and minerals required in small amounts by the body for proper growth and development.

Zinc deficiency can have a number of negative health consequences, affecting the central nervous, immune, reproductive, and skeletal systems. Zinc is an essential nutrient for growth and recovery deficiencies can therefore stunt growth, increase susceptibility to disease and infection, increase recovery time, or in some cases, impair recovery, reduce mental capacity, and increase the prevalence of maternal, new born and child complications higher morbidity and mortality in mothers (Prasad *et al.*, 2013). Generally,

recommended dietary allowance for Zn is around 15 mg per day (National Research Council 1989). About 2.5 billion world population suffer from Zinc deficiency (Oliver and Gregory, 2015).

About 400 million people have vitamin A Deficiency, 1.6 billion populations suffer from Iron deficiency globally and anaemia affects more people than any other health problem. Anaemia has important implications for general productivity and development, reducing the work capacity of individuals by up to 20 percent (Welch and Graham 2015).

India is one of the countries having problem of malnutrition more than 52% of women, 44% of children below 5 years are underweight and 38% are stunted. As per India state hunger index (ISHI), all the states are with serious to alarming, in which Madhya Pradesh most alarming. The World Bank estimates that India is one of the highest ranking countries in the world for the number of children suffering from malnutrition. As per the Global Hunger Index, 2018 (GHI), published on October 10, 2018, the level of hunger and under nutrition worldwide fell to 20.9, down from 29.2 in the year 2000. As per the index, India was ranked 103rd out of 119 qualifying countries (World Bank).

### **Importance of crop biofortification**

Biofortification on staple foods could be a more sustainable approach, also suitable for remote regions. Biofortified crops can potentially provide iron, zinc and vitamin A to people with limited connection to commercial markets (Mayer *et al.*, 2008).

The suitability of biofortification for the poor, who mainly eat staples that are not commercially processed and sold but depend on household produced crops, is the most notable advantage (Tanumihardjo *et al.*,

2008). Thus, biofortification has the potential to reduce the preponderance of micronutrient deficiencies and lower the number of people requiring intercession such as fortification and supplementation to increment of nutritional quality in diets (Bouis and Welch, 2010). In contrast to dietary diversification, no behavioural changes are required from the consumers. However, the target crop has to be select carefully, following the dietary patterns of the consumers (Qaim *et al.*, 2007).

The acceptance of the newly developed crop by the targeted population is a major issue for biofortification to be successful. To be accepted and cultivated by the farmers, the new variety must exhibit a high yield and resistance against disease and pests, in short be profitable. Characteristics of the newly developed plant such as yield, micronutrient concentration and disease and pests resistance should be stable over different environments and climatic zones. Moreover, the level of micronutrients must have the potential to significantly improve human health and ensure an adequate mineral bioavailability (Nestel *et al.*, 2006; Bouis and Welch, 2010).

Aims of micronutrient levels for biofortified crops are amount of Fe, Zn and Vitamin A required, breeding target is combination of baseline and increment that is set to meet the specific dietary require of women and children, based on current consumption patterns. Biofortification conduct a solution in the hands of farmers, combining the micronutrient trait with other agronomic and consumption traits that farmers prefer.

After achieve the household's food needs, surplus biofortified crops make their manner into rural and urban retail outlets. Genotypic differences in keeping and concentrations of compounds that inhibit or enhance micronutrient bioavailability are considered.

## **Pathway for biofortification**

For biofortification to accomplish some factors have to be considered, beginning with the identification of the targeted population and finishing with the improvement of the nutritional status of this population. The "impact pathway for fortified crops" as suggested by Harvest Plus is divided into the following three stages: (1) Discovery, (2) Development and (3) Dissemination of the newly developed plant variety.

### **Discovery**

The first stage starts with the identification of targeted populations, for that the biofortified crop should be developed. The targeted populations are not sometimes restricted to only one country and scatter over effects to other countries or areas have to be taken into consideration. The selection should be done with regard to the prosperity of micronutrient deficiencies, the production and consumption of the targeted crop and the proportion and importance of self and locally produced plants (Ortiz-Monasterio *et al.*, 2007). Incomplete or missing data from national health surveys complicates the recognition of populations effected by micronutrient deficiencies in many cases (Zapata-Caldas *et al.*, 2009). Moreover, to correctly approach the consumption of the targeted crop in a population, the availability of representative and dependable dietary intake data has to be implicit (Hotz and McClafferty, 2007).

