

INVITED ARTICLE

Is Biofortification of Zinc a Sustainable Approach to Deal with Micronutrient Malnutrition in Humans?: A Crop Physiologists Perspective

Past and present scenario

During green revolution the agriculture productivity is boosted (enhanced) by 2 major inputs, one is inorganic fertilizer and second one is irrigation facilities. Because of the continuous sustained effort, the production and productivity increased from 196.8 mt (2000) to 308.6 mt in 2020-21 and the productivity from 1626 kg/ha to 2386 kg/ha. However the projected food production for the year 2050 is 333mt (Agricultural statistics, 2021). But, to feed the huge increasing population (projected to be from 6.7 to 8.5 billion by 2030), a large increase in cereal production needs to be enhanced by 40%. To achieve this, the bottle neck is decreased soil productivity due to erosion, nutrient depletion, water scarcity, acidity, salinization, depletion of organic matter and poor drainage (Scherr, 1999). And variation in rainfall pattern and temperature due to global warming. Apart from drought, flooding is also going to be a serious concern in the future with regard to agricultural production. It can be concluded that both quantity and quality (2q) has to be sustained to feed hungry people and improve the human health.

Though some review papers published in the year 2022 alone and they are exhaustive and give the complete picture of the current status and future scenario. Yet, this review aims at focussing on the role of physiologists on addressing the malnutrition in humans, in holistic manner.

Malnutrition in humans is because of two reasons.

1. Inadequate intake of food by Below Poverty Line (BPL) families.
2. And adequate food but with less nutrient status in food.



Dr. A.G. SHANKAR

**(PDF, Sydney University, Australia)
ICAR-Emeritus Professor (2022-25)**

Dr. A. G. Shankar at present working as Professor (ICAR-Emeritus), worked as Professor (HAG and former Head, University Head, Dept. of Crop Physiology,, Former Dean (PGS&DSW). UAS, GKV, Bengaluru-560 065, INDIA. He is post doctoral fellow from Sdney University of Australia..

He Published more than 45 papers in International and National Journals. And he Visited- Japan, Australia, USA, Malaysia, Denmark, France, Germany for academic purpose to attend conferences, seminars, workshops and project collaboration work. Three students guided by him awarded gold medals at M.Sc. and Ph.D. level. Operated worth of more than 2 crore projects as PI and worth of 4 crore projects as co PI funded by ICAR DBT and DST. He is instrumental in developing the infrastructure facilities like Nutritional labor. Green house facilities, soil mixing unit and ain out shelter worth of 7 crores obtained from DST and DBT.

He got Appreciation certificate from USA (United States Department of Agriculture) in PL-480 project, DBT fellow to Copenhagen University, Denmark. And Life time achievement award from society for scientific development in agriculture and technology.

Dr. A. G. Shankar is a fellow of Indian society of Plant Physiology-1983, Society for advancement of breeders and researchers in Asia and Oceania (SABRO), Japan-1989 and Life time member of SABRAO, Japan & The Indian Science Congress Association, India

He is a University gold medalist in sports. Represented the state and the University in Kabaddi and Kho-Kho. And Served as coach and member of the University sports and council for last 20 years. Under his leadership, UAS teams won All India Championship 5 times in the All India Inter-Agri Sports meet.

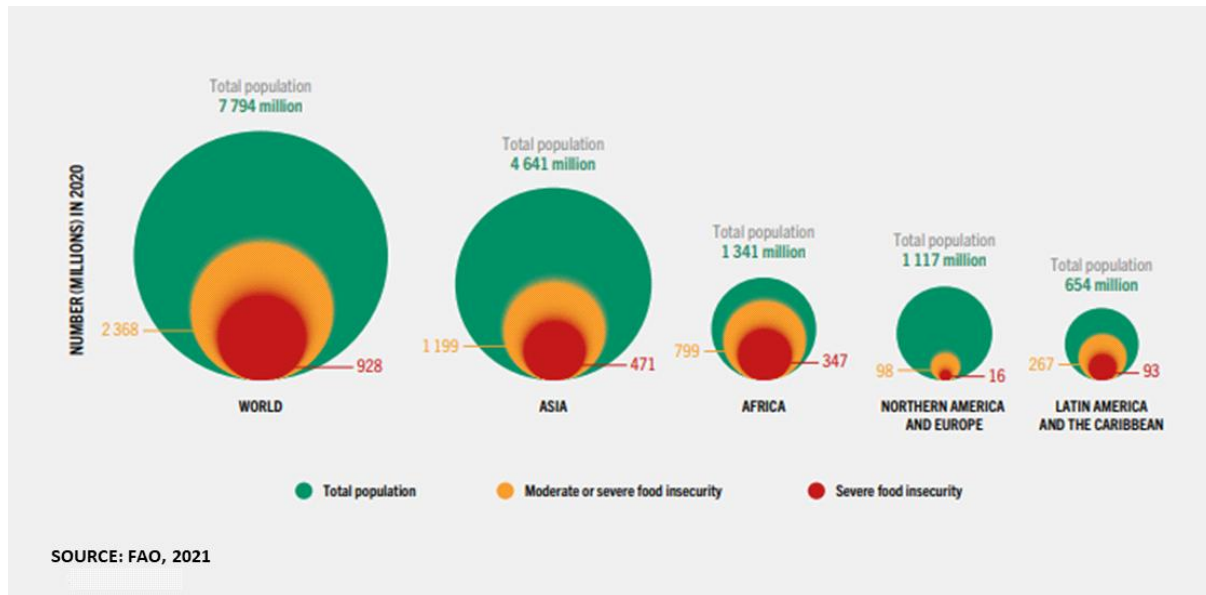


Fig. 1. The concentration and distribution of food insecurity by severity differs greatly across the regions of the world

Food security and nutritional security (FSNS) are the important issues to be addressed globally.

Indian scenario:

Around 70% of Indians can't afford healthy meals (FAO report, 2021) and another study reported 1.7 million die every year due to diseases caused by poor diet in India. According to the statistics available, globally 42% and in Srilanka (49%), Bhutan (53%), India (70.5%), Bangladesh (73.5%), Pakistan (83.5%) and Nepal (84%) of people unable to afford a healthy diet.

