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Ensuring nutritional security through zinc biofortification of rice grain in Indian scenario: A review

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Abstract

The developing countries like India are facing serious consequences of nutritional inadequacy for the ever-increasing population masses. Consumption of large scale cereal-based foods with small concentrations and low bioavailability of Zn is the major reason behind this problem. Low grain Zn concentrations is highly related with the Zn deficient soils (accounting to more than 40-50% of Indian soils) where they are grown. The common strategies for sustaining Zn bioavailabilty include food fortification, dietary diversification, and medical supplementation. Several limitations have emerged regarding nutritional diversification and food enrichment, which has favored Zn biofortification as a perpetual solution of malnutrition. The agronomic and genetic biofortification processes emerge as the fore-runner in this scenario. The current review thereby focuses on the role of Zn in plants and human health, chalks out the uptake and translocation of Zn in cereal grains, and more specifically tries to pave out the paths for nutritional security driven by various agronomic, breeding and biotechnological approaches. The review enunciates that the adoption of amenable strategy combined with better cultivation practices can be the only future pathway for human welfare.

Keywords: Zinc, rice, biofortification, nutritional security, agronomic, genetic, transgenic

Introduction

Post green revolution period has been enunciated as a story of agricultural development (Dhaliwal *et al*., 2015; Rada, 2016) [42, 143] through the transformation of subsistence to sustainable farming and deficiency in food grain production to its sufficiency (Sengupta and Dey, 2019) [167]. To augment the production hitherto, there has been a continuous increase in fertilizer use and consumption in India (Jaga and Patel, 2012) [86] while on the other hand this indiscriminate application culminated into several nutrient deficiencies occurring in soil (Singh, 2008). The mining of nutrients from the soil on continuous basis has robbed off the inherent soil fertility status (Majumdar *et al*., 2016) [107] coupled with inadequate and imbalanced use of fertilizers caused increasing deficiencies of secondary and micronutrients which are limiting crop response to use of primary nutrients N, P and K (Goud *et al*., 2013) [59]. The deficiencies of B, Fe, Mn, S and Zn in soil are now cropping up on a wide scale in the country (NAAS, 2018) [120].

The deficiency of Zinc (Zn) has global connotation owing to its necessity in human dietary system (Singh and Prasad, 2014) [175]. The deficiency Myers through the dietary dilemma may profoundly influence one third of the world’s population (*et al*., 2014; Saha *et al*., 2017; Bhattacharya, Sengupta and Halder, 2019) [159, 168]. The prevalence is more in children under 5 years of age because of large demand for Zn to support growth and development (Wessells & Brown, Krebs 2012) [215] and results in mortality of about half a million annually (Black *et al*., 2008; *et al*., 2014; Cakmak and Kutman, 2018) [17, 29]. Deficiencies Zinc has diverse biochemical and physiological functions in biological systems especially regarding critical structural, functional and regulatory roles including enzyme activation, protein synthesis, starch, auxin and nucleic acid metabolism and pollen development (Cakmak, 2000; Chang *et al*., 2005) [26, 32]. The deficiency of Zn in human system is associated with serious health complications like defective immune system, physical growth, learning capabilities, risk of infections, damage to DNA and cancer (Gibson 2006; Zaman *et al*., 2018) [54, 230].

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A close inspection suggests that Zn deficiency in human beings are predominant in areas where there is deficiency in soils as the availability through the major dietary pathways are curtailed (Singh and Prasad, 2014) [175]. In the Indian context, the consumption of cereal grains especially rice deserves special mention. Rice (*Oryza sativa* L.) is one of the major staple foods, contributing to half of the world population's dietary intake (Sunusi *et al*., 2019) [190]. It is grown in more than 100 countries, predominantly in Asia and contributes to about 21% of the global energy and 15% protein requirements (Maclean *et al*., 2002; Depar *et al*., 2011) [106, 41]. Rice productivity is often severely jeopardised by several abiotic hindrances. Zinc deficiency is one of the prime abiotic factors limiting the rice productivity and associated human availability (Rehman *et al*., 2012) [151]. This assumes greater significance since the major obstacle to improve tolerance to zinc deficiency in rice is not fully understood and a wide range of soil conditions affect its availability (Wissuwa *et al*., 2006) [219].

The common approaches to alleviate human micronutrient deficiency involves food fortification, dietary diversification and medical supplementation (Bouis *et al*., 2011) [21] which is difficult to adopt for poor rural residents of the developing countries (Zhang *et al*., 2018) [232]. Biofortification, which intends to increase the micronutrient content in plant edibles is thereby attracting and ever increasing attention (Wani *et al*., 2015; Chattha *et al*., 2017) [209, 33]. Genetic and agronomic biofortification are two important agricultural approaches that could be helpful in improving cereal grains to optimum Zn concentrations (Das *et al*, 2019) [37, 39].

With this background, in this review, we have made a modest attempt to discuss the functions of Zn in rice, Zn dynamics in the soil to unearth the Zn deficiency in soils which in turn affect its bioavailability. Furthermore, we have discussed agronomic management and breeding options to improve Zn intake and partitioning into rice grains for the improvement in yield and quality and ultimately help to ensure food and health safety.

Role of Zn in plant growth and nutrition

The imperative micronutrient zinc has several vital functions to play in the plant systems (Das and Green, 2016) [38]. Acting as a cofactor, Zn activates different hormones e.g. auxin required for growth and development of plants (Begum *et al*. 2016) [12]. Moreover, numerous biochemical processes, such as nucleotides production, auxin metabolism, enzyme activation, chlorophyll formation, pollen fertilization are all contributed by tissue Zn content (Zaman *et al*., 2018) [230]. Apart from these, membrane function, photosynthesis, gene expression, protection against drought and pathogens are also influenced by the Zn content (Hefferon, 2019) [74]. Zinc is usually absorbed as Zn^{2+} ion, and is involved with all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases) especially acting as a constituent of carbonic anhydrase, alcoholic dehydrogenase and superoxide dismutase (Romheld and Marschner, 1991; Rattan, 2017) [56, 149].

Role of Zn in human health and physiology

Zn deficiency is ranked as the 5th leading risk factor for diseases (e.g. diarrhoea and pneumonia in children) in the developing countries (WHO, 2002). Zinc plays a significant role in diverse physiological functions in biological systems by interacting with a large number of enzymes and proteins in the body and performing critical roles in structural, functional and regulatory systems (Cakmak and Kutman, 2018) [29]. Its role in the structural and functional integrity of biological membranes and detoxification of highly aggressive free radicals is immense (Cakmak, 2000) [26]. It acts as an effective antioxidant and anti-inflammatory agent (Rattan, 2017) [149]. The deficiency of Zn is attributed to any alteration in Zn homeostasis or decrease in Zn concentration of human body may result in wide range of health problems such as growth retardation, loss of appetite, impaired immune function, hair loss, diarrhoea, eye and skin lesions, weight loss, delayed healing of wounds, and mental lethargy (Hotz and Brown 2004; Prasad 2004; Wang and Busbey 2005; Swamy *et al*., 2016) [76, 175, 206, 195].

The dietary allowable limits of Zn for infants is 3–5 mg/day, for children of 1–10 years it is 10 mg/day for adults, 15 mg/day for men and 12 mg/ day for women and 16–19 mg/day for lactating women (WHO, 1996) [216]. The murky picture however is that these intake limits are hardly met (Singh and Prasad, 2014) [175]. Over 25% of the total population in India is at the risk of inadequate Zn intake (Ray *et al*., 2016) [150] and the current burden of Zn deficiency related anomaly amounts to 2.8 million disability-adjusted life years (DALYs) lost, 2.7 million from mortality and 1,40,000 from morbidity, 70% of which occur among infants (Stein, 2014) [186].

The dynamics of Zn in soil

The total zinc concentration of the lithosphere is approximately 80 mg kg^{-1} (Brennan, 2005) [22] and in soil it ranges approximately from 7 to 1000 ppm (Havlin *et al*., 2005) [73]. In agricultural soil, it varies from 4.65 to 427.8 mg kg^{-1} with an average of 117.35 mg kg^{-1} (Su *et al*., 2014) [188]. Available (DTPA extractable) Zn ranged between 0.12 and 2.80 mg/kg soil (Katyal and Sharma, 1991) [95]. Unbound Zn occurs in soil as Zn^{2+} because of having a typical complete $3d^{10}4s^2$ outer electronic configuration contributing to a fixed oxidation state of +2 (Barker & Pilbeam, 2015) [11].

There are five major pools of zinc in the soil: (a) zinc in soil solution; (b) surface adsorbed and exchangeable zinc; (c) zinc associated with organic matter; (d) zinc associated with oxides and carbonates; and (e) zinc in primary minerals and secondary alumino-silicate materials (Shuman, 1991; Moreira *et al*., 2006; Moreira *et al*., 2016) [174, 117, 188].

Zinc present in soil solution represents only a small fraction of the total metal content in soil (Shukla & Anshumali, 2018) [173]. It is regulated by a number of factors such as moisture, soil reactions, temperature, redox potential (Eh), fertilizer additions, and plant uptake (Sun and Zhang, 2017) [189]. Zn containing minerals like franklinite ($ZnFe_2O_4$) also affects the zinc concentration in soil solution via equilibrium solubility (Wisawapipat *et al*., 2017) [217]. Surface adsorbed and exchangeable fraction together with the soil solution zinc generally accounts for less than 2% of the total zinc present in soil (Emmerson *et al*., 2000) [43]. It involves weakly adsorbed metals attached to the solid surface by relatively weak electrostatic interaction. Remobilisation of metals can occur in this fraction due to adsorption desorption reactions and lowering of pH (Ahnstrom & Parker 1999; Narwal *et al*., 1999) [3, 124]. The exchangeable fractions include weak acid soluble carbonates and exchangeable pools of micronutrients (Adamo *et al*., 2018) [2]. Exchangeable and adsorbed ions i.e weakly adsorbed (on nonspecific sites) and strongly held ions (on specific sites) respectively are grouped as one pool and are extracted together inspite of having separate impact on the zinc chemistry of soil (Shukla & Anshumali, 2018) [173].