### **Development**

In the development stage mainly focuses on the development and testing of biofortified crops. An overview of crops directly undergoing the biofortification process the identification of promising lines by breeders is followed by mapping of genotypic differences. New varieties are developed by

crossing promising lines and selecting those with favourable characteristics over many generations (Grusak and Cakmak, 2004). The performances of the newly developed biofortified varieties are then tested over different environments, to evaluate genetic and environment (GxE) interactions. It is suggested that the variability of micronutrients in the germplasm depends on the genotype, the environment and GxE interaction, but the impact of the various factors differs between the micronutrients and crops. Formerly the desired variety is developed; the consumer acceptance in terms of taste, look and cooking quality is evaluated (Khoshgofarmanesh *et al.*, 2010) (Fig. 1).

### **Dissemination**

In dissemination step the performance of the new variety in terms of micronutrient retentively is tested, followed by the examination of micronutrient bioavailability in humans. If results from these preliminary tests are superior, the performance of the new variety is investigated in an efficacy trial in human subjects, which is usually execute as a follow up study to an absorption study. Efficacy trials aim at investigation whether an intervention produces the expected results under ideal conditions. This is why efficacy trials are very closely monitored, well-controlled and conducted by highly trained specialists (Hallfors *et al.*, 2006). They require an exacting research design including a specified and standardized treatment within standardized settings (Flay *et al.*, 2005). Subjects often belong to a narrowly defined, homogenous group, who should be part of the targeted population. It has to be that the participants accept and fulfil with the treatment (Glasgow *et al.*, 2003).

If the outcome of the efficacy trial is positive, in this step, the impact of the new variety on human health status is evaluated in an

effectiveness trial. In this type of study, the beneficial effects of the crop are tested under conditions assume reality (Gartlehner *et al.*, 2006). This is generally done among a broadly defined population which is representative for the targeted population.

The food is prepared and eaten in traditional ways within the usual household environment. Standardization only takes places in terms of accessible and availability of the biofortified crop among the population. To be certain that a crop is ready for dissemination, an effectiveness trial should be implemented since the outcome might be different from the efficacy trial and hidden difficulties, such as lack of proper implementation or weak acceptance might be uncovered. It is arguable whether efficacy trials earlier to effectiveness studies are necessary if the latter meet the standards of efficacy trials.

### **Approaches for biofortification**

Biofortification is a strategy that involves the use of plant breeding or agronomic practices to increase the density of essential nutrients in the edible part of staple crops.

Agronomic biofortification is a fertilizer-based approach that relies on soil and foliar application of micronutrients either alone or in combination with other fertilizers. It is well established that a Zn fertilizer strategy is an effective way to biofortify cereal crops with Zn, but recurrent cost is involved (Cakmak and Kutman, 2018).

Genetic biofortification is a strategy that uses plant breeding techniques to produce staple food crops with higher micronutrient levels, reducing levels of anti-nutrients and increasing the levels of substances that promote nutrient absorption.

### **Agronomic biofortification**

The soils of the world are highly diverse, ranging from some of the oldest soils to relatively young volcanic soils in the Great Rift Valley that splits East and Southern Africa and alluvial soils along rivers. Many soils in the world suffer from multiple micronutrient deficiencies, due both to their poor inherent soil properties and to continuous cropping without nutrient restore. Contemporary fertilization programmes in many countries, primarily focus on NPK fertilizers, but many soils are non-responsive to NPK due to (multiple) micronutrient deficiencies. Soil amendment with small amounts of (multiple) micronutrients has been suggested as a sustainable strategy to increase yields and nutritional quality of crops (Voortman and Bindraban, 2015; Manzeke *et al.*, 2012).

In the prevail paragraphs we discuss the impact of different fertilization approaches on agronomic biofortification, as well as the interactions of micronutrients with NPK fertilizers and the importance of Integrated Soil Fertility Management (ISFM).

### **Impact of different fertilization techniques**

Foliar fertilization with micronutrients often restorative more nutrient uptake and efficient allowance in the edible plant parts than soil fertilization, especially with cereals and leafy vegetables (Lawson *et al.*, 2015). Seed priming and seed coating with fertilizers are other approach for exact micronutrient application, that can encourage plant development and increase yields, but increased nutritional values of grains are infrequently found (Duffner *et al.*, 2014). The combination of soil and foliar application is often the most effective method (Phattarakul *et al.*, 2012; Cakmak, 2010). The downside of foliar application is that fertilizers can easily

be washed off by rain and are costlier and difficult to apply (Garcia-Banuelos *et al.*, 2014).