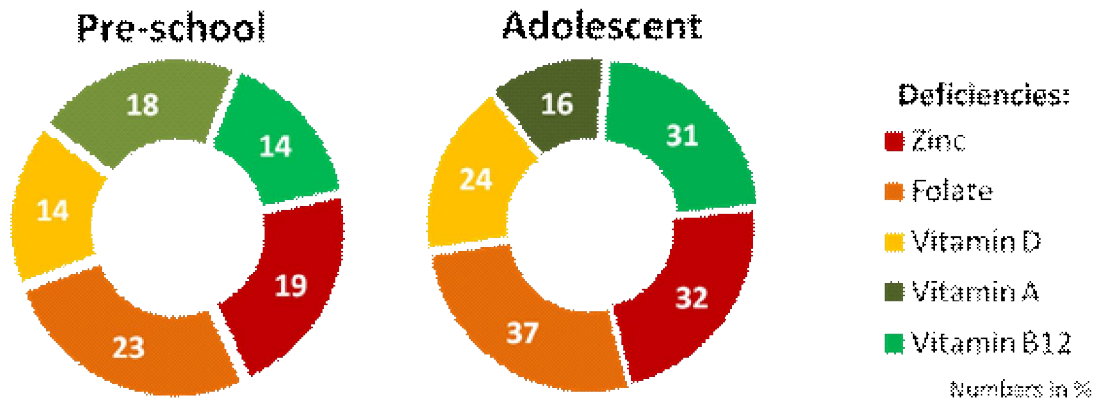
Healthy diets are out of reach for 3 billion people (2019). In 2020, between 720 and 811 million people faced hunger.

To overcome the malnutrition, Indian government through different states provide, public distribution system(PDS) to 60% of Indians comprising mainly 2 staple food grains Rice and Wheat but, there is no mechanism in vogue to provide diversified food (vegetables, fruits and meat) under the present program. Of the 49 known essential nutrients that are essential for human growth and development except Vit D and B12, the prime source of all these nutrients comes from "Agri-products". Of these, Vit A, D, B12, Fe, Zn, Proteins, Iodine and Folate are deficient from the point of human health. Especially Fe and Zn are predominantly deficient, though their requirement is very small, for eg: RDA of Fe is 10 mg/ day for male and 15 mg/ day for female and Zn is 15mg/day for male and 12 mg/ day for female. Yet both are deficient and Zn deficiency alone



Source: FAO report, 2021 and Anura kurpad, 2022

Micronutrient deficiency is common among children- The incidence of anaemia has increased significantly in all age groups.



Source: FAO report, 2021 and Anura kurpad, 2022

globally affecting 3 to 4 billion people. Among the micronutrient deficiencies (Zn, Folate, Vit A, Vit D, Vit B₁₂), 19 % preschool and 32 % adolescents are having Zn deficiency. And WHO (2004) reports that trillions of dollars are being spent globally and in India alone US \$ 12 billion annually to correct this deficiency. In recent years, it is proven beyond doubt, nutrient stress is the most important constraint next to moisture in limiting crop productivity and also micronutrients are deficient (Zn & Fe) from the point of human health.

“Micronutrients are delivered by diverse foods, not mono-foods. For a majority of those who receive the PDS, it diverts their diet into a monotonous cereal-based menu, which can actually cause risk for other problems that are linked to increased weight” – **Dr. Anura Kurpad (2022)**.

Concerns about PDS



- 1** Distributed in many communities without their knowledge or consent
- 2** Inadequate warning about impact on people with pre-existing conditions
- 3** Not proven to be effective in treating anaemia
- 4** Pilot studies of fortified rice in India not completed or evaluated

Why they (Fe & Zn) are deficient in food?

The reason being in case of Fe, though it constitute 3% of earth's crust, it is not present in available form Fe²⁺, since it exists in Fe³⁺ form, which is not a preferable form for plants to take it up and translocate to edible parts. In contrast, Zn²⁺ content as such is low in cultivated soils (0.1 to 0.6 ppm). Of

these, only 5-20 % available for plants. But, one should be careful with Zn fertilization to soil because Zinc is a heavy metal and > 100 ppm is toxic. Yet it can reduce the crop yield by 20-40 %, which is termed as “Hidden Hunger” and also causes many health disorders in humans.

Another contrast between these 2 elements is, the deficiency of Fe^{2+} throws out interveinal chlorosis symptoms in plants and anaemic conditions in humans. Zn deficiency in plants may not manifest to the level of visual diagnosis (stunted growth, unfilled berries or quality change in cob) nor in humans. Only at chronic level, it shows stunted growth in children, mortality due to diarrhoea in children, mid age depression and many more health disorders.

Zn has an important role in both plant and human nutrition

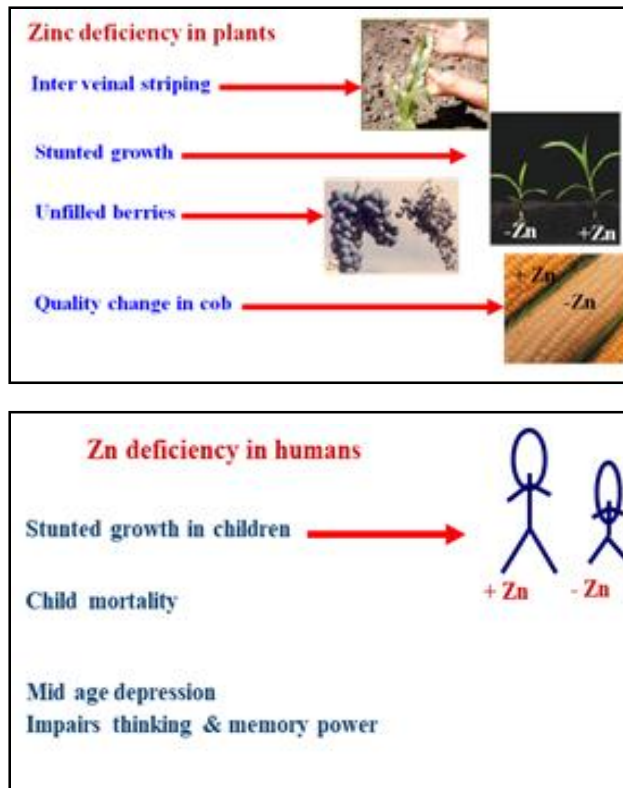


Fig. 2. Zn impact on plant and human health

Since, the plant themselves do not get sufficient amount of nutrients (Zn and Fe), so the economical part of food (grains, vegetables, fruits) and animal foods (egg, milk and milk based products) also contain less amount of these elements from the point of human health.