Zinc may be associated with soil organic matter, which includes water-soluble and organic compounds. Zinc is bound *via* incorporation into organic molecules, exchange, chelation, or by specific and nonspecific adsorption (Shuman, 1991; Moreira *et al*., 2006) [174, 117]. Under oxidizing conditions, decompostion of organic matter results into release of zinc bound to this component. The organic fraction released through this reaction is not considered to be bioavailable as those are associated with stable high molecular weight humic substances that slowly releases zinc (Filgueiras *et al*., 2002) [47]. Zinc is associated with hydrous oxides and carbonates *via* adsorption, ion exchange, surface complex formations, incorporation into the crystal lattice and co-precipitation. Some of these reactions fix zinc rather strongly and are believed to be instrumental in controlling the amount of zinc in the soil solution. In heavy metal contaminated acid soils of the southeastern United States, high rates of soil-applied zinc may be responsible for the elusive mouse-ear symptom (Itanna & Coulman 2003) [85] possibly due to competition with nickel ions (Grill *et al*., 1985) [64].

A majority of the micronutrient metals are found in the crystal structures of the highly resistant primary and secondary minerals which comprise the residual fraction after all other extractants have been employed. These forms usually are less resistant to extraction. These exist predominantly in two forms (a) sulfides for Zn, Cu, and Fe; and (b) carbonates for Zn, Cu, Mn and Fe (Krauskopf, 1972; Sposito, 1983) [98, 184]. Franklinite ($ZnFe_2O_4$), smithsonite ($ZnCO_3$), Sphalerite (ZnS) and Willemite (Zn_2SiO_4) are common minerals which contain Zn (Havlin *et al*., 2005) [73]. In non-contaminated areas, soil zinc is predominantly concentrated in crystalline primary and secondary minerals (Minkina *et al*., 2015) [116].

Waterlogging may initiate a suite of reactions that affect Zn mobility in contrasting ways. Under acidic soil condition, submergence leads to neutrality in the pH value which ultimately contributes to Zn sorption on organic matter possibly due to reduction in the concentration of major competing H^+ ions. In contrast, reductive dissolution of iron oxides and hydroxide can mobilize metals absorbed on or embedded in iron oxides (Chuan *et al*., 1996) [36]. For Fe, crystalline minerals such as Fe_3O_4 (magnetite) or $FeCO_3$ (siderite) can be formed via conversion of an amorphous iron mineral to a crystalline mineral if the maximum Fe solubility is exceeded. The formation of these new minerals leads to reduction in the bio availability possibly due to re-absorption of zinc as substituting ion or an inherent component such as franklinite-like solids ($ZnFe_2O_4$) (Renault *et al*., 2009; Weber *et al*., 2009) [154, 211].

Magnitude of Zn deficiency in Indian soils

The phenomenon of zinc deficiency is spread over a wide range of countries throughout the world (Naik and Das, 2008; Sadeghzadeh, 2013) [122, 158] and reported long back, especially India (Nene, 1966) [126], Japan (Yoshida and Tanaka, 1969) [227], Philippines (Yoshida *et al*., 1973) [228], Pakistan (Yoshida and Tanaka, 1969) [227], Taiwan (Yoshida *et al*., 1973) [228], USA and Brazil (Deb, 1992) [40]. The tropical regions are more prone to such deficiency especially for highly weathered soils, semi arid calcareous soils, sandy and acid soils (Rattan, 2017) [149]. 40-50% of the Indian soils on an average are responsive to Zn application (Rattan, 2015) [147]. An interesting observation was evident from the study of Katyal and Rattan (1993) [94] that the average Indian soils contain 0.57 mg/kg of Zn in the bioavailable form, which is only 1/100th of the total Zn content. Such lower mea estimates were observed for all soils (Katyal and Sharma, 1991) [95] as evident from DTPA extractable Zn content (mg/kg) of Ultisols (0.28), Aridisols (0.38), Alfisols (0.55), Oxisols (0.86), Vertisols (0.41), Entisols (0.44) and Inceptisols (0.66). On that basis, coarse-textured, calcareous or alkaline low in organic matter alluvial soils (Entisols and Inceptisols) of Indo-Gangetic plains of North India; fine textured calcareous black soils (Vertisols) of Deccan Plateau; and highly leached rice-growing red and other associated soils (Alfisols, Oxisols and Ultisols) are major Zn deficient soils in India (Rattan *et al*., 2008) [148]. The deficiency of Zn is predominant mostly in cereal grown areas; covering nearly 50% of the cereal-grown areas in the world (Graham and Welch, 1996; Cakmak, 2002) [60, 25] and in India as well (Ray *et al*., 2016) [150]. Thus to augment dietary Zn availability manipulation of soil status and plant uptake mechanisms are important.

Uptake mechanism of Zn in rice plant

To manipulate the Zn availability in rice, better understanding of the physiological basis of Zn uptake, its translocation, the maintenance of Zn homeostasis, Zn partitioning within and between different plant parts and within rice grain, internal allocation, re-allocation, re-mobilization, and efficient loading into grain is essential (Stomph *et al*., 2011; Olsen and Palmgren, 2014) [187, 128]. The loading of Zn in the rice grain is mainly based on three different hindrances: 1) soil-to-root barriers; 2) root-to-shoot barriers; and 3) barriers in loading Zn into grains.

Root uptake, the first step towards the accumulation of Zn in rice grains is influenced by root architecture, root hairs, crown root development, root surface area, root anatomical structures and modification of rhizosphere chemistry that involves exudation of protons, which can change soil pH, thereby improving the solubility of Zn in the soil and facilitate its diffusion to the root surface (Rose *et al*. 2013; Swamy *et al*., 2016) [157, 195]. Although only negligible amount of Zn cross the root and succeed to reach the xylem using an apoplastic pathway, it is mostly the symplastic pathway is responsible for transportation of Zn across the roots to the xylem (Broadley *et al*. 2007) [24]. The main source of Zn in rice grains occurs by direct root uptake, remobilizations from vegetative tissues or combination of both of these two approaches are the main source of Zn in grains (Impa *et al*. 2013) [79]. Usually, Zn can be obtained in the form of Zn phytosiderophore complex or as Zn^{2+} ions (Broadley *et al*. 2007) [24]. Some Ca^{2+} channels in the plasma membrane and predominantly ZIPs (ZIP1, ZIP3, and ZIP4) (Palmgren *et al*., 2008) [132] mediate Zn^{2+} uptake, while yellow stripe-like (YSL) proteins helping the uptake of Zn–phytosiderophore complexes in gramineae family (Suzuki *et al*. 2006) [192]. Guerinot (2000) [66] opined about the role of ZRT/IRT-like proteins and ZIP like transporters (AtZIP1, AtZIP2, AtZIP3 and AtZIP4) for Zn uptake into the roots.

A continuous xylem flow from root to grain enabled by transpiration pull can directly transport Zn to grains (Krishnan and Dayanandan 2003) [100]; however the barriers in root-shoot transfer and the internal allocation and re allocation of Zn within and between vegetative and reproductive tissues (Swamy *et al*. 2016) [195], can hinder Zn uptake and its load in grain (Jiang *et al*. 2008) [79]. The major root-to-shoot barriers include suberin constituent of the cell wall, casparian strips, Zn sequestration in cytoplasm and vacuoles, as well as anatomical variations that exists in the root-shoot junction (Yamaguchi *et al*. 2012; Yamaji *et al*. 2013) [222, 223]. From the roots, Zn is transported to above ground parts of the plant through xylem as well as in phloem, mostly chelated by nicotianamine (NA) (Von Wirén *et al*. 1999) [205].

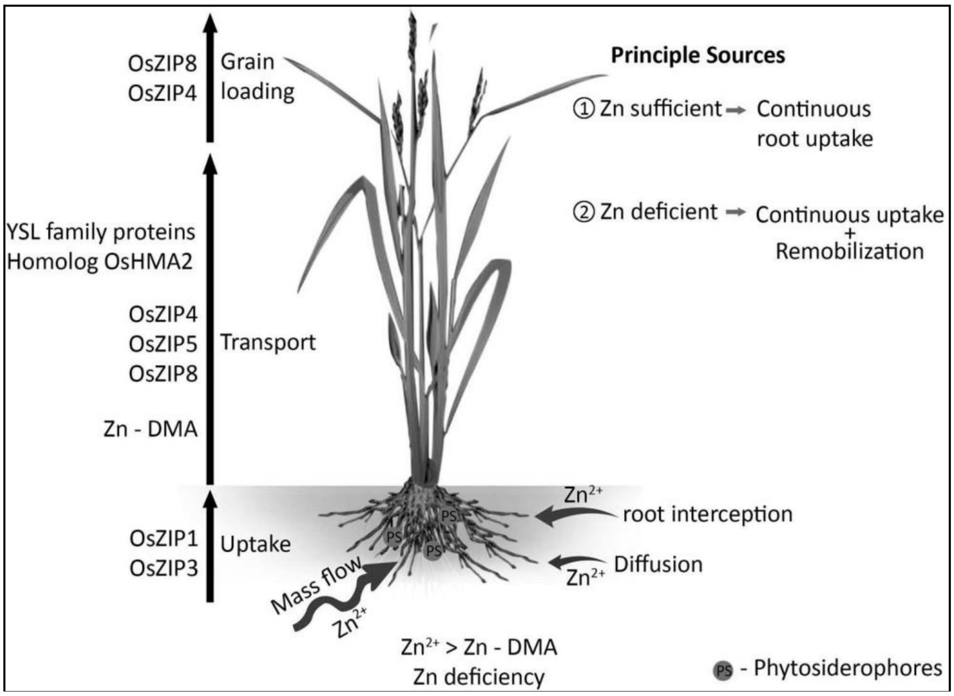

Fig 1: Zn uptake and transport in the root grain (Nakandalage *et al*. 2016; Zaman *et al*., 2018) [123, 230].