### **Impact in combination with NPK fertilization**

Combination of micronutrients with macronutrients can influence the success of agronomic biofortification (Prasad *et al.*, 2014). This results in increased micronutrient uptake and concentrations in the edible parts of the crop, as shown in wheat experiments, where high N application increased Zn and Fe concentrations in the grain endosperm (Kutman *et al.*, 2011; Shi *et al.*, 2010). Rao *et al.*, (2012) observed that nutrient uptake (N, P, Zn, B, S) and productivity of sorghum and finger millet were increased significantly by fertilization with combine of mineral NPK plus Zn, B and S. However, P fertilization can also decrease micronutrient concentrations due to a dilution effect when plants grow productive (Singh *et al.*, 1988). As indicated above, addition of P fertilization can also disclose incipient Zn deficiency by precipitation of insoluble Zn phosphate. Proper N and P management is important for the effectiveness of micronutrient fertilization and indicates the importance of a more integrated soil fertility management.

### **Impact of integrated soil fertility management**

A consistently strategy suggested to optimize soil conditions is Integrated Soil Fertility Management which is defined as “a set of soil fertility management practices that necessarily include the use of mineral fertilizer, organic inputs and improved germplasm” (Vanlauwe *et al.*, 2010). The combination of mineral fertilizers and organic inputs is beneficial, because they have complementary functions and enhance mutual effectiveness. Organic resources (plant

residues and animal manure) help to sustain soil organic matter with multiple benefits in terms of enhanced soil structure, cation exchange capacity and water holding capacity (Van Noordwijk *et al.*, 1997).

The application of micronutrient-enriched fertilizers should have no significant negative environmental effect when used at appropriate rates and generally has agronomic benefits as it improves soil fertility and crop health. Agronomic biofortification can be effective in increasing yields and nutritional quality for certain crop-micronutrient combinations, especially Zn and Se on wheat and maize, whereas Fe has shown little potential to era (Hussain *et al.*, 2010).

### **Genetic biofortification**

Conventional breeding and genetic engineering techniques both are approaches that may be used to increase micronutrient like iron, zinc and vitamin in biofortify crops (Tiwari *et al.*, 2010). Cereals are the most important source of calories in human's body. Rice, wheat and maize provide about 23%, 17% and 10%, respectively, of the calories acquired globally (Khush, 2003). To effectively target biofortification of cereals, five key steps can be targeted. These are (a) enhanced uptake from soil, (b) increased transport of micronutrients to grains, (c) increased sequestration of minerals to endosperm rather than husk and aleurone, (d) reduction in anti-nutritional factors in grains and (e) increase in promoters of mineral bio-availability in grains. Biofortified crops are also a good source of reaching rural populations who may have limited access to diverse diets or other micronutrient interventions. The Consultative Group on International Agricultural Research (CGIAR) through its HarvestPlus initiative has been exploring the genetic variability, heritability of mineral traits, stability over different

environments, genetic studies and breeding strategies to enhance the mineral content in major edible crops such as wheat, rice, maize, beans and cassava (CIAT/IFPRI, 2002).

Biofortification conventional plant breeding and genetic engineering both involve changing the genotype of targeted crops with the target of developing plants containing genes that support the accumulation of bio-available minerals. The way of reaching this goal differs between the both approaches (Gomez-Galera *et al.*, 2010). Currently work done on traditional plant breeding techniques which are exploiting the variability of micronutrient concentrations found in different germplasm (Qaim *et al.*, 2007). In selective plant breeding programs search for variation in the character of desired, for example, higher iron content, within existing varieties of the crop. This characteristic is then bred into cultivated varieties by crossing (advisedly interbreeding) and selecting those individual plants with the desired characteristics. (1) Seed banks - collections of seeds usually collected in the past, which may have greater genetic variation than current varieties. (2) Mutagenesis - a chemical or physical induction of genetic mutations used to create new variation. (3) Wide crosses – inter-breeding between a cultivated species and another, normally closely-related species.

Genetic engineering, used when crops have not the genetic potential to meet desired micronutrient levels with traditional plant breeding, and therefore it has to be applied to achieve sufficient improvements (Borg *et al.*, 2009). A step change in our ability to biofortify crops has come from a much better understanding of how plants take up and distribute micronutrients, mainly through the identification of genes for mineral transport and the biosynthesis of organic metal chelators. This knowledge has been exploited in modern biotechnology approaches,

demonstrating that it is possible to increase iron and zinc levels, not only in the whole grain but also specifically in the starchy endosperm. The proof-of-concept of transgenic approaches was initially demonstrated in rice. Increased expression of NAS-3, one of three genes encoding nicotianamine synthase (NAS), led to a 2.2-fold increase in the concentration of zinc and a 2.9-fold increase in the concentration of iron in the grain (Fig. 2).