There is high amount of variability in Zn content in seeds or grains across sps. Dicots contain relatively high Zn in their seeds compared to monocots, among dicots pulses are known to accumulate high Zn and cereals and millets have low Zn. Unfortunately fruits do not contain detectable level of zinc.

Although lot of information is present for micronutrient variation in bean (Beebe *et al.*, 2000; Islam *et al.*, 2002), rice (Gregorio *et al.*, 2000), wheat (Monasterio and Graham, 2000), maize (Kovacevic *et al.*, 2004), only a few were related to QTL analysis for the micronutrient concentrations (Waters and Grusak 2008). Availability of useful variability in the germplasm and understanding of its genetic architecture are the prerequisites for a breeding programme aimed at biofortification of crop plants. Realizing the importance of biofortification several studies were undertaken for the evaluation of germplasm and advanced breeding lines for grain Zn content in particular (Cakmak *et al.*, 2000; Yamunarani, *et al.*, 2016; Nagarathna, *et al.*, 2010). In our earlier study, progress had been made with respect to screening for genetic variability for Zn concentration in seeds and leaves of pigeonpea.

Hence, both from the point of plant and human nutrition perspective, it is a challenge to plant scientists in general (and in particular plant physiologists and soil scientists) to develop a strategy to improve crop varieties with high nutrient efficiency crops for Fe and Zn. Though several approaches are being made in this direction, some progress is being made but it is not sustainable to achieve the target in the long run. In view of this, few short-term interventions are being recommended and attempted namely Supplementation, Fortification and diversification of food.

Nutrition imprinting.....

Zn status in edible parts of our crops

Cereals	Zn (mg/100g)
Maize	2.98
Wheat	2.87
Setaria	2.52
Proso	1.99
Rice	1.91
Jowar	1.78
Ragi	3.74

Pulses	Zn (mg/100g)
Bengal gram (white)	3.56
Bengal gram (Brown)	3.49
Redgram	3.41
Green peas	2.84
Field bean	2.52
Green gram	2.44
Cowpea	2.37
Horse gram	1.88

Oilseeds	Zn (mg/100g)
Sunflower	3.16
Groundnut	2.38
Soybean	2.17
Black gingelly	6.00-10.5
GingellyE8	10.25
GingellyD84	9.25
Gingelly local	11.5
White gingelly	4.25-10.00
Mint	6.50
Mustard	8.50
Coriander	4.00

Zn content among selected vegetables & green leafy vegetables

Vegetables	Mean Zn (mg/100g)	Green leafy Vegetables	Mean Zn (mg/100g)
Beans	6.62 ± 1.2	Amaranthus	5.72 ± 1.3
Carrot	2.71 ± 0.1	Fenugreek	5.38 ± 1.1
Beetroot	4.24 ± 0.4	Coriander	6.90 ± 0.9
Tomato	7.50 ± 1.1		
Drumstick	2.17 ± 1.2		
Pumpkin	1.49 ± 0.2		
Brinjal	3.74 ± 0.1		
F - Value	23.85*	F - Value	1.59 NS
SEm ±	0.3021	SEm ±	0.6325
CD at 5 %	0.9164	CD at 5 %	-

* Significant at 5 % level NS : Non-Significant

Interventions:

Supplementation

In Supplementation program, the cost involved and effective implementation on daily basis (either in the form of pill or syrup) for the entire population is an unrealistic proposal. And also due to the bottle neck, that, because of interaction between micronutrients. For ex. Fe intake in these forms, may reduce Zn²⁺ absorption (Krebs, 2000) due to antagonistic behaviour.

Zinc supplementation studies in humans

Several studies were undertaken globally, especially in developing countries including India in the last 2-3 decades.

An extensive studies on children with 6 to 35 months of age by administering 50 ml of oral rehydration solution per kg of body weight and given packets of oral hydration salts of Zn, and the findings suggests by reducing the duration and severity of diarrhoea in these groups, both diarrheal illness, child mortality can be improved. In India, the research group headed by M.K.Bhan (Former DBT Secretary and Professor of AIIMS, New Delhi) conducted a study on school children (600 students), where they were given oral pill supplements (10 mg elemental Zinc in the form of Zinc Sulphate) 6 days a week for 5-6 months shown to improve growth in these groups compared to placebo group who were not administered. Zn supplementation studies elsewhere also had shown a positive effect on the incidence of diahorrea and pneumonia in children less than one year age (Tanden and Arya, 2020, Sur D *et.al*, 2003) and in school going children.

Zinc sulphate, Zinc gluconate and Zinc acetate supplementation found to be beneficial for common cold (Mc Elroy *et. al*, 2002). Supplementation program for children at school level is also risky if not properly administered (both in terms of quantity and the form in which it is administered).

Fortification

It appears to be an easy method to address this problem, but again synergistic and antagonistic behaviour of nutrients and their bioavailability make the task difficult. Except Iodine which is blended with salt, where the iodine content and bio availability is the same, other nutrients have problem with bioavailability. The bioavailability is the amount of elements in food consumed, of which, how much is absorbed in the guts, intestine and digested. Especially Zn is not stored in any tissue or organ, and the extra amount will be thrown out through urine and faecal matters. Hence, there is a need to supply on daily basis. It is really a good metabolism, since zinc is a heavy metal. This is corroborated with studies conducted elsewhere and in our own laboratory, based on histological studies of piglets (Fig.3); it didn't accumulate in animal vital organs namely heart, lungs, liver, kidney and spleen.