Although the cytoplasm of a plant cell comprises of several Zn^{2+} holding proteins, but overall Zn^{2+} concentration is very small (Broadley *et al*. 2007) [24]. Zn transport in the xylem may occur as Zn^{2+} or as a complex with organic acids, nicotianamine or histidine and collected in the vacuole as an organic acid complex (Leitenmaier & Küpper 2013) [102]. The Zn^{2+} influx to the leaf portion of the plant through the phloem by either ZIP family (Ishimaru *et al*. 2005) [84] or YSL proteins (Zaman *et al*., 2018) [230]. From the vacuolar pool, NRAMP protein transporters enable remobilization (Thomine *et al*. 2003) [196]. However, the increased expression of *OsNAS3*, *OsNAAT1*, and *OsDMAS1* genes in rice shoot can severely curtail Zn uptakes (Suzuki *et al*. 2008; Ishimaru *et al*., 2011) [193, 82]. Nakandalage *et al*. (2016) [123] presented mass flow of Zn intake and transport for loading into the rice grain. The selective phloem transport of Zn from old to new tissues and grain lower the Zn content in reproductive tissues (Wu *et al*., 2010; Impa *et al*., 2013) [221, 79]. Even the flag leaf, which plays an important role in photosynthesis and grain yield, was found to have a little contribution to grain Zn (Sperotto *et al*. 2013) [183]. Even higher Zn uptake through roots and shoots were not found to significantly contribute to grain through internal translocation emerging as the major bottleneck in this research (Stomph *et al*. 2014; Yin *et al*. 2016) [226] and emerged as the major basis to over express genes such as *OsZIP1*, *OsZIP4*, *OsZIP8*, *OsZIP8a*, *OsYSL8*, *OsYSL9*, *OsFRO2A*, *OsNAS1*, *OsNAS2*, *OsNAS3*, *OsArd2*, *OsIRT1*, *OsNRAMP1* and *OsHMA2* for augmenting Zn uptake by transgenic approaches (Ishimaru *et al*. 2005, 2007, 2011; Chandel *et al*. 2010; Sasaki *et al*. 2014) [84, 83, 82, 30, 31].

Expression of Zn deficiency in plants (rice)

Zn is considered to be intermediate or conditionally mobile by several researchers and because of that its deficiency symptom is first seen in the younger leaves (Marschner, 1995; Epstein and Bloom, 2005) [109, 44]. Acute Zn deficiency is manifested through stunted growth, shortened internodes and petioles and 'little leaf' (small malformed leaves) which was reported to be the classic 'rosette' symptom in new tender growth of dicotyledons (Snowball and Robson, 1983; 1986) [179, 180] and 'fan shaped stem' in monocotyledons (Grundon, 1987) [65].

These leaves remain small, cup upwards and develop interveinal chlorosis with veins remaining green in mild to moderate deficient conditions and on the upper leaf surfaces necrotic spots appear which later forms brown necrotic and brittle patches (Brennan *et al*., 1993; Shukla *et al*., 2016) [23, 172]. Zinc deficiency in rice termed as “khaira” disease was first reported by Nene (1966) [126]. Symptoms appear both in nursery and in main field 2-4 weeks after transplanting. Symptoms include dusty brown spots on upper leaves of stunted plants, uneven plant growth, poor seedling establishments, chlorotic midribs and ultimately spikelet sterility in rice (Nene, 1966; Prasad & Shivay, 2018) [126, 140, 171].

Biofortification: a tool to combat Zn malnutrition

To avail Zn to the human community and curtail malnutrition, augmenting Zn load in food edibles especially rice grain, has become a serious necessity. Biofortification is the most acclaimed method to effectuate this (Zhang *et al*., 2018) [232]. It may be defined as an agricultural strategy aimed to increase the content of micronutrients in edible parts of major staple food crop for better human uptake (Hotz, 2009; Cakmak and Kutman, 2018) [77, 29] in a more practical, sustainable, and cost-effective approach and combat malnutrition problems (Hess and Brown, 2009; Bhullar and Gruissem, 2013; Wang *et al*., 2016) [75, 16, 208]. Production of nutritious and safe foods, sufficiently and sustainably, is considered the ultimate goal of biofortification (Saltzman *et al*, 2013) [161]. Biofortification or biological fortification of cereals for zinc ideally aims at increasing the Zn content in grains by 40-60 mg/kg, although for rice a value is not obtained (Rattan, 2017) [149]. A fixed target concentration of 28 m/kg has been envisaged for rice (Harvest Plus, 2014) [71]. Apart from improving human health, it can also impart tolerance to abiotic stress, provide higher yield and even improve resistance to insects, pests and diseases (Cakmak, 2008) [28]. The process of biofortification can be achieved through modern bio-technology techniques, conventional plant breeding, and agronomic practices (Garg *et* *al*., 2018) [53]. While agronomic biofortification is usually done through soil application of efficient Zn sources at the right rate, time, and stage (Saha *et al*., 2017; Zaman *et al*., 2018) [159, 230] and improving soil phytoavailability, the process of genetic biofortification involves development of new crop cultivars with relatively higher accumulating ability for essential nutrients such as Zn in grains (Zhao and McGrath, 2011; Velu *et al*., 2012; Palanisamy, 2018) [237, 204, 131]. Such processes of biofortification have been elucidated here.

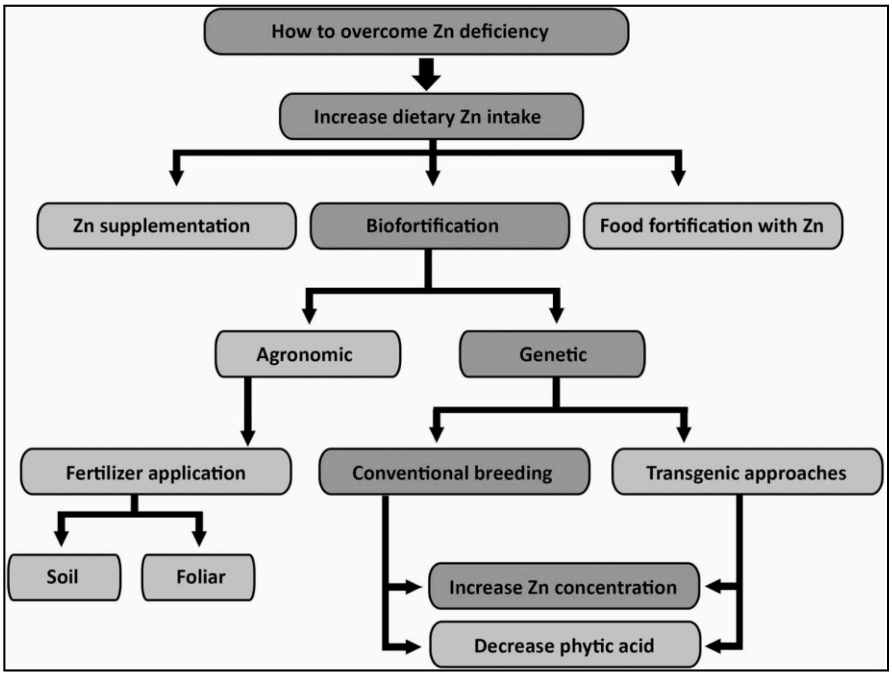

Fig 2: Approaches of zinc biofortification (Nakandalage *et al*. 2016; Zaman *et al*., 2018) [123, 230].

Agronomic Biofortification of zinc in rice

Among the different approaches that are involved in improving the zinc availability in the grain the easiest one is the agronomic biofortification.This agronomic processes involve strategies, such as fertilizer soil application, foliar applications, and seed dipping which are practically and effectively employed to increase the tissue micronutrient content of rice and other crops in a short period of time (Cakmak *et al*., 2010; Yuan *et al*., 2013; Wang *et al*., 2016) [26, 229, 208]; however, the repeated applications involves higher costs of labor and extra relevant expense, thus is a comparatively expensive measure (Velu *et al*., 2014; Zhang *et al*., 2018) [201, 203, 232]. The most common method of application is root-applied Zn (Jiang *et al*., 2007) [89], although foliar application is much more efficient (Yilmaz *et al*. 1997; Cakmak *et al*., 2010; Zou *et al*., 2012; Jan *et al*. 2016) [225, 26, 239, 87]. This can also be explained more categorically as (i) soil applied zinc, which is absorbed by roots followed by xylem pathways in the storage tissues and finally the grains by phloem (Pottier *et al*., 2014; Cakmak and Kutman, 2018) [29] winning over the impediments of high pH of phloem sap and chelation tendency (Impa and Johnson-Beebout, 2012) [80] and (ii) foliar applied easily translocated Zn in the plants based on plant nutritional status, germplasm and phenological stage (Waters *et al.*, 2009; Kutman *et al.*, 2012; Sperotto, 2013; Saha *et al*., 2017) [210, 101, 183, 159].

Soil Zn application

The most widely employed method of Zn application is its soil based application which can be executed through broadcasting, band placement, or fertigation (Zaman *et al*., 2018) [230]. However, the efficiency of Zn fertilizers applied varies considerably for waterlogged and aerobic rice system (Velu *et al*., 2014) [201, 203]. The timing of application is also vital (Rehman, 2012) [151]. It was interesting to note that a shift in rice cultivation from flooded to dry sowing aggravated the Zn deficiency problem as the involvement of a large number of factors come into foreground like N transformations, root growth, mycorrhizal inoculation, metabolites and root exudates, and soil factors *viz*., pH, anions and redox conditions (Gao *et al*., 2006; 2012) [51, 50]. The fertilizers with higher solubility (e.g. Zn- EDTA and $ZnSO_4$) usually transport greater Zn to the roots compared with insoluble ZnO or fritted Zn; however research from Harvest Zinc project (www.harvestzinc.org), reported very little effect of soil Zn applications at the time of sowing on the concentration of Zn in the grain under field conditions (Cakmak and Kutman, 2018) [29]. Even research carried out in India suggests that soil application had increased grain yield but could not contribute significantly to grain Zn load irrespective of type of fertilizer applied (Naik and Das, 2008; Ram *et al*., 2015; Rattan, 2017) [122, 145, 149].