So, the genetic modification is an excellent approach to gain high micronutrient concentrations (Bouis *et al.*, 2010) and that genetically modified organisms (GMO) have the potential for increased agricultural productivity. A positive factor is the fast development and stable expression of GMO traits, to receive the desired new variety far fewer breeding generations are needed with genetic engineering compared to traditional plant breeding. Additionally, genetic engineering is more precise since single genes can be introduced in the targeted plants. But usually patented inventions are associated with the developed GMOs, making them inaccessible for researchers in developing countries and unaffordable for farmers (Pardey *et al.*, 2000). Aside from numerous regulatory and political restrictions, transgenic plants often have to face social and ethical considerations causing a certain resistance to them (WHO, 2005).

Transgenic breeding could be another option in improving food crops with micronutrients. Generally, impressive progress is being made in developing transgenic plant genotypes with increased concentrations of Zn and Fe. Evidence is available showing a potential role of ZIP family that is Fe and Zn transporter proteins in improving micronutrient density in grain (Schachtman and Barker, 1999; Eide, 2006). These proteins are involved in uptake and transport of cationic micronutrients in

cells. In most cases, the genes encoding the Fe and Zn transporter proteins are expressed in response to Fe and Zn deficiencies, respectively. However, the role of these transporter proteins in genotypic variation for Zn deficiency tolerance or grain Zn accumulation is not clear.

**Harvest Plus:** It was launched in 2004 with funding from the Bill and Melinda Gates Foundation the UK Department for International Development (DFID) and others. In this focusing on increasing levels of iron, zinc and beta-carotene in seven staple crops, grown in areas of high subsistence farming, namely sweet potato, bean, pearl millet, cassava, maize, rice and wheat. Main target of HarvestPlus is to obtain a new wheat variety by crossing high micronutrient wheat varieties with modern wheat (short stems and husk free). The newly developed wheat plant is expected to contain 40- 50% more iron and zinc than currently cultivated varieties (HarvestPlus, 2006). However, depending on wheat intake targeted levels might be lower than the levels recommended by WHO for wheat flour fortification. To improve iron status flour fortification levels should deliver about 6 mg additional iron in the form of ferrous sulphate. Biofortified wheat varieties would deliver only about 1 mg additional iron per 100 g wheat flour (40% losses due to milling).

## **Wheat**

A breeding program goals development of new genotypes with high Zn concentration first requires actuality of useful genetic variation for Zn accumulation in grain. However, as indicated above, cultivated wheat contain very low levels of Zn and shows a narrow genetic variation for Zn. But the wild and primitive wheat cultivars represent a better and more promising genetic resource for high Zn concentrations. Among

wild relatives tested so far, the collections of wild emmer wheat (*Triticum turgidum ssp. dicoccoides*,) showed massive genetic variation and the highest concentrations of Zn (14 to 190 mg Zn kg<sup>-1</sup>). Very recently, new wild emmer wheat accessions have been identified showing simultaneously very high concentrations of Zn (up to 139 mg kg<sup>-1</sup>), Fe (upto 88 mg kg<sup>-1</sup>) and protein (up to 380 g kg<sup>-1</sup>) in seeds and high tolerance to drought stress and Zn deficiency in soil (Peleg *et al.*, 2008). In addition, synthetic wheats derived from *Aegilops tauschii* have also a high genetic potential for increasing grain Zn concentration of cultivated wheat (Calderini and Ortiz-Monasterio, 2003).

More than 3000 lines have been screened for Fe and Zn with concentrations for iron ranging from 25 µg to 56 µg per g wheat and for zinc ranging from 25 µg - 65 µg/ g wheat. They observed high 16 Biomedicine and Biotechnology G X E Interactions for zinc and iron and low G x E interactions for Mg and suggested that breeding for high concentrations of Fe and Zn might be difficult. Substantial impact of G X E interactions on mineral concentration was confirmed by a recently conducted study in India (Joshi *et al.*, 2010), and it is suggested that genetic factors for zinc and iron concentration in the wheat plant are of minor importance. Recreated synthetics, wild and landraces are being used as Progenitor for high Zn/Fe Limited backcross approach to introgression high Zn genes into elite wheat Selected bulk scheme- Most effective method, non-destructive XRF machine being used for fast-track Zn/Fe analysis.

## **Biofortified varieties in wheat:**

**WB 02:** It is pure line variety, developed by ICAR- Indian Institute of Wheat and Barley Research, Karnal. Contain high iron (40 ppm) and zinc (42 ppm) as compared to 32 ppm

zinc and 28-32 ppm iron in popular varieties. Grain yield 51.6 q/ha, maturity 142 days, it is timely sown irrigated variety. It adapted in Punjab, Haryana, Rajasthan and UP etc.