Fig. 3. Fortification study in Piglet

The study was carried out in collaboration with veterinarians. Such studies have driven to fortify food as an intervention. Now there are attempts to fortify salt, bread, few more food items and drinking water with Zn.

Fortification has some limitations also. Some nutrients are lost during processing of food grains because they are stored almost exclusively in the husk and aleurone layer, therefore they are lost during milling

and polishing (Cakmak, 2002). For eg: Iron content in rice after milling is around $1.5-6.1 \text{ } \mu\text{g g}^{-1}$ against the target of $13 \text{ } \mu\text{g g}^{-1}$ (Bouis *et. al.*, 2011). Despite the use of iodized salt, an estimated 2 billion people are at risk of Iodine deficiency (Pearce *et. al.*, 2013).

Diversification of food

It is very crucial to eat enough vegetables, pulses, fruits in addition to cereals and also it is important to blend vegetarian food with non vegetarian. People below the poverty line (BPL) families can't diversify food. In fact, in India the staple food grains are supplied to these families at subsidised rate through PDS, but they don't have enough buying power to meet the purchasing power to other food items. Coming to blending meat with veg food is also doesn't seem to work, because of the religious reasons and no buying (economic) power. And also since both content and bio availability of consumed food is low- It's difficult to meet it by only diversifying plant based diet. When the BPL families can't afford 2 square a meal a day, how they can afford to buy non-vegetarian food which are very expensive. PDS to BPL families just with monotonous food grain distribution without complementing with pulses, vegetables, fruits and meat is like something better than nothing.

Disclaimer: I am not advising vegetarians to become non- vegetarians, but nevertheless fact remains.

It is proven that, it is necessary to blend vegetarian food with animal based diets (NIN, Hyderabad) to meet the total micronutrients requirement for humans.

Globally, Western/or developed countries meet their nutrient requirements because, their diet is more balanced with meat and dairy based products. The reason being the feed to produce ratio 1kg of meat varies in animal sources; the beef (16kg), pork

(6 kg), sheep (4-6 kg), goat (4.5-5 kg), eggs and chickens (3 kg), suggesting that animal based diet will have more Zinc. In contrast, in developing countries people are not in a position to afford diversified food, nor the Govt. can implement supplementation and fortification, because they are neither practically feasible nor economically cost effective.

The consensus was none of the mentioned short term nutritional interventions and diversification of food by itself is sufficient to eliminate nutrient deficiencies as a public health problem. Best solution is biofortification of our food grains.

Bioavailability

Phytic acid, a storage of phosphorous, which is very important and broken to release the energy during germination, the moment dry seeds are put in contact with moisture. In addition to the content of nutrients in food, bioavailability (the amount finally digested, after intake and absorption) is also very crucial. The major hurdle in this regard is presence of Anti nutritional factors (ANF) in not only in cereal grains and also in pulses and vegetables.

It is estimated that in cereals, which are consumed in large quantity by humans (Rice, Wheat, Maize and Sorghum) on daily basis, contain 78% of Zn and 72% Fe, present together by binding with phytate, so bioavailability is reduced. To overcome this to certain extent, eating of pre-germinated seeds (sprouts) is advised because phytate will break down when dry seeds imbibe water to start germination.

The next question is, are there any promoters of Zn absorption in plant food sources. The answer is yes, they are Ascorbic acid, histidine, cysteine, methionine, fumaric acid, palmitic acid, but cereals contain very low levels of these substances. Some approaches are being made to enhance these substances in food crops (White and Broadley, 2005).

Initially the proposal to reduce phytic acid (ANF) content as such in food is opposed, since it is also an anti-carcinogenic. However, over certain period of time the human specialists (physiologists/ nutritionists) came to a decision that it is acceptable to reduce it by 50% from human nutrition perspective. Hence, attempts are made to reduce to an extent of 50% of phytic acid in food grains was one of the approach to do so. Elsewhere and in our own lab, we have demonstrated phytate can be reduced to a certain extent by down regulating a key enzyme (MIPS) of phytic acid biosynthesis (Rajashekar reddy, 2014).

Earlier, piglets and mice are used as experimental material to study bioavailability. Now, the International nutrition committee has made it mandatory to use Caco-2 cells as model to study the micronutrient bioavailability (Buchanan *et. al.*, 2000).

Is Zinc jinxed from human nutrition perspective- Biofortification as an agricultural intervention and a sustainable approach for fighting micro nutrient malnutrition?

The answer seems to be yes, because even scientists and researchers (human nutritionists) involved in short term interventions strongly suggest that these nutrients coming through natural food is a sustainable approach. So it is the challenging task for Agri. Scientists especially plant physiologists.

In 2001, Steve Beebe coined the term biofortification. In 2003, CGIAR's biofortification challenge program was renamed as Harvest plus till 2018. In Brazil, India and China, several biofortified crops are used in what is known as a "Food Basket" approach. In 2016, Bouis was awarded by the World Food prize for his groundbreaking work on biofortification. H. E. Howday Bouis started working on solution for micronutrient malnutrition in the early 1990's. It is strongly recognized that biofortification

is economical and sustainable agriculture strategy for improving malnutrition (Aggarwal *et. al.*, 2020).

Biofortification strategies for micronutrient enhancement of food crops.

- 1) Agronomic (Physio-agronomic)
- 2) Conventional - Genetic breeding:
Time consuming and limited new genotypes.
- 3) Transgenic- Gene manipulation
- 4) Nano-biofortification

Is Bio-fortification a cost-effective intervention?

- Any interventions or research proposals are also should be linked into cost effectiveness.

Sources	Cost per person per year(\$)
Bio- fortified varieties	0.02- 0.03
Commercial fortification	0.12
Supplementation	3.00

Several biofortified crop varieties developed through conventional or molecular breeding approaches including India, are listed in the review paper (Sheoran *et. al.*, 2022).

Agricultural interventions – is it a best option available?