Seed treatment of Zn fertilization

Zn seed treatments were initiated in the early 1970s but to limited success (Giordano and Mortvedt, 1973; Mengel *et al*., 1976; Haghighat and Thompson, 1982) [56, 114, 67]. Applying Zn in the seed as a starter-fertilizer can effectuate good crop yield, but cannot increase the Zn load; thus application of seed based Zn fertilizer at sowing as well as specific stages is necessary to enhance both grain yield and grain Zn content (Stomph *et al*. 2011) [187]. The seed treatment process for categorizing Zn application can be practiced by two ways: seed priming and seed coating (Farooq *et al*. 2012) [46]. The seed priming is a simple and low-cost technique of soaking seeds in solutions of desired nutrients for a certain period of time (Zaman *et al*., 2018) [230]. The seed treatment is comparatively better equipped than soil application owing to smaller quantity of requirements and ease of application. It seldom improves Zn concentration in rice grain, although is associated with improved germination and seedling growth under stressful environment (Shivay *et al*., 2008) [171]. In an experiment, Slaton *et al.* (2001) [178] reported that rice seed treatment with $ZnSO_4$ solution or liquid 9% Zn-EDTA chelate (wt./wt.) was not able to improve Zn load sufficiently in edible rice grains.

Seedling root dipping of Zn

Although seedling root dipping is a cheaper solution to the worldwide phenomenon of Zn induced malnutrition to the human races, it is still not widely carried out till now (Johnson *et al*., 2005; Cakmak, 2008; Zaman *et al*., 2018) [92, 28, 230]. The dominant mechanism of such transport is believed to be diffusion although the efficiency to improve the plant growth and yield parameters *viz.* number of panicle bearing tillers, panicle length, number of grains per panicle and test weight was much lower (Rashid, 2001) [146]. Dipping of rice seedlings in ZnO slurry before transplanting or application of Zn solution of different sources can be carried out to combat Zn deficiency (Robson, 2012). Thus the process of seedling root dipping is an easy and economical method of Zn treatment but comparative less efficient and unsuitable for biofortification purpose (Imran *et al*., 2015) [81].

Foliar Zn application

The discovery of Zn as an essential micronutrient for plants (Sommer & Lipman, 1926) [182] ushered the research on fertilization of crop plants with Zn fertilizers either through soil or foliar application (Cakmak and Kutman, 2018) [29]. The basis of the application is that zinc finds its way through leaf stomata to the vascular system when it is applied as a foliar spray (Zaman *et al*., 2018) [230] or more categorically foliar-applied Zn is phloem-mobile and can be readily translocated into developing grains (Haslett *et al*., 2001; Erenoglu *et al.*, 2011) [72]. A number of Zn sources, namely $ZnSO_4$, $Zn(NO_3)_2$, and Zn-EDTA, have been used on many crops (McBeath & McLaughlin 2014; Sharifianpour *et al*. 2015) [111, 170]. The foliar application of Zn has been proved to be an effective technique to increase grain Zn concentration to overcome Zn deficiency (Stomph *et al*. 2011; Zaman *et al*., 2018) [187, 230] even to the tunes of 2.5-3.5 times higher value (Yilmaz *et al*., 1997; Ram *et al*., 2015) [225, 145]. Zhou *et al.* (2012) [238] and Phattarakul *et al.* (2012) [137] reported Zn applied in the form of foliar application of $ZnSO_4$ improved the grain Zn by 27%. Significant increase in plant parameters like grain yield, straw biomass and grain Zn content were observed with foliar application of Zn as Zn-EDTA and $ZnSO_4$ (Benedicto *et al*. 2011) [14]; at proper time (Welch *et al*., 2013) [214] especially at the time of flowering (Pandey *et al*., 2013) [134] or grain-filling stage (Cakmak *et al*., 2010; Boonchuay *et al*., 2013; Abdoli *et al*., 2014) [26, 19, 1] because of low binding of Zn in soil. However, the agronomic biofortification is only supplemental for short term benefits, the longer duration viable strategies can be only obtained through plant breeding and genetic engineering approaches (Rattan, 2017; Zhang *et al*., 2018) [149, 232].

Evaluation of agronomic biofortification

The simplest means to estimate Zn biofortification is by collecting the grains as a whole or separating them into *un-husked* (whole grain with husk), *brown* (whole caryopsis with husk removed by hand) and *white* (outer layers of the caryopsis including pericarp, testa, nucella and part of the aleurone layer along with the embryo removed by polishing in standard laboratory mill) at harvest, rinsing with distilled water, oven drying, acid digestion and analysis for the zinc content by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Phattarakul *et al*., 2012) [137] or Atomic Absorption Spectrophotometry (AAS) (Saha *et al*., 2017) [159] or Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Seth *et al*., 2017) [168].

The enrichment of Zn in grains can also be assessed through changes in other important nutritional traits of grain (Saha *et al*., 2017) [159] *viz*., concentrations of Fe (Giordano and Mortvedt, 1972) [57] and phytic acid, the phytic acid: Zn molar ratio (Cakmak *et al*., 2010; Hussain *et al*., 2012) [26], protein (Cakmak, 2008) [28] etc. A decrease in the phytate content in grains can increase the bioavailability of the mineral micronutrient (Zhao and Shewry, 2011; Tyagi *et al*., 2018) [237, 19]. Based on the molecular weight of phytic acid = 660 Da, zinc = 65 Da, it is computed as,

$$\text{PA/Zn molar ratio} = \frac{(\text{Phytic acid content in mg/kg})/660}{(\text{Zinc content in mg/kg})/65}$$

This can also be carried out using a model Zn biofortification in the rice grain will be further analysed using a model factoring in Zn and phytate concentration (Miller *et al*., 2007)

$$TAZ=0.5[A_{max}+TDZ+K_R(1+TDP/K_P)^2-\{(A_{max}+TDZ+K_R(1+TDP/K_P))^2-4A_{max}+TDZ\}^{1/2}]$$

Where, TAZ = total daily absorbed Zn (m mol day^{-1}), TDP = total daily dietary phytate (m mol day^{-1}), TDZ = total daily dietary Zn (m mol d^{-1}), A_{max}= maximum Zn absorption (0.091), K_R= equilibrium dissociation constant of Zn-receptor binding reaction (0.033), K_P= equilibrium dissociation constant of Zn-phytate binding reaction (0.680) developed through isotope studies in gastrointestinal tract of humans (Hussain *et al*., 2012; Saha *et al*., 2017; Rehman *et al*., 2018; Wang *et al*., 2019; Das *et al*., 2019; Maqbool and Beshir, 2019) [159, 207, 37, 39, 152].

Genetic biofortification of zinc in rice

The genetic strategy, due to its highly cost-effectiveness, seems like an optimal way to tackle malnutrition originated due to the deficiency of Zn in diet (Khoshgoftarmanesh *et al*., 2010; Meenakshi *et al*., 2010) [97, 113]. For long term and stable benefits to be accrued to combat malnutrition, genetic manipulations to augment Zn load in rice endosperm deserve special emphasis (Swamy *et al*., 2016) [195] inspite of the complexity such as lack of a target gene, interactions between genotypes and environments, potential food safety risks etc (Solymosi and Bertrand, 2012) [181]. Different transgenic and conventional breeding approaches have already been made to have better and stable Zn density traits (Graham and Welch, 1996; Graham *et al*., 1999, Goto *et al*. 1999; Gregorio *et al*. 2000) [60, 61, 58, 63].

Genetic basis of grain Zn

The zinc distribution within the rice grain has a varying pattern, where the aleurone layer has around 25-30% of the grain zinc while the endosperm posseses 60-75% 0f the grain zinc which is retained even after polishing (Hansen *et al*. 2009) [69]. So it is obvious that the major goal of rice biofortification is increasing the bioavailable zinc in the endosperm.

The genetic basis of high grain Zn in brown/polished rice is rather complex and requires a better understanding for the systematic utilization of rice germplasm in Zn biofortification programs. Moderate to high broad sense heritability is reported for grain Zn and it can be improved by breeding (Norton *et al*. 2010; Zhang *et al*. 2014) [127, 188], Additive and dominant genetic effects were confirmed from the reports which showed narrow sense heritability for the grain Zn. Reports suggest that grain Zn has been found to be significantly influenced by environmental factors (Gregorio 2002; Chandel *et al*. 2010; Anuradha *et al*. 2012a) [62, 30, 31].

Genetic characterization of grain Zn in several Recombinant Inbred Lines (RILs) and also in rice germplasm collections has shown significant Phenotypic Co-efficient of Variation (PCV), Genotypic Co-efficient of Variation (GCV), broadsense Heritability and Genetic Advance (GA) as depicted in Table-1.

Table 1: Genetic parameters for grain Zinc concentration in rice (Swamy *et al*., 2016) [195].