**HPBW 01:** Pure line variety developed at Punjab Agricultural University, Ludhiana. It contains high iron (40) and zinc (40.6) in comparison to 28-32 ppm iron and 32 ppm zinc in popular varieties. It also adapted in Punjab, Rajasthan, Haryana, HP and Uttarakhand (Ref. ICAR New Delhi 2017).

### **Pearl millet**

India, which has the largest pearl millet area about >9 million hectare in the world. Pearl Millet, as a species, has higher levels of Fe and Zn densities than other major cereal crops. Thus, there is a need to increase the cultivation of hybrids having higher Fe and Zn levels and enhance their consumption to better address various health problems associated with the deficiencies of these micronutrients. High-yielding open pollinated varieties (OPVs) and hybrids with higher levels of Fe and Zn densities than those found in most of the commercial cultivars otherwise not bred for these micronutrients as target traits have been developed and are available for commercialization (Rai *et al.*, 2014).

Breeding lines and germplasm with still higher levels of Fe and Zn densities have been identified. Their utilization in breeding has the potential to enable development of hybrids with >75 ppm Fe density and >55 ppm Zn density. The primary focus of pearl millet biofortification is on improving Fe density with Zn density as an associated trait. Depending on the genotypic composition of the trials, moderate to high correlations between Fe and Zn densities have been observed, indicating good prospects of simultaneous genetic improvement for both traits, but perhaps also the need to make

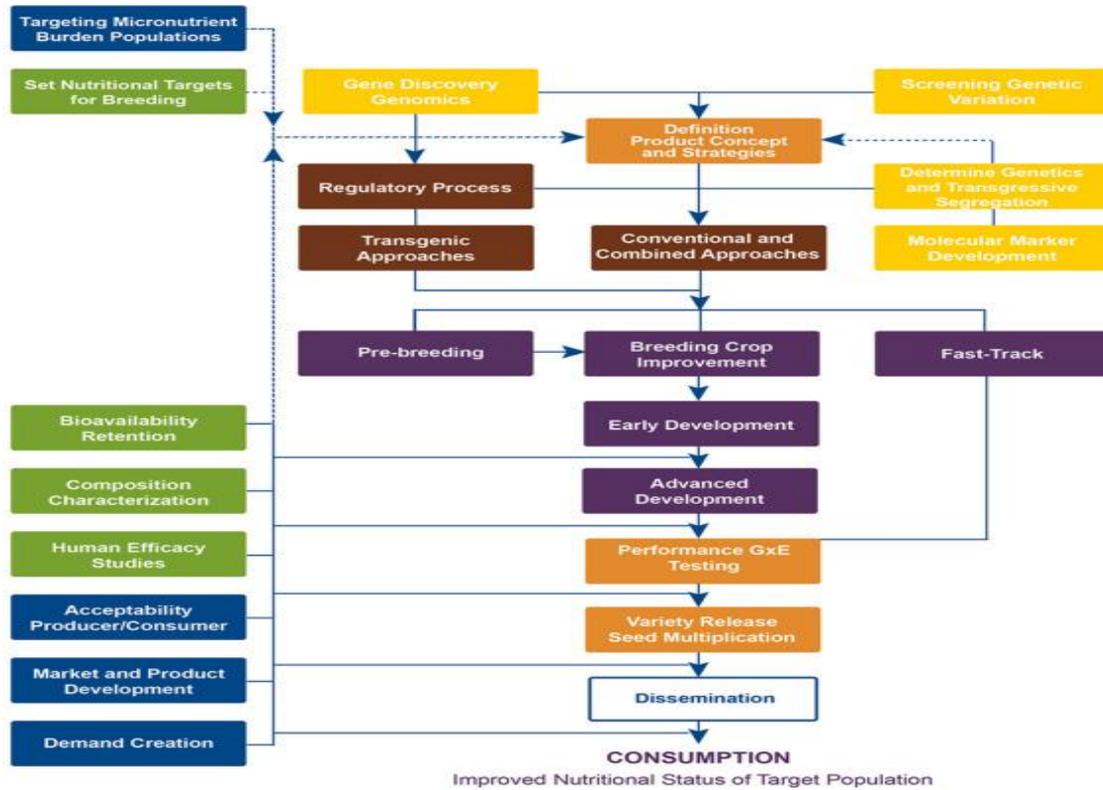
conscious selection for Zn density along with Fe density. Lack of association of Fe and Zn densities with grain size showed that both micronutrients can be improved without compromising on seed size. The association of Fe and Zn densities with grain yield was weak and negative, but not always significant, indicating that both micronutrients can be improved without significantly compromising grain yield by using large segregating populations.