YES, the agri. strategies should aim at;

- “ Increasing micronutrient availability in soils, altering pH and organic acid levels,-The role to be played by soil scientists and agronomists.
- “ Reducing content of ANFs (Role of plant physiologists and plant breeders)
- “ Enhancing facilitators of absorption (Role of plant physiologists and plant breeders)
- “ Increasing micronutrient uptake by plants (Role of physiologists)

Current status and future goals

Role of physiologists in addressing Zn deficiency in plants and humans

As stated earlier biofortification of food is the only long lasting solution to address Zn deficiency in humans.

Approaches are:

1. Assessing the genetic variability in a given crop.
2. Effect crossing of high Zn type with leading high yielding variety of given species.
3. Over expression of Zn transporters (Transgenic or Inplanta technique).
4. Down regulating rate limiting enzyme in phytic acid biosynthesis- myo-inositol 3-phosphate synthase (MIPS) to reduce the ANF (Anti nutritional factors) phytate (phytic acid) content.
5. Nano fertilizers usage.

Zn fertilization studies on crops

In several crop species whenever zinc is supplied externally to soil, seed priming and foliar application resulted in higher Zn content in economical/ edible part of crops.

Sources for homeostasis, uptake and translocation of nutrients in plants

Thlaspi caerulescens and *Arabidopsis halleri* are wonder plants – hyperaccumulators of heavy metals can accumulate 3% Zn in shoot (where as > 100 ppm is toxic to other crop plants) without any toxicity symptoms and complete their life cycle.

An interesting experimental model system to study mechanisms of heavy metal transport and accumulation in plants. This facilitates to identify candidate genes and to use them to enhance Zn in plants and economic parts.

The long journey from Soil to Grain (Zn)

Zn is acquired from soil solution primarily as Zn^{2+} , to some extent *via* complexed with organic ligands. Roots contribute to making metal ions more available for uptake by 2 strategies. First, depending on the nutrient status of the soil by acidifying the rhizosphere and secondly, roots actively secrete organic acids and phytosiderophores (Palmgren *et al.*, 2008) depending on the species. During the period of grain filling, metals are also remobilized from leaves, exported and transported *via* the phloem to the grain/fruit. Nicotinamide act as binding partner for Zinc for the intercellular movement of Zn in leaves.

Seed or Grain phytic acid content in finger millet

Phytic acid is ubiquitous to prokaryotes and eukaryotes, it is the primary storage form of phosphate and inositol in seeds, can account for more than 60% of the total seed phosphorous. There are 2 ways to enrich micronutrients in seed. By conventional breeding, crossing the low phytic acid genotypes with the agronomically superior variety or produce transgenic crops by over expressing phytase enzyme or develop mutants with low phytic acid. There was 7 to 8 fold variation in phytic acid in 532 core germplasm lines, indicating phytic acid governed by many genes, it is a quantitative trait. There is a huge scope for molecular characterization among the contrasting genotypes and development of lpa mutants (Mahesh *et al.*, 2015).

In depth studies were conducted in these areas, elsewhere and in India. Three main crops were focused in our lab but Rice is not included because, micronutrients are stored predominantly in husk and aleurone layer, and are lost during polishing and processing of grains.

So, Ragi (finger millet), pigeon pea and tomato were chosen because, Ragi is the only grain that contains 330 mg Ca for every 100 gm of grain, pigeon

pea contains high amount (22g/100g) of protein (considered as poor man's meat) and Tomato, an important vegetable consumed daily and both contain less amount of Zn.

Proof of concept

The experiments were carried in our laboratory (by physiologists) and the results are summarized as follows.

In this study, in 341 genotypes of finger millet, the Zn content ranged from 1 to 8 mg/100 g of seeds. 88 % of them fall in the range of 1 to 4 mg/ 100g, suggesting that huge genetic variability in seed Zn content. Seeds containing more than 7 mg/ 100 gm are very less. Nevertheless they can be used in conventional breeding. Another study demonstrated that over expression of osZIP1 a Zinc transporter responsible for the uptake and translocation, helps in improving Zn content in edible parts (Yamunarani *et al.*, 2013).

Phytic acid, poly phenols lectins oxalates *etc.*, are considered as ANF's (White and Broadley, 2005). Phytate is the more important ANF, accounts maximum upto 90% phosphorous content in cereal grains, legumes, nuts and oilseeds. Phytic acid can contribute to nutritional and environmental problem, as it reduces the bioavailability of cations and leading to malnutrition in humans and monogastric animals. In this study both breeding and transgenic, approaches are attempted.

The Zinc and phytic acid estimation was done in 530 germplasm lines of **finger millet** (Mahesh *et al.*, 2015). There was 7-8 fold difference between the lowest and highest phytic acid content. The ten low phytic acid types contained in the range of 70 to 109 mg per 100g, whereas high phytic acid types had 503 to 517 mg per 100 g of seeds. The low phytic acid types can be introgressed with agronomically superior varieties.

In this an attempt was made to detect QTLs, and the composite interval mapping resulted in identifying the specific regions on the pegenopea genome that contributed to seed Zn content and several other physiological traits. Major effect QTLs have been identified governing all the traits considered in this study. These QTLs can be effectively used in breeding to improve seed Zn content. However it would be more appropriate to saturate these QTL regions by using additional markers and validating their relevance. bZIP transcription factor (ASSR20) known to regulate Zn is found to be linked to high seed Zinc content (Basavarajeswari *et al.*, 2015; Pavithra *et al.*, 2016). The study conducted in collaboration with NIN, Hyderabad using Caco-2 cells, suggested the availability of Zn enhanced in grains.

Tomato

Here the approach was to over express Zinc transporter (osZIP 1) Zn in tomato, both by Tissue culture method of *Agrobacterium* mediated transformation and Inplanta transformation methods (Matapathi, 2009). This Inplanta method of floral dip experiments was found to be successful in various plant sps. *eg. Arabidopsis thaliana* (Bent, 2000), apple, pear, tomato, peach, strawberry (Spolare *et al.*, 2001), citrus (Ahmad and Mirza, 2005) and tomato (Desfeux *et al.*, 2000; Abida *et al.*, 2009; Basavarajeswari *et al.*, 2018).