Sl. No	Population	PCV (%)	GCV (%)	Heritability (%)	Genetic advance (% mean)	Reference
1	ADT 37 × IR68144-3B-2-2-3	19.2	18.6	94.2	37.2	Sala *et al*. 2013 [160]
2	ADT 43 × IR68144-3B-2-2-3	15.6	15.2	94.1	30.4	Sala *et al*. 2013 [160]
3	TRY (R) 2 × Mapillaisamba	9.3	9.2	96.8	18.6	Sala *et al*. 2013 [160]
4	TRY (R) 2 × IC 255787	17.2	17.0	98.0	34.8	Sala *et al*. 2013 [160]
5	Rice land races	21.9	18.4	70.6	31.9	Thongbam *et al*. 2012 [197]
6	Rice hybrids	11.7	10.8	85.8	20.7	Babu *et al*. 2012 [7]
7	BPT5204 × HPR14	26.1	26.0	99.4	53.6	Samak *et al*. 2011 [162]
8	Rice genotypes	25.5	21.1	94.0	30.1	Bekele *et al*. 2013 [13, 48]
9	IRRI38 × Jeerigesanna	18.4	17.0	85.6	32.5	Gande *et al*. 2013 [48]
10	F_2 population	-	-	96.9	-	Zhang *et al*. 2004 [233]
11	BIL mapping population	10.8	-	76.4	-	Susanto 2008 [191]
12	Azucena × Moromutant	40.1	36	80.6	66.6	Bekele *et al*. 2013 [13, 48]
13	Bala × Azucena	-	-	>60	-	Norton *et al*. 2010 [127]
14	Teqing × *O rufipogon*	-	-	41	-	Garcia-Oliveira *et al*. 2009 [52]

The data registered from the above mentioned reports suggests suuficient variation for grain Zn concentration along with moderate to high heritability and genetic advance. The combining ability analysis obtained from the diallele crosses comprising seven specific rice varieties with different levels of grain Zn revealed the additive genetic effects to be more important for grain Zn, whereas the coefficient of variation (CV) for grain Zn sundry significantly among the entries over the years and locations, thereby hinting significant genotype and environment interactions (G x E) (Zhang *et al*. 1996; Sharifi 2013) [234]. In another study by Zhang *et al*. 2004 [233] involving black pericarp indica rice it was reported that genetic and cytoplasmic effects influenced final grain Zn content, however the genetic effect was stronger. Single plant selection was suggested as an effective approach fror improving Zn content as it was observed that the heritability for the seed geetic effect was highly significant as well as the narrow sense heritability registered very high values. Positive correlation was filed between grain Zn and the other grain characteristics like grain weight, grain length and width of the grain, so the grain Zn content can be indirectly selected by considering these grain traits. However platykurtic and skewed distributions were observed for grain Zn in a RIL population, which indicated presence of minor genes with duplicate gene actions. (Banu and Jagadeesh 2014) [70].

Some reports also minuted positive heterosis for grain Zn. In a line X tester analysis which involved six lines and eight testers with a total of 48 hybrids, it was reported that 14 out of those 48 hybrids showed significant positive heterosis for grain Zn content over the standard chech Chittimutyalu. Among these 14 crosses two crosses (PR116×Chittimutyalu, MandyaVijay×Jalamagna) registered more than 50% heterosis for grain Zn (Babu *et al*. 2012) [7]. Transgressive seggregants were also recovered for grain Zn content. (Stangoulis *et al*. 2007) [185].

Some studies reveal that grain zinc is highly linked with aroma while there is no pleiotropic effect of grain Zn on any other character. (Welch and Graham 2004; Gregorio 2002) [212, 62]. Some researchers reported epistatic interactions for grain Zn (Lu *et al*. 2008; Norton *et al*. 2010) [127]. It has been registered that in some cases, the genetic factors that increase the available Zn also co-segregate with the genetic factors involved in increasing Fe and other mineral elements (Gregorio 2002; Jiang *et al*. 2007) [62, 79]. Some reports also suggest that the grain quality and grain Zn are correlated to each other (Anandan *et al*. 2011; Zhang *et al*. 2004) [4, 233]. All the grain quality traits and other minerals with which the grain Zn content are associated should be taken into consideration while breeding for high grain Zn content. One of the pivotal aspect while breeding for high grain Zn content is the relation of grain Zn and grain yield. Several studies have registered a significant negative correlation between grain Zn concentration and grain yield in rice (Gao *et al*. 2006; Jiang *et al*. 2008; Norton *et al*. 2010; Wissuwa *et al*. 2007) [51, 90, 127], but contradictingly a positive correlation between grain yield and grain Zn concentration was observed under Zn deficient soil conditions (Gregorio 2002) [62]. No significant correlation was observed between grain Zn concentration and grain yield under Zn sufficient soil conditions for different panel of aromatic rice and land races. (Gangashetty *et al*. 2013; Sathisha 2013) [49, 164]. This report is also supported by the study of Rai *et al*. (2012) which stated non-significant correlation between grain yield and Zn in other cereal crops like pear millet. Therefore it is safe to conclude that it is possible to develop high Zn rice varieties having good yield potential. Evidence for the possibility of combining high grain Zn concentration and high yield potential in rice (Harvest Plus 2014) [71] can be assured from the identification of high Zn donor lines with high yield, transgenic rice lines with high Zn concentration (Johnson *et al*. 2011; Trijatmiko *et al*. 2016) [91, 198] and the recently released high Zn rice lines from Bangladesh.

Conventional Breeding as a tool for Zn Biofortification

Genetic biofortification is a strategy that uses plant breeding techniques to increase the micronutrient levels in staple food crops (Harvest Plus 2014) [71]. Production of high yielding rice varieties has been the major focus of rice breeding programs and selection of rice with high grain micronutrient concentrations has largely been ignored as a breeding objective, since the breeders gave major focus on traits such as size, shape, appearance of grain, milling quality and cooking features (Graham and Welch, 1996) [60]. However recently emphasis has been given on nutritional aspects since micronutrient deficiency especially of Zn and Fe has become globally well recognized. (Welch, 1993; Seneweera *et al*., 1996; Kennedy *et al*., 2002; Seneweera, 2011; Kant *et al*., 2012; Myers *et al*., 2014) [213, 165, 96, 166, 93, 119]. Genetic biofotification helps to develop mineral enriched crops by utilizing the natural genetic variation present in the germplasm and therby providing sustainable solution to malnutrition problems (Bouis 2003; Pfeiffer and McClafferty 2008) [61, 136]. Gregorio *et al*. (2000) [63] and Prom-u-thai *et al*. (2007) [142] reported considerable amount of genetic variation for grain Zn concentration in rice germplasm. In a study it was reported that Indica rice had almost two fold higher Zn concentration whereas slightly lower Fe concentration than Japonica (Yang *et al*., 1998. Anandan *et al*., 2011) [224, 4] in his study reported significantly lower Zn content in the seeds of modern rice cultivars compared to the landraces. Reports suggest that some wild species of rice like *O. nivara*, *O. rufipogon*, *O. latifolia*, *O. officinalis* and *O. granulata* contains around two- three fold higher Zn concentration than in cultivated rice. The Zn concentration ranges from 37 mg/kg to 55 mg/kg in non polished grains (Cheng *et al*. 2005; Banerjee *et al*. 2010; Anuradha *et al*. 2012) [10, 30, 31, 34]. Gregorio (2002) [62] registered that aromatic rice contained higher Zn concentration compared to no aromatic rice cultivars.

Breeders always prefer simple and precise phenotyping to identify high Zn rice variety. Fast, accurate and inexpensive methods are required since breeding programs handles large population which requires inch-perfect screening. Seed sampling, hulling, and milling procedures without any metal contaminations have already been standardized for rice (Stangoulis and Sison 2008). Atomic absorption spectrometry (AAS) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) are used for elemental analysis (Zarcinas *et al*. 1987) [231]. Even though these methos are precise to the core but they are expensive and require highly skilled analysts, extensive samples and contamination free reagents (Velu *et al*. 2014) [201, 203]. Colorimetric approaches have been developed for several cereal crops to analyse Zn and Fe, however these methods are semi-quantitative and laborious when applied in large scale (Prom-u-thai *et al*. 2003; Ozturk *et al*. 2006; Choi *et al*. 2007; Velu *et al*. 2008) [141, 129, 35, 202]. In a study it was established that non-destructive determination of Zn and Fe concentration to discard low Zn enriched rice lines can be served best by X-Ray Fluoroscence (XRF). It is also registered that the high Zn lines obtained by this method can be validated with (Paltridge *et al*. 2012) [133]. It is reported that most of the biofortification programs aiming to increase the Zn concentration in rice are following XRF for metal analysis.

Breeding strategies for implementing Zn biofortifications

The genotypic variation present in rice germplasm can be exploited through breeding to enhance the grain Zn concentration. Since the genetic basis of grain Zn is complex with the involvement of multiple small effect genes/QTLs and significantly influenced by the environment, the choice of appropriate breeding methods, crossing programs, individual plant selections and field evaluation processes are critical for the successful development of high-Zn rice. Previously high Zn donor was crossed with popular cultivar which possessed low grain Zn. The selection was based on favourable agronomic traits from the segregating population with final fixed lines tested for grain Zn and yield in replicated large scale plots. However this method was time consuming and the resultant lines showed moderate increase in grain Zn content along with moderate yield potential. Recently a more convenient breeding method involving the cross between high Zn donors with acceptable yield potential and popular high yielding, highly adapted but low Zn rice varieties has hastened the process of high Zn variety development if early selection for Zn testing from F4 generation onwards along with selection for acceptable agronomic traits are performed. It has been reported that multiple crosses involving several donors and recipient parents such as three way, four way crosses along with reciprocal crosses has enhanced grain Zn content in rice genotypes. Multi-parent Advanced Generation Inter-Cross (MAGIC) is also an attractive method for pooling the genes for high Zn, and at IRRI several MAGIC populations such as MAGIC-indica, MAGIC-japonica and MAGIC-global (utilizing crosses between indica and japonica MAGIC lines) have been developed (Bandillo *et al*. 2013) [9] and these are a good resource for selecting high Zn lines and also provides an opportunity to select transgressive segregants for high Zn. Nagesh *et al*. 2012 reported that there is substantial amount of heterosis for grain Zn in rice. Wild relatives of rice such as *O. nivara*, *O. rufipogon*, *O. barthii*, and *O. longistminata*, and African cultivated rice *O. glaberrima* are found to have higher level of Zn in the grains and these are a potential source of high Zn donors (Garcia-Oliveira *et al*. 2009; Sarla *et al*. 2012) [52, 5]. These wild relatives can be exploited following advanced backcross breeding to combine high Zn and high yield potential. Mutation breeding is also gaining importance with respect of increasing grain Zn content in rice. A number of IR64 mutants produced by the treatment with Sodium azide were reported to have high Zn (Jeng *et al*. 2012) [88]. Three IR64 mutant lines *viz*; M-IR-180, M-IR-49, and M-IR-175 had more than 26 mg kg −1 Zn in polished rice as against 16 mg kg $^{-1}$ in IR64.