Breeding Approach, the pearl millet biofortification breeding program at ICRISAT has taken a three-pronged breeding Phase-I, II and III. The first phase is a short-term approach dealing with traits genetics, germplasm screening and creating genetic variability. The second phase is the medium-term approach consisting of validating identified high-iron and zinc breeding lines and hybrid parents from the regular breeding program to develop fast-track biofortified variety/hybrids. The third phase consisted of long-term objective- development high-Fe/Zn breeding lines and hybrid parents and its genetic diversification through steady mainstreaming micronutrient traits at ICRISAT and NARS breeding programs (Govindaraj and Rai 2016,). Currently, the pearl millet biofortification is in a gradual transition from fast-track breeding (breed in current breeding efforts in the public and private sector are towards hybrid development and indeed supported by ICRISAT through the development and dissemination of a large number and diverse range of improved breeding lines and hybrid parents through Pearl Millet Hybrid Parent Research Consortia (PMHPRC). By utilizing identified high-Fe hybrid-parents from amongst those initially not bred for high-Fe as a target trait, several high-yielding and high-Fe hybrids have been developed, which are at various stages of testing. One such hybrid has been identified and designated as ICMH 1201,

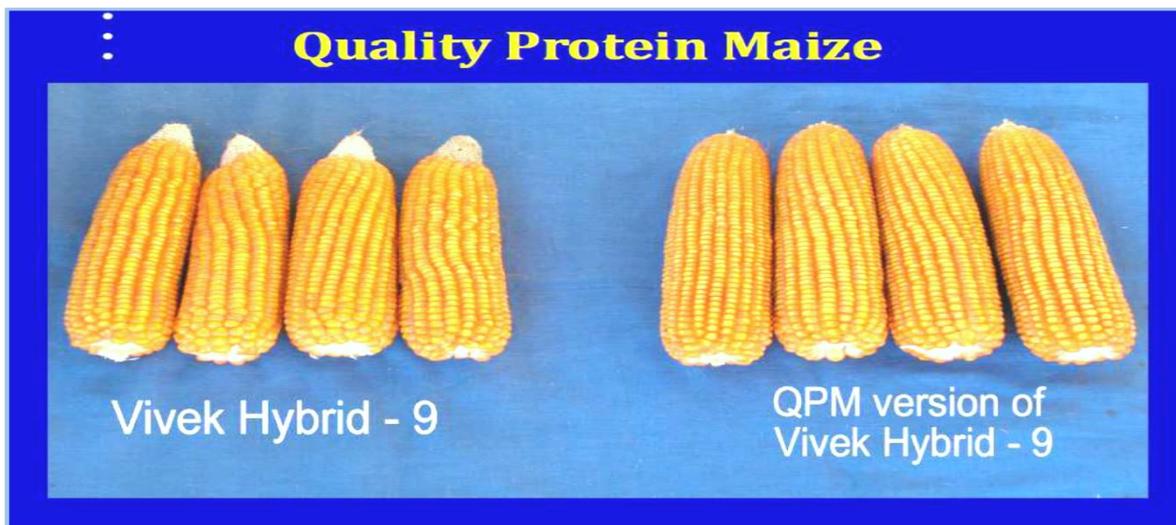
which in 48 field trials during 2011–2013 gave a 75 mg per kg Fe density (similar to

ICTP8203) but had 3.6 t ha<sup>-1</sup> grain yields (38% higher than ICTP 8203).

**Fig.1** Crop development framework. (Source: Harvest Plus)



**Fig.2** Characteristics of the original and reconstituted version of hybrid. A: Vivek Hybrid-9; B: Improved Vivek Hybrid-9 (Ref. Researchgate.net)



At ICRISAT, biofortified fast-track hybrids/varieties are developed through the XRF machine. Currently ICRISAT demonstrated the combining micronutrients and grain yield by conventional breeding approaches.

### **Biofortified hybrid varieties in pearl millet**

**HHB 299:** Developed at CCS-Haryana Agricultural University, Hisar in collaboration with ICRISAT. Contains high iron (73 ppm) and zinc (41 ppm) as compared to 45-50 ppm iron and 30-35 ppm zinc in popular varieties/hybrid. Grain yield 32.7 q/ha, dry fodder yield 73 q/ha, maturity 81 days.

**AHB 1200:** It developed, by Vasant Rao Naik Marathwada Krishi Vidyapeeth Parbhani with ICRISAT. Rich in iron (73 ppm) in comparison to 45-50 ppm in popular hybrids. Grain yield 32 q/ha, dry fodder yield 70 q/ha, maturity 78 days (Ref. ICAR 2017).

### **Rice**

Rice (*Oryza sativa* L.), one of the most important food crops in the world, forms the staple diet of over 50% of the world population. Billions of people in developing countries suffer from a lack of micronutrients in their daily food, a form of hunger also known as hidden hunger.