Past, present and future of Biofortification

Apart from the hunger faced by population, the malnutrition is met to some extent by bio fortification. To enhance the availability several biofortified products (Zn, Vitamin A and Fe) are released by different countries including India. For more details refer to Bouis and Saltzman, 2017.

In India, open pollinated pearl millet varieties (Dhanashakti) and hybrids ICMH 1202, 1203 and

1301 with high content of iron (70-75 mg/kg) and Zinc (35-40 mg/ kg) have been introduced (Govindaraj *et al.*, 2019).

Biofortification of non-cereals

Common beans (*Phaseolus vulgaris*) is an essential grain legume, consumed by humans in all parts of the world. It has been shown that in common beans the Fe concentration can be enhanced by 60-80% and Zinc concentration by around 50%. The orange sweet potato developed by harvest plus (consortium funded by Bill gates and Melinda) has already shown a remarkable impact on nutrition and food security in Africa, which was acknowledged by world Food prize, 2016. Foliar application of Zn fertilizers showed an increased Zinc content significantly. It was also reported that Zinc sulfate and Zn oxide were more productive than Zinc nitrate through foliar application in potato. Harvest plus with International Institute of Tropical Agriculture (IITA) produced 6 Vit A fortified varieties (Cassava) in Nigeria. It has been introduced in many more countries.

Yet another study was done with cocktail of Zinc, Iodine, Fe and Selenium at 21 field sites during 2015-2017 in Brazil, China, India, Pakistan and Thailand. Brown Rice Zn increased from 21.4 mg to 28.1 mg/kg with cocktail spray at all sites except in 3 sites. Both Iodine and Selenium showed increased content (Prom-U-Thai *et al.*, 2020).

Collaborative research efforts involving breeders, biotechnologists, physiologists, biochemists, human nutritionists, need to strengthen biofortification programs to meet the challenge of attaining nutritional security.

Nanotechnology -Nanofertilizers

Conventional fertilizers have nutrient utilization efficiency of just 30–35 per cent, 18–20 per cent,

and 35–40 per cent for nitrogen, phosphorus, and potassium respectively. For the previous few decades, the data has remained consistent, and study efforts have not yielded productive outcomes. Nanofertilizers provide higher nutrient utilization, lower cost, and lower volume by weight (vol/wt) than traditional fertilizers due to their minute size, increased surface area, and unique properties. In this context, the nanofertilizer was prepared and applied to maize cultivar and response is evaluated. Using nano fertilizers is a promising approach for improving sustainability of food production and for the biofortification of food crops. Nano technology could be the most sustainable way to enhance food production, productivity, crop protection and eradicating micro nutrient deficiencies in human (Chugh *et al.*, 2022). As we are aware IFFCO, has already released nano-urea fertilizer.

There are several studies conducted using nano-urea and nano zinc in several crop species in different countries. For the moment in most of the studies have used Hydroponics (Li *et al.*, 2016) and they have applied to roots (Rui *et al.*, 2016), seed priming (Li *et al.*, 2013), foliar (Rostamizadeh *et*

al., 2021) and fertigation (Palmqvist *et al.*, 2017). The concentration varied from 20 mg/kg to 1000 mg/kg depending on the crop. All these studies have demonstrated that there is increase in yield and increase in Fe and Zinc content in grains. Nano fertilizers of N and Zn under field condition for the cereal *i.e.*, Wheat, pearl millet and oil seed crops *i.e.*, mustard and sesame in 160 field demonstrations comprising 1225 acre area showed increase in yield in wheat (5.35%), in sesame (22.24%), in pearl millet (4.2%) and 84% in mustard. Seed Priming with Nano Boron Nitride Increases the Performance of Sunflower (*Helianthus annuus* L.) seedlings (Geetha *et al.*, 2018) and seed yield (Kavita *et al.*, 2018).

Nano particles are normally prepared by chemical and physical methods but, very recently green synthesis of nanoparticles is reported (Ahmed *et al.*, 2022; Ramesh Raddy *et al.*, 2018, 2017a, 2017b; Pruthvi *et al.*, 2022). The results obtained are encouraging and may help in preventing soil and water contamination.

Table 1: Examples of major food groups, their role in nutrition, and examples of fortification (Gani *et al.*, 2018)

Food Commodity	Disadvantages	Example
Cereals	Consumed in large quantities, throughout the year, and by all members of society, as part of typical diet but micronutrient concentrations usually low.	Wheat flour fortification with iron (Chile)
Fats, oils, and margarines	Intake generally not sufficient to supply 100% of recommended intake.	Margarine fortification with vitamins A and D (e.g., in Europe).
Dairy products	Favors mothers and children, but poor rural populations usually have limited access.	Fortification of milk with iron (Argentina).
Condiments	Sugar, spices, starches, sauces consumed regularly through the population, particularly SE Asia, but usually in small amounts.	Fish sauce fortification with iron (Thailand), sugar fortification with vit. A (Guatemala).
Value added products	May be consumed only sporadically by populations with deficiencies.	Water, bread, juices, bread fortified with Zn, Fe, and vitamins (e.g., in the United States).

Future line of work

In nutshell, physio- agronomic approaches are proved to be useful and specific genes are identified for Zn uptake, translocation from root to shoot and from shoot to grains/ vegetables/ fruits and few methodologies are also standardized and protocols are also available. Now it is up to the crop physiologists to choose the choice of their crop, identify and access the candidate genes to improve the enhanced Zn content in edible parts from human nutrition perspective. By doing so, we can demonstrate that the crop physiologists have a role to play directly for the benefit of farmers (in terms of high productivity) and improve the quality of grains from consumer point of view. So, both Quantity and quality (2q) can be achieved.

The adage 'health comes from the farm, not the pharmacy' is the heart of ongoing international biofortification research and breeding programs.

“THINK GLOBALLY AND ACT LOCALLY”- is the (Successful mantra) need of the hour.