Marker assisted breeding for high Zn rice using major effect grain Zn QTLs is also a more faster and precise approach. Several major effect grain Zn QTLs with a high PV (>10%) and also gene-specific markers for grain Zn have been reported in rice, but use of these markers to assist breeding efforts to improve Zn concentration in rice has not been reported. Marker Assisted Recurrent Selection (MARS) and Genomics Assisted Breeding approaches are worth trying to develop high-Zn rice (Swamy *et al*., 2016) [195]. SNPs are widely used as marker for various breeding programmes. The cheaper, faster and high throughput SNP assays made it possible the routine use of markers in the breeding programs (McCouch *et al*. 2010; Swamy and Kumar 2013; Singh *et al*. 2015) [112, 194]. Varshney *et al*. 2009 [200] reported that next generation sequencing (NGS) and third generation sequencing (TGS) have revolutionalised breeding to many folds. It can be safely stated that rice varieties with high Zn can be developed with these methods. In rice, 3000 accessions have been sequenced and efforts are ongoing to sequence 10,000 accessions (Li *et al*. 2014) [103].

Some antinutritional element especially phytate limits the bioavailability of Zn. In rice Zn is preferentially stored along with phytate which is a strong chelator of divalent cations (Bohn *et al*. 2008; Hambidge *et al*. 2010; Petry *et al*. 2012) [18, 68, 135]. Hence, selections should be made for low phytate content. Recently by mutation breeding, several mutants with low phytate content have been developed and are good resources as low-phytate donors in breeding programs (Liu *et al*. 2007) [104]. The world's first Zn enriched rice variety was released in 2013 by the Bangladesh Rice Research Institute (BRRI dhan 62), which is claimed to contain 20–22 mg Zn kg^{-1} for brown rice. Nonetheless this is short of the target of 30 mg Zn kg^{-1} set by the HarvestPlus program (Shahzad *et al*., 2014) [20].

Modern biotechnological (transgenic) approach towards Zn biofortification

Crops such as rice, cassava, oilseeds and potatoes are used to develop transgenics. Advanced biotechnological tools are used to increase micronutrients like Zn, Fe and Vitamin A. Researches are also going on to improve other nutritional factors such as essential amino acids and fatty acids, as well as reduced antinutritional factors (such as cyanogens and phytates) (Gilani *et al*. 2007) [55] Masuda *et al*. (2013) [6] increased the accumulation of the iron storage protein ferritin as well as enhanced iron translocation by over expressing the iron(II)-nicotianamine transporter OsYSL2 in rice endosperm. While yield remained similar to conventional rice, the transgenic lines produced higher levels of iron (6-fold in the green house and 4.4-fold in the paddy) and zinc (1.6-fold). Masuda *et al*. (2013) [6] successfully increased iron and zinc accumulation even more by enhancing the uptake and transport of iron using the ferric iron chelator, mugineic acid. Targeting Fe deficiency in rice can also result in an increased accumulation of Zinc. Aung *et al.* (2013) developed a transgenic line commonly used in mayanmar where surrounding area area is iron deficiet. These plants were developed so that they can accumulate nore concentrations of Fe and Zn. It was observed that the transgenic lines accumulated 3-4 times higher Fe concentration whereas they accumulated 1.3 folds more Zn contration compared to the nontransgenic lines. Banaker *et al*. (2017) reported transgenic plants expressing nicotinamine and 2'- deoxymugenic acid (DMA) to enhance the accumulation of Fe and Zn in rice endosperm. The transgenic lines developed showed 2 fold increases in Zn accumulation and 4 fold increases in Fe accumulation in the endosperm of the rice lines.

Conclusion

The issue of malnutrition throughout the world cannot be undermined. It is more severe in developing countries like India where it has imposed massive health and economic burden in the form of surge in the cases of mortality, morbidity, impaired physical and neurological development, a rapid drop in financial productivity and hike in health care expenses which is an alarming situation to the human race especially the poorer section of the society. Researches are underway to find the most suitable and viable option to face this crisis. The widely acclaimed process of biofortification can play a key role in this case. Its preeminent capacity to curb malnutrition for human welfare is not unknown. The main focus of this method is increasing the content of specific nutrient in rice edibles to augment the nutritional availability through the mostly consumed dietary consumable. The genetic biofortification emerges the most successful long term solution but its complexity in adoption can efficiently be complemented by agronomic biofortification processes. The future goal of this research emphasizes on development of most efficient method for Zn application, finding most viable fertilizer source through use of chelated Zn fertilizers and adopting strategies amenable to need based nutrient solubility and mobility; and adopting more efficient genetic, breeding, molecular and biotechnological approaches; to serve the basic nutrient needs of the teeming millions.

References

1. Abdoli M, Esfandiari E, Mousavi SB, Sadeghzadeh B. Effects of foliar application of zinc sulfate at different phenological stages on yield formation and grain zinc content of bread wheat (cv. Kohdasht). Azarian Journal of Agriculture. 2014; 1:11-16.
2. Adamo P, Agrelli D, Zampella M. Chemical speciation to assess bioavailability, bioaccessibility and geochemical forms of potentially toxic metals (PTMs) in polluted soils. In Environmental Geochemistry, Elsevier, 2018, 153-194.
3. Ahnstrom ZS, Parker DR. Development and assessment of a sequential extraction procedure for the fractionation of soil cadmium. Soil Science Society of America Journal. 1999; 63(6):1650-1658.
4. Anandan A, Rajiv G, Eswaran R, Prakash M. Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. Journal of food science. 2011; 76(4):H122-H130.
5. Anuradha K, Agarwal S, Rao YV, Rao KV, Viraktamath BC, Sarla N. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. Gene. 2012; 508(2):233-240.
6. Aung MS, Masuda H, Kobayashi T, Nakanishi H, Yamakawa T, Nishizawa NK. Iron biofortification of Myanmar rice. Frontiers in Plant Science. 2013; 4:158.
7. Babu VR, Shreya K, Dangi KS, Usharani G, Nagesh P. Genetic variability studies for qualitative and quantitative traits in popular rice (*Oryza sativa* L.) hybrids of India. International Journal of Scientific and Research Publications. 2012; 2(6):1-5.
8. Banakar R, Alvarez Fernandez A, Díaz-Benito P, Abadia J, Capell T, Christou P. Phytosiderophores determine thresholds for iron and zinc accumulation in biofortified rice endosperm while inhibiting the accumulation of cadmium. Journal of experimental botany. 2017; 68(17):4983-4995.
9. Bandillo N, Raghavan C, Muyco PA, Sevilla MAL, Lobina IT, Dilla-Ermita CJ *et al*. Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. Rice. 2013; 6(1):11.
10. Banerjee S, Sharma DJ, Verulkar SB, Chandel G. Use of in silico and semiquantitative RT-PCR approaches to develop nutrient rich rice (*Oryza sativa* L.), 2010.
11. Barker AV, Pilbeam DJ. Handbook of plant nutrition. CRC press, 2015.
12. Begum MC, Islam M, Sarkar MR, Azad MAS, Huda AN, Kabir AH. Auxin signaling is closely associated with Znefficiency in rice (*Oryza sativa* L.). J Plant Interact. 2016; 11:124-129.
13. Bekele DB, Naveen GK, Rakhi S, Shashidhar HE. Genetic evaluation of recombinant inbred lines of rice (*Oryza sativa* L) for grain Zinc concentrations, yield related traits and identification of associated SSR markers. Pak J Biol Sci. 2013; 6:1714-1721.
14. Benedicto A, Hernández-Apaolaza L, Rivas I, Lucena JJ. Determination of Zn distribution in navy bean (*Phaseolus vulgaris* L.) after foliar application of ^{67}Zn–

lignosulfonates using isotope pattern deconvolution. J Agricult Food Chem. 2011; 59:8829-8838.