Micronutrient malnutrition especially Fe and Zn deficiency is recognized as a massive and rapidly growing public health issue mainly among poor people living on an unbalanced diet dominated by a single cereal such as rice (Brar *et al.*, 2011).

The natural variation of iron in rice is quite low and milling and polishing usually results in a loss of up to 80% since iron is mainly stored in the aleurone layer and not in the endosperm. Iron and zinc concentrations in

rice of different genotypes (n= 1138) were found to range between 6.3- 24.4 µg/ g and 13.5- 58.4 µg/ g, respectively, suggesting that there is at least some genetic potential to successfully breed high mineral rice. Golden Rice was an important breakthrough in this direction as an effective source of provitamin A (beta-carotene) with a significant potential to reduce disease burden by expressing genes encoding PSY and carotene desaturase (Beyer *et al.* 2002). The level of beta-carotene precursor, *i.e.*, phytoene, has been enhanced up to 23-fold by targeting gene encoding carotene desaturase. Folic acid (vitamin B9) is important for normal pregnancy and anaemia. Rice has been genetically modified to increase folate content (up to 150-fold) by overexpressing genes encoding Arabidopsis GTP-cyclohydrolase I (GTPCHI) and amino deoxychorismate synthase [ADCS], (Datta *et al.* 2003)]. The 100 g of modified rice was found to be sufficient to meet daily folate requirements of an adult individual.

Breeding for high Zinc 3500 rice accessions, 100 popular lines have been screened, 14 genotypes with high Zn content in polished grains have been identified. Selection and phenotyping of 40 rice genotypes are under multi-location trials (Paine *et al.*, 2005).

The development of the 'golden rice' proved that, it is possible to redirect a complete biosynthetic pathway of carotenoids by genetic engineering of multiple genes encoding key enzymes of the pathway. So, Golden Rice is such a biofortified it was developed in the year 2000 (Burkhardt, *et al.*, 1997). To obtain a functioning provitamin A (β-carotene) biosynthetic pathway in rice endosperm, we introduced in a single, combined transformation effort the cDNA coding for phytoene synthase (psy) and lycopene β-cyclase (β-lcy) both from *Narcissus pseudo narcissus* and both under the control of the endosperm-specific glutelin

promoter together with a bacterial phytoene desaturase (*crtI*, from *Erwinia uredovora* under constitutive 35S promoter control). This combination covers the requirements for  $\beta$ -carotene synthesis and, as hoped, yellow  $\beta$ -carotene-bearing rice endosperm was obtained in the T<sub>0</sub>-generation. Additional experiments revealed that the presence of  $\beta$ -lyc was not necessary, because *psy* and *crt I* was able to drive  $\beta$ -carotene synthesis as well as the formation of further downstream xanthophylls. Plausible explanations for this finding are that these downstream enzymes are constitutively expressed in rice endosperm or are induced by the transformation, e.g., by enzymatically formed products.

Results using *N. pseudo-narcissus* as a model system led to the development of a hypothesis, our present working model, that trans-lycopene or a trans-lycopene derivative acts as an inductor in a kind of feedback mechanism are stimulating endogenous carotenogenic genes.

### Varieties of biofortified rice

**CR Dhan 310** It is a pure line variety and contains high protein (10.3%) in polished grains average grain yield is 45q/ha (quintal/hectare), it matures in 125 days. This biofortified variety has been developed by ICAR-National Rice Research Institute, Cuttack, and Odisha.

**DRR Dhan 45:** It is a pure line variety and possesses high zinc (22.6 ppm) in polished grain. Its average grain yield is 50q/ha; it matures in 125-130. This biofortified variety has been developed by ICAR-Indian Institute of Rice Research, Hyderabad, and Telangana.

**DRR Dhan 49:** It is a pure line variety with high zinc (25.2 ppm) in polished grain its average grain yield is 50q/ha. It matures in 125-130 days. This biofortified variety has

been developed by ICAR-Indian Institute of Rice Research, Hyderabad, and Telangana (Ref. ICAR 2017).

### Maize

Maize is the staple food for more than 1.2 billion people worldwide, particularly in Latin America, Sub-Saharan Africa, and many of the South East Asian countries, including India. Maize lacks lysine and tryptophan necessary for protein synthesis. QPM contains a naturally-occurring mutant (*opaque2*) maize gene that increases the amount of those two essential amino acids. It produces 70-100% more of lysine and tryptophan than the most modern varieties, it reduces the incidence of pellagra, QPM is a product of conventional plant breeding *i.e.*, it is not genetically modified (Atlin *et al.*, 2011). Development of micronutrient-enriched staple plant foods through breeding holds significant promise for sustainable food-based solutions (Banziger and Long 2000, Graham *et al.*, 2001, Pfeiffer and McClafferty, 2007).