LITERATURE CITED

- Abida yasmeeen, Bushra mirza Samia inayatullah, Naila safdar, Maryam Jamil, Shawkat Ali and M Fayyaz Choudhry 2009** Inplant Transfoirmation of Tomato. *Plant Mol. Biol. Rep.*, 27: 20-28.
- Aggarwal N, Upadhyay P and Tigadi S B 2020** Biofortification to improve nutrition: a review. *International Journal of Current Microbiology and Applied Sciences*, 9:763-779.
- Agricultural Statistics at a Glance 2021** (www.agricoop.nic.in & https://desagri.gov.in)
- Ahmad M and Mirza B 2005** An efficient protocol for transient transformation of intact fruit and transgene expression in Citrus. *Plant Mol Bio Rep.*, 23:419a–k.
- Ahmed S, Qasim S, Ansari M, Shah A A, Rehman H U, Shah M N, Ghafoor U, Naqvi S A H, Hassan M Z, Rehman S and Ahmad F 2022** Green synthesis of zinc nanoparticles and their effects on growth and yield of *Pisum sativum*. *Journal of King Saud University-Science*,102132.
- Anura Kurpad 2022** Tacking malnutrition will be a challenge Crop diversity vital to good diets published in Deccan Herald, 1ty July, 2022.
- Basavarajeshwari R M, Geetha K N, Rajashekar Reddy B H, Monoa L And Shankar A G, 2019** Genetic Diversity And Phylogenetic Behavior Of 30 Pigeon Pea Genotypes. *Intl. J. Genetics*, 11(7); 618-621.
- Basavarajeshwari R M, Yamunarani R, Ramegowda V, Geetha K N And Shankar A G 2018** Expression Profiling Of Zinc Transporter Genes In Tomato Grown Under Different Concentrations Of Zinc, *Intl. J. Micro. Res.* 10(6):1252-1255.
- Basavarajeshwari R M, Yamunarani R, Geetha K N and Shankar A G 2015** Sub-cloning of Zinc Transporter Gene for Genetic Transformat ion to Improve Zinc Nutrient Status in Crop Plants, *Intl. J. Bio-resource Stress Mnt.*, 6(3):396-401.
- Beebe S, Gonzalez A V and Rengifo J 2000** Research on trace minerals in the common bean. *Food Nutr. Bull.*, 21:387–391.
- Bent AF 2000** *Arabidopsis* in planta transformation. Uses, mechanisms, and prospects for transformation of other species. *Plant Physiol.*, 124:1540–7.

- Bouis H E and Saltzman A 2017** Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Glob. Food Secur.*, 12:49–58.
- Bouis H E, Hotz C, McClafferty B, Meenakshi J V and Pfeiffer W H 2011** Biofortification: a new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin*, 32:S31–S40.
- Buchanan B B, Gruissem W And Jones R L 2000** Biochemistry and Molecular Biology of plants, The American Society of Plant physiologists, Rock-ville, MD.
- Cakmak I 2002** Plant nutrition research: priorities to meet human needs for food in sustainable ways. *Plant and Soil*. 247: 3-24.
- Cakmak I 2000** Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.*, 146: 185–205.
- Desfeux C, Clough S J And Bent A F 2000** Female reproductive tissues are the primary target of Agrobacterium- mediated transformation by the Arabidopsis floral-dip method. *Plant Physiol.*, 123:895-904.
- FAO** The State of Food Security and Nutrition in the World 2021. In Building Climate Resilience for Food Security and Nutrition; *Food and Agriculture Org.*: Rome, Italy, 2021.
- Gani G, Gulsar B, Bashir O, Bhat T A, Naseer B, Qadri T and Jan N 2018** Hidden hunger and its prevention by food processing: A review. *Int. J. Unani. Integr. Med.*, 2:1–10.
- Gaurav Chugh, Kadambot H M, Siddique and Zakaria M Solaiman 2022** Iron fortification of food crops through nanofertilisation. *Crop and Pasture Science*, 73(7–8), 736–748. doi:10.1071/CP21436.
- Geetha K N, Kavita Mahadev Goudar N N, Lingaraju Ramesh Raddy and A G Shankar 2018** Seed Priming with Nano Boron Nitride Increases the Performance of Sunflower (*Helianthus annuus* L.) Seedlings, *Int.J.Curr.Microbiol.App.Sci* , 7(11): 503-508.
- Govindaraj M, Rai K N, Cherian B, Pfeiffer W H, Kanatti A and Shivade H 2019** Breeding Biofortified Pearl Millet Varieties and Hybrids to Enhance Millet Markets for Human Nutrition. *Agriculture*, 9:106.
- Gregorio G B, Senadhira D, Htut T and Graham R D 2000** Breeding for trace mineral density in rice. *Food Nutr. Bull.*, 21: 382-386.
- Islam F M A, Basford K E, Jara C, Redden R J and Beebe S E 2002** Seed compositional and disease resistance differences among gene pools in cultivated common bean. *Genet. Resour. Crop Evol.*, 49: 285–293.
- Kavita Mahadev Goudar, Geetha K N, Lingaraju N N, Shankar A G and Ramesh Raddy 2018** Response of sunflower (*Helianthus annuus* L.) to nano boron nitride fertilization. *International journal of Chemical Studies*, 6(5): 2624-2630.
- Kovacevic V, Banaj D, Brkic I, Antunovic M and Petosic D 2004** Fertilization impacts on the yield and nutritional status of maize (*Zea mays* L.). *Cereal Res. Commun.*, 32(3): 403-410.
- Krebs N F 2000** Overview of zinc absorption and excretion in the human gastrointestinal tract. *The Journal of nutrition*, 130(5): 1374S-1377S.
- Li J, Chang P R, Huang J, Wang Y, Yuan H and Ren H 2013** Physiological effects of magnetic iron oxide nanoparticles towards watermelon. *Journal of Nanoscience and Nanotechnology*, 13:5561–5567.
- Li J, Hu J, Ma C, Wang Y, Wu C, Huang J and Xing B 2016** Uptake, translocation and