15. Bhattacharya P, Sengupta S, Halder S. Customized, Fortified and Nano Enabled Fertilizers-Prioritizing and Profiteering Sustainability in Agriculture. In: Advances in Agriculture Sciences; Vol-19 (R.K. Naresh, Eds.), Akinik Publications, New Delhi, 2019, 69-97. (ISBN-978-93-5335-728-3).
16. Bhullar NK, Gruissem W. Nutritional enhancement of rice for human health: the contribution of bio-technology. Biotechnol Adv. 2013; 31(1):50-57.
17. Black RE, Lindsay HA, Bhutta ZA, Caulfield LE, de Onnis M, Ezzati M *et al*. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008; 371:243-260.
18. Bohn L, Meyer AS, Rasmussen SK. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. Journal of Zhejiang University Science B. 2008; 9(3):165-191.
19. Boonchuay P, Cakmak I, Rerkasem B, Prom–U–Thai C. Effect of different growth stages on seed zinc concentration and its impact on seedling vigor in rice. Journal of Soil Science & Plant Nutrition. 2013; 59:180-188.
20. Bouain N, Shahzad Z, Rouached A, Khan GA, Berthomieu P, Abdelly C *et al*., Phosphate and zinc transport and signalling in plants: toward a better understanding of their homeostasis interaction. Journal of Experimental Botany. 2014; 65(20):5725-5741.
21. Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH. Biofortification: a new tool to reduce micronutrient malnutrition. Food and nutrition bulletin. 2011; 32(1):S31-S40.
22. Brennan RF. Zinc application and its availability to plants (Doctoral dissertation, Murdoch University), 2005.
23. Brennan RF, Armour JD, Reuter DJ. Diagnosis of zinc deficiency. In ‘Zinc in soil and plants’. (Ed. AD Robson), 1993, 168-181.
24. Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. Zn in plants. New Phytol. 2007; 173:677-702.
25. Cakmak I. Plant nutrition research: Priorities to meet human needs for food in sustainable ways. Plant Soil. 2002; 247:3-24.
26. Cakmak I, Pfeiffer WH, McClafferty B. Review: biofortification of durum wheat with zinc and iron. Cereal Chem. 2010; 87:10-20.
27. Cakmak I. Role of Zn in protecting plant cells from reactive oxygen species. New Phytol. 2000; 146:185-205.
28. Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant and Soil. 2008; 302:1-17.
29. Cakmak I, Kutman UB. Agronomic biofortification of cereals with zinc: a review. European Journal of Soil Science. 2018; 69(1):172-180.
30. Chandel G, Banerjee S, See S, Meena R, Sharma DJ, Verulkar SB. Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. Rice Sci. 2010; 17:213-227.
31. Chandel G, Banerjee S, See S, Meena R, Sharma DJ, Verulkar SB. Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. Rice Science. 2010; 17(3):213-227.
32. Chang HB, Lin CW, Huang HJ. Zinc-induced cell death in rice (*Oryza sativa* L.) roots. Plant Growth Regul. 2005; 46:261-266.
33. Chattha MU, Hassan MU, Khan I, Chattha MB, Mahmood A, Nawaz M *et al*. Biofortification of wheat cultivars to combat zinc deficiency. Frontiers in plant science. 2017; 8:281.
34. Cheng ZQ, Huang XQ, Zhang YZ, Qian J, Yang MZ, Wu CJ *et al*. Diversity in the content of some nutritional components in husked seeds of three wild rice species and rice varieties in Yunnan Province of China. Journal of Integrative Plant Biology. 2005; 47(10):1260-1270.
35. Choi EY, Graham RD, Stangoulis JCR. Rapid semi-quantitative screening methods for determination of iron and zinc in grains and staple foods. Journal of Food Composition and Analysis. 2007; 20(6).
36. Chuan MC, Shu GY, Liu JC. Solubility of heavy metals in a contaminated soil: Effects of redox potential and pH. Water, Air, and Soil Pollution. 1996; 90(3-4):543-556.
37. Das A, Singh SK, Kumar M, Kumar O. Evaluation of Different Methods of Zinc Application on Growth, Yield and Biofortification of Zinc in Rice (*Oryza sativa* L.). Journal of the Indian Society of Soil Science. 2019; 67(1):92-102.
38. Das S, Green A. Zinc in crops and human health. In Biofortification of food crops. Springer, New Delhi, 2016, 31-40
39. Das S, Chaki AK, Hossain A. Breeding and agronomic approaches for the biofortification of zinc in wheat (*Triticum aestivum* L.) to combat zinc deficiency in millions of a population: a Bangladesh perspective. Acta Agrobotanica, 2019, 72(2).
40. Deb DL. Development of soil and plant analytical methods for micronutrients and sulphur in Srilanka. GCPF/SRI/047/NET field document 1992, 11
41. Depar N, Rajpar I, Memon MY, Imtiaz M. Mineral nutrient densities in some domestic and exotic rice genotypes. Pakistan Journal of Agriculture: Agricultural Engineering Veterinary Sciences (Pakistan). 2011; 27:134-142.
42. Dhaliwal GS, Jindal V, Mohindru B. Crop losses due to insect pests: global and Indian scenario. Indian Journal of entomology. 2015; 77(2):165-168.
43. Emmerson RHC, Birkett JW, Scrimshaw M, Lester JN. Solid phase partitioning of metals in managed retreat soils: field changes over the first year of tidal inundation. Science of the Total Environment. 2000; 254(1):75-92.
44. Epstein E, Bloom AJ. Mineral nutrition of plants: principles and perspectives, 2nd Edn. Sinauer Assoc. Inc., Sunderland, UK, 2005.
45. Erenoglu EB, Kutman UB, Ceylan Y, Yildiz B, Cakmak I. Improved nitrogen nutrition enhances root uptake, root-to-shoot translocation and remobilization of zinc (^{65}Zn) in wheat. New Phytologist. 2011; 189:438-448.
46. Farooq M, Wahid A, Siddique KHM. Micronutrient application through seed treatments: a review. J Soil Sci Plant Nutr. 2012; 12:125-142.
47. Filgueiras AV, Lavilla I, Bendicho C. Chemical sequential extraction for metal partitioning in environmental solid samples. Journal of Environmental Monitoring. 2002; 4(6):823-857.
48. Gande NK, Soman R, Kundur PJ, Ambati R, Bekele DB,

Shashidhar HE. Evaluation of recombinant inbred lines of rice (*Oryza sativa* L.) for grain zinc content, yield related traits and identification of Transgress ant lines grown under aerobic conditions. Asian J Exp. Biol Sci. 2013; 4:567-574.

49. Gangashetty PI, Salimath PM, Hanamaratti NG. Genetic variability studies in genetically diverse non-basmati local aromatic genotypes of rice (*Oryza sativa* (L.). Rice Genomics and Genetics. 2013; 4(1).
50. Gao X, Hoffland E, Stomph T, Grant CA, Zou C, Zhang F. Improving zinc bioavailability in transition from flooded to aerobic rice. A review. Agron Sustain Dev. 2012; 32:465-478.
51. Gao X, Zou C, Fan X, Zhang F, Hoffland E. From flooded to aerobic conditions in rice cultivation: consequences for zinc uptake. Plant Soil. 2006; 280:41-47.
52. Garcia-Oliveira AL, Tan L, Fu Y, Sun C. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. Journal of Integrative Plant Biology. 2009; 51(1):84-92.
53. Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V *et al*. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Frontiers in nutrition. 2018; 5:12.
54. Gibson RS. Zinc: the missing link in combating micronutrient malnutrition in developing countries. Proc Nutr Soc. 2006; 65:51-60.
55. Gilani GS, Nasim A. Impact of foods nutritionally enhanced through biotechnology in alleviating malnutrition in developing countries. Journal of AOAC International. 2007; 90(5):1440-1444.
56. Giordano PM, Mordvedt JJ. Zinc sources and methods of application for rice. Agron. J. 1973; 65:51-53.
57. Giordano PM, Mortvedt JJ. Rice response to zinc in flooded and non-flooded soil. J Agron. 1972; 64:521-524.
58. Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F. Iron fortification of rice seed by the soybean ferritin gene. Nature biotechnology. 1999; 17(3):282.
59. Goud BR, Nanjappa HV, Ramachandrappa BK. Influence of Doses of Customized Fertilizer on Nutrient Uptake and Yield of Finger millet (*Eleusine coracana* (L.) Gaertn.). Indian Journal of Dryland Agricultural Research and Development. 2013; 28(2):77-79.
60. Graham RD, Welch RM. Breeding for staple food crops with high micronutrient density Intl Food Policy Res Inst, 1996, 3.
61. Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field crops research. 1999; 60(1, 2):57-80.
62. Gregorio GB. Progress in breeding for trace minerals in staple crops. Symp. Plant Breeding: A New Tool for Fighting Micronutrient Malnutrition. In Journal of Nutrition, 2002, 500S-502S.
63. Gregorio GB, Senadhira D, Htut H, Graham RD. Breeding for trace mineral density in rice. Food and Nutrition Bulletin. 2000; 21(4):382-386.
64. Grill E, Winnacker EL, Zenk MH. Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science. 1985; 230(4726):674-676.
65. Grundon NJ. Hungry crops: A guide to nutrient deficiencies in field crops. Brisbane, Australia: Queensland Government, 1987, 246.
66. Guerinot ML. The ZIP family of metal transporters. Biochim Biophys Acta. 2000; 1465:190-198.
67. Haghighat NG, Thompson LF. Zinc seed coating studies with rice. p. 74-75. In Proc. Rice Tech. Working Group, 19th, Univ. of Arkansas, Hot Springs, AR. 23-25 Feb. 1982. Texas Agric. Exp. stn. Texas A&M Univ., College Station, 1982, TX 77843-2124.
68. Hambidge KM, Miller LV, Westcott JE, Sheng X, Krebs NF. Zinc bioavailability and homeostasis. The American journal of clinical nutrition. 2010; 91(5):1478S-1483S.
69. Hansen TH, Laursen KH, Persson DP, Pedas P, Husted S, Schjoerring JK. Micro-scaled high-throughput digestion of plant tissue samples for multi-elemental analysis. Plant Methods. 2009; 5(1):12.
70. Harshiya B, Jagadeesh BN. Genetic analysis of skewness and Kurtosis for yield and its parameters, total grain protein, macro and micro nutrients in F7 generation of rice (*Oryza sativa* L.). Environment and Ecology. 2014; 32(1A):381-385.
71. Harvest Plus. Biofortification progress briefs, 2014. www.HarvestPlus.org
72. Haslett BS, Reid RJ, Rengel Z. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. Annals of Botany. 2001; 87:379-386.
73. Havlin JL, Beaton JD, Tisdale SL, Nelson WL. Soil fertility and fertilizers. New Jersey: Pearson, 2005, 515.
74. Hefferon K. Biotechnological Approaches for Generating Zinc-Enriched Crops to Combat Malnutrition. Nutrients. 2019; 11(2):253.
75. Hess SY, Brown KH. Impact of zinc fortification on zinc nutrition. Food Nutr Bull. 2009; 30(1):S79-S107.
76. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 2004; 25:94-204.
77. Hotz C. The potential to improve zinc status through biofortification of staple food crops with zinc. Food and Nutrition Bulletin. 2009; 30(1):S172-S178.
78. Hussain S, Maqsood MA, Rengel Z, Aziz T. Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. Plant Soil. 2012; 361:279-290.
79. Impa SM, Morete MJ, Ismail AM, Schulin R, Johnson-Beebout SE. Zn uptake, translocation, and grain Zn loading in rice (*Oryza sativa* L.) genotypes selected for Zn deficiency tolerance and high grain Zn. J Exp Bot. 2013; 64:2739-2751.
80. Impa SM, Johnson-Beebout SE. Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. Plant Soil. 2012; 361:3-41.
81. Imran M, Kanwal S, Hussain S, Aziz T, Maqsood MA. Efficacy of zinc application methods for concentration and estimated bioavailability of zinc in grains of rice grown on a calcareous soil. Pakistan Journal of Agricultural Sciences. 2015; 52(1).
82. Ishimaru Y, Bashir K, Nishizawa N. Zn uptake and translocation in rice plants. Rice. 2011; 4:21-27.
83. Ishimaru Y, Masuda H, Suzuki M, Bashir K, Takahashi M, Nakanishi H *et al*. Over expression of the OsZIP4 zinc transporter confers disarrangement of zinc distribution in rice plants. J Exp Bot. 2007; 58:2909-2915.
84. Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S *et al*. OsZIP4, a novel Zn-regulated Zn transporter in rice. J Exp Bot. 2005; 56:3207-3214.