Micronutrient-enriched or biofortified maize would not only serve as the logical vehicle for providing Fe and Zn in the diets of the people (Long *et al.*, 2004) but also shall be a cost-effective and sustainable approach to alleviate micronutrient deficiencies (Bouis, 2002). Development of micronutrient-rich cultivars would produce higher yield in a micronutrient-deficient soil than the micronutrient inefficient variety.

Kernel iron (Fe) and zinc (Zn) concentrations were evaluated in a set of 30 diverse maize genotypes during rainy (kharif) season of 2006, 2007 and 2008 (Prasanna *et al.*, 2011). The ranges of kernel Fe and Zn concentrations were 11.28–60.11 mg/kg and 15.14–52.95 mg/kg, respectively, across the three years. Based on the performance of the entries across the years, four highly promising

inbreds and three landrace accessions were identified as highly promising for kernel Fe concentration, including a HarvestPlus line, HP2 (42.21 mg/kg).

Similarly, for kernel Zn concentration, three inbreds and one landrace were identified as highly promising, including V340 (43.33 mg/kg). No significant association was found between kernel Fe and Zn concentrations indicating the need for independent selection for enhancing the concentration for these traits.

Development of vitamin A-rich cereals can help in alleviating the wide spread problem of vitamin A deficiency. We report here significant enhancement of kernel  $\beta$ -carotene in elite maize genotypes through accelerated marker-assisted backcross breeding (Muthusamy *et al.*, 2014). A favourable allele (543 bp) of the  $\beta$ -carotene hydroxylase (*crtRB1*) gene was introgressed in the seven elite inbred parents, which were low (1.4 mg/g) in kernel  $\beta$ -carotene, by using a *crtRB1*-specific DNA marker for foreground selection.

About 90% of the recurrent parent genome was recovered in the selected progenies within two backcross generations. Concentration of  $\beta$ -carotene among the *crtRB1*-introgressed inbreds varied from 8.6 to 17.5 mg/g – a maximum increase up to 12.6-fold over recurrent parent.

The reconstituted hybrids developed from improved parental inbreds also showed enhanced kernel  $\beta$ -carotene as high as 21.7 mg/g, compared to 2.6 mg/g in the original hybrid. These constituted hybrids evaluated at two locations possessed similar grain yield to that of original hybrids. These  $\beta$ -carotene enriched high yielding hybrids can be effectively utilized in the maize biofortification programs across the globe.

## Varieties of maize

### **Pusa Vivek QPM9 improved**

It is possessing high provitamin-A (8.15 ppm). It also contains high tryptophan (0.74%) and lysine (2.67%) in endosperm protein and known as 'quality protein maize' (QPM). This biofortified variety has been developed by ICAR-IARI, New Delhi.

### **Pusa HM4 improved**

It is a QPM hybrid that possesses high tryptophan (0.91%) and lysine (3.62%) in endosperm protein. Its average grain yield is 64.2 q/ha and it matures in 87 days. This biofortified variety has been developed by ICAR-IARI, New Delhi.

### **Pusa HM8 improved**

It is a QPM hybrid that possesses high tryptophan (1.06%) and lysine (4.18%) in endosperm protein. Its average grain yield is 62.6 q/ha and it matures in 95 days. This biofortified variety has been developed by ICAR-IARI, New Delhi (Ref. ICAR 2017).

In conclusion the biofortification of crops is a feasible and most economical approach for overcoming 'hidden hunger'. Increasing the concentration of minerals in edible portions of cereals involves better uptake from soil and improved translocation to grains from leaves and finally enhanced sequestration to endosperm. Genetic diversity can be utilized to enhance micronutrient composition through conventional and modern breeding approaches. The most promising work plan to successfully alleviate micronutrient malnutrition will be to increase mineral content in the crops and simultaneously enhance their bioavailability by reducing anti-nutritional compounds and enhancing concentration of mineral absorption

promoters. To effectively combat hidden hunger through biofortification, even after the development of biofortified varieties, it will be essential to address various socio-economical and socio political challenges to popularize their cultivation by farmers and ultimately their consumption by the end users. A multi-tier coordinated strategy will play a pivotal role in overcoming hidden hunger. Given the severity of mineral malnutrition in humans worldwide, bio-fortification of micronutrients, especially Fe and Zn, in cereals must be encouraged. Although agronomic strategies (e.g., fertilizer strategy) can also increase micronutrient concentrations in cereal grains, however, there are still many challenges.

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