- physiological effects of magnetic iron oxide (α -Fe₂O₃) nanoparticles in corn (*Zea mays* L.). *Chemosphere*, 159:326–334.
- Mahesh S, Pavithra G J, Parvathi M S, Rajashekara Reddy and Shankar A G 2015** Effect of processing on phytic acid content and nutrient availability in food grains. *Int. J. Agril. Res.*, 5(5): 771-777.
- McELroy B H and Miller S P 2002** Effectiveness of zinc gluconate glycine lozenges (Cold Eeze) against the common cold in school-aged subjects: a retrospective chart review. *AM J Ther.*, 9(6):472 5.
- Monasterio J L and Graham R D 2000** Breeding for trace minerals in wheat. *Food Nutr. Bull.*, 21: 392-396.
- Nagarathna T K, Shankar A G and Udayakumar M 2010** Assessment of genetic variation in zinc acquisition and transport to seed in diversified germplasm lines of rice (*Oryza sativa* L.) *J Agri. Techn.*, 6: 171-178.
- Palmgren M G, Clemens S, Williams L E, Kramer U, Borg S, Schjorring J K and Sanders D 2008** Zinc biofortification of cereals: problems and solutions. *Trends in Plant Science*, 13: 464-473.
- Palmqvist N G M, Seisenbaeva G A, Svedlindh P and Kessler V G 2017** Maghemite nanoparticles acts as nanozymes, improving growth and abiotic stress tolerance in *Brassica napus*. *Nanoscale Research Letters*, 12:631.
- Pavithra G J, Mahesh S, Pervathi M S, Basavarajeshwari R M, Nataraja K N, Pearce E N, Andersson M and Zimmermann M. B 2013** Global iodine nutrition: where do we stand in 2013? *Thyroid*, 23:523–528.
- Prom-U-Thai C, Rashid A, Ram H, Zou C, Guilherme L R G, Corguinha A P B, Guo S, Kaur C, Naeem A, Yamuangmorn S and Ashraf M Y 2020** Simultaneous biofortification of rice with zinc, iodine, iron and selenium through foliar treatment of a micronutrient cocktail in five countries. *Frontiers in Plant Science*, 11:589835.
- Pruthviraj N, Geetha K N, Prakash S S, Jayadeva H M, Pushpa K and Shankar A G 2022** Impact of different methods of nano fertilizers application on soil chemical properties and fertility status in sunflower growing soils. *Mysore Journal of Agricultural Sciences*. 56(1): 25-284.
- Rajashekar Reddy B H, Raju B R, Udayakumar M and Shankar A G 2014** Assessment of genetic variability for seed phytic acid content in finger millet (*Eleusine coracana* L. Gaertn) mini-core germplasm accessions Abstract submitted for Mini-symposia at Plant Biology Conference 2014 at Portland, Oregon, USA. Organised by ASPB.
- Rameshraddy, Mahesh Salimat H K N Geetha and A G Shankar 2018** ZnO nanoparticle improves maize growth, yield and seed zinc under high soil pH condition. *International journal of current microbiology and applied science* 7(12): 1593-1601.
- Rameshraddy, Pavithra G J, Rajashekar Reddy B H, Salimath M, Geetha K N And Shankar A G 2017** Zinc oxide nanoparticles increases Zn uptake, translocation in rice with positive effect on growth, yield and moisture stress tolerance. *Indian J. Plant Physiol.*, 22 (3): 287–294.
- Rameshraddy, Pavithra G J, Salimath Mahesh, Geetha K N And Shankar A G 2017** Seed

- priming and foliar spray with nano zinc improves stress adaptability and seed zinc content without compromising seed yield in ragi (*Finger millet*). *Int. J. Pure App. Biosci.*, 5 (3): 251-258.
- Rostamizadeh E, Iranbakhsh A, Majd A, Arbabian S and Mehregan I 2021** Physiological and molecular responses of wheat following the foliar application of iron oxide nanoparticles. *International Journal of Nano Dimension*, 12:128–134.
- Rui M, Ma C, Hao Y, Guo J, Rui Y, Tang X, Zhao Q, Fan X, Zhang Z, Hou T and Zhu S 2016** Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Frontiers in Plant Science*, 7:815.
- Scherr S J 1999** Soil degradation, a threat to developing-country food security by 2020? Food, agriculture, and environment discussion paper 27. International Food policy research institute. Washington, DC.
- Shankar A G 2016** Comparative growth responses and transcript profiling of zinc transporters in two tomato varieties under different zinc treatment. *Indian Journal of Plant Physiology*, 21(2) : 208-212
- Sheoran S, Kumar S, Ramtekey V, Kar P, Meena R S and Jangir C K 2022** Current Status and Potential of Biofortification to Enhance Crop Nutritional Quality: An Overview. *Sustainability*, 14:3301.
- Spolaore S, Trainotti L and Casadoro G 2001** A simple protocol for transient gene expression in ripe fleshy fruit mediated by *Agrobacterium*. *J Exp Bot.*, 52:845–50.
- Sur D, Gupta D N, Mondal S K, Ghosh S, Manna B, Rajendran K and Bhattacharya S K 2003** Impact of zinc supplementation on diarrheal morbidity and growth pattern of low birth weight infants in kolkata, India: a randomized, double-blind, placebo-controlled, community-based study. *Pediatrics*, 112(6):1327-1332.
- Tandon V K and Gaurav Arya 2020** Role of zinc supplementation in school aged children suffering from common cold: A clinical trial. *MedPulse International Journal of Pediatrics*, 14(2):04-07.
- Waters B M and Grusak M A 2008** Quantitative trait locus mapping for seed mineral concentrations in two Arabidopsis thaliana recombinant inbred populations. *New Phytol.*, 179: 1033–1047.
- White P J and Broadley M R 2005** Biofortifying crops with essential mineral elements. *Trends in plant science*. 10(12): 586-593.
- WHO REPORT 2004** global database on anaemia. World Health Organization, Geneva.
- Yamunarani R, Govind G, Ramegowda V, Thammegowda H V and Guligowda S A 2016** Genetic diversity for grain Zn concentration in finger millet genotypes: potential for improving human Zn nutrition. *The Crop Journal*, 4(3):229-234.
- Yamunarani R, Venkategowda R, Jagadish P, Govind G, Reddy R H, Makarla U, and Guligowda S A 2013** Expression of a rice Zn transporter, OsZIP1, increases Zn concentration in tobacco and finger millet transgenic plants. *Plant Biotechnology Reports*. 7:309-319.