85. Itanna F, Coulman B. Phyto-extraction of copper, iron, manganese, and zinc from environmentally contaminated sites in Ethiopia, with three grass species. Communications in soil science and plant analysis. 2003; 34(1-2):111-124.
86. Jaga PK, Patel Y. An overview of fertilizers consumption in India: determinants and outlook for 2020-a review. Int J Sci Eng Technol. 2012; 1(6):285-291.
87. Jan M, Anwar-ul-Haq M, Tanveer-ul-Haq AA, Wariach EA. Evaluation of soil and foliar applied Zn sources on rice (*Oryza sativa* L.) genotypes in saline environments. Int J Agricul Biol. 2016; 18:643-648.
88. Jeng TL, Lin YW, Wang CS, Sung JM. Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the indica type cultivar IR64. Journal of food composition and analysis. 2012; 28(2):149-154.
89. Jiang W, Struik PC, Lingna J, van-Keulen H, Ming Z, Stomph TJ. Uptake and distribution of root applied or foliar applied Zn after flowering in aerobic rice. Ann Appl Biol. 2007; 150:383-391.
90. Jiang W, Struik PC, Van Keulen H, Zhao M, Jin LN, Stomph TJ. Does increased zinc uptake enhance grain zinc mass concentration in rice? Annals of Applied Biology. 2008; 153(1):135-147.
91. Johnson AA, Kyriacou B, Callahan DL, Carruthers L, Stangoulis J, Lombi E *et al*. Constitutive overexpression of the Os NAS gene family reveals single-gene strategies for effective iron-and zinc-biofortification of rice endosperm. PloS one. 2011; 6(9):e24476.
92. Johnson SE, Lauren JG, Welch RM, Duxbury JM. A comparison of the effects of micronutrient seed priming and soil fertilization on the mineral nutrition of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) in Nepal. Experimental Agriculture. 2005; 41(4):427-448.
93. Kant S, Seneweera S, Rodin J, Materne M, Burch D, Rothstein SJ *et al*. Improving yield potential in crops under elevated CO_2: integrating the photosynthetic and nitrogen utilization efficiencies. Frontiers in Plant Science. 2012; 3:162.
94. Katyal JC, Rattan RK. Distribution of zinc in Indian soils. Fertiliser News. 1993; 38(12):15-26.
95. Katyal JC, Sharma BD. The DTPA extract-able and total Zn, Cu, Mn and Fe in Indian soils and their association with some soil properties. Geoderma. 1991; 49:165-169.
96. Kennedy G, Burlingame B, Nguyen VN. Nutritional contribution of rice and impact of biotechnology and biodiversity in rice-consuming countries. The international rice commission. Twentieth session, Bangkok, Thailand, 2002.
97. Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M. Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture: a review. Agron Sustain Dev. 2010; 30(1):83-107.
98. Krauskopf KB. Geochemistry of micronutrients. Micronutrients in agriculture, 1972.
99. Krebs NF, Miller LV, Hambridge KM. Zinc deficiency in infants and children: a review of its complex and synergistic interactions. Paediatrics & International Child Health. 2014; 34:279-288.
100. Krishnan S, Dayanandan P. Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L). J Bioscience. 2003; 28:455-469.
101. Kutman UB, Kutman BY, Ceylan Y, Ova EA, Cakmak I. Contributions of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and nitrogen nutrition. Plant and Soil. 2012; 361:177-187.
102. Leitenmaier B, Küpper H. Compartmentation and complexation of metals in hyperaccumulator plants. Front Plant Sci. 2013; 4:374-380.
103. Li JY, Wang J, Zeigler RS. The 3,000 rice genomes project: new opportunities and challenges for future rice research. GigaScience. 2014; 3(1):8.
104. Liu QL, Xu XH, Ren XL, Fu HW, Wu DX, Shu QY. Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.). Theoretical and Applied Genetics. 2007; 114(5):803-814.
105. Lu L, Tian S, Liao H, Zhang J, Yang X, Labavitch JM *et al*. Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-ray fluorescence imaging of Zn, Fe, K, Ca, and Mn. PLoS One. 2013; 8(2):e57360.
106. Maclean JL, Dawe DC, Hardy B, Hettel GP. Rice almanac. 3rd edn. Wallingford, Oxon, 2002.
107. Majumdar K, Sanyal SK, Dutta SK, Satyanarayana T, Singh VK. Nutrient mining: Addressing the challenges to soil resources and food security. In Biofortification of food Crops Springer, New Delhi, 2016, 177-198.
108. Maqbool MA, Beshir A. Zinc biofortification of maize (*Zea mays* L.): Status and challenges. Plant breeding. 2019; 138(1):1-28.
109. Marschner H. Mineral Nutrition of Higher Plants Academic Press London, 1995, 889.
110. Masuda H, Ishimaru Y, Aung MS, Kobayashi T, Kakei Y, Takahashi M *et al*. Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. Scientific reports. 2012; 2:543.
111. McBeath TM, McLaughlin MJ. Efficacy of zinc oxides as fertilisers. Plant Soil. 2014; 374:843-855.
112. McCouch SR, Zhao K, Wright M, Tung CW, Ebana K, Thomson M *et al*. Development of genome-wide SNP assays for rice. Breeding Science. 2010; 60(5):524-535.
113. Meenakshi JV, Johnson NL, Manyong VM *et al*. How cost-effective is biofortification in combating micronutrient malnutrition? an ex ante assessment. World Dev. 2010; 38(1):64-75.
114. Mengel DB, Leonards WJ, Sedberry Jr JE. Effect of zinc oxide seed treatment on stand establishment, leaf zinc concentrations, and yield of Saturn rice. 68th Annual Progress Report, Rice Exp. Stn. Crowley, Louisiana. Louisiana State Univ. Agric. Exp. Stn. Crowley, Louisiana. Louisiana State Univ, Baton Rouge, 1976, 62-63.
115. Miller LV, Krebs NF, Hambidge KM. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. J Nutr. 2007; 137:135-141.
116. Minkina TM, Bauer TV, Batukaev AA, Mandzhieva SS, Burachevskaya MV, Sushkova SN *et al*. Transformation of Technogenic Cu and Zn Compounds In Chernozem. Environmental Engineering & Management Journal (EEMJ). 2015; 14(2).
117. Moreira SG, Prochnow LI, de Castro Kiehl J, Neto LM, Pauletti V. Formas químicas, disponibilidade de manganês e produtividade de soja em solos sob semeadura direta. Revista Brasileira de Ciência do Solo. 2006; 30(1):121-136.

118. Moreira SG, Prochnow LI, de Castro Kiehl J, Pauletti V, Martin-Neto L. Chemical forms in soil and availability of manganese and zinc to soybean in soil under different tillage systems. Soil and Tillage Research. 2016; 163:41-53.
119. Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ *et al*. Increasing CO_2 threatens human nutrition. Nature. 2014; 510(7503):139.
120. NAAS. Soil Health: New Policy Initiatives for Farmers Welfare. Policy Brief No. 3, National Academy of Agricultural Sciences, New Delhi, 2018, 19.
121. Nagesh VR, Usharani G, Reddy TD. Heterosis studies for grain iron and zinc content in rice (*Oryza sativa* L.). Annals of Biological Research. 2012; 3(1):179-184.
122. Naik SK, Das DK. Relative performance of chelated zinc and zinc sulphate for lowland rice (*Oryza sativa* L.). Nutrient cycling in agroecosystems. 2008; 81(3):219-227.
123. Nakandalage N, Nicolas M, Norton RM, Hirotsu N, Milham P, Seneweera S. Improving rice zinc biofortification success rates through genetic and crop management approaches in a changing environment. Front Plant Sci. 2016; 7:764.
124. Narwal RP, Singh BR, Salbu B. Association of cadmium, zinc, copper, and nickel with components in naturally heavy metal-rich soils studied by parallel and sequential extractions. Communications in Soil Science and Plant Analysis. 1999; 30(7-8):1209-1230.
125. Neelamraju S, Mallikarjuna Swamy BP, Kaladhar K, Anuradha K, Venkateshwar Rao Y, Batchu AK *et al*. Increasing iron and zinc in rice grains using deep water rices and wild species–identifying genomic segments and candidate genes. Quality Assurance and Safety of Crops & Foods. 2012; 4(3):138-138.
126. Nene YL. Symptoms, cause and control of Khaira disease of paddy. Bull Indian Phytopathol Soc. 1966; 3:97-191.
127. Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, Price AH. Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant and soil. 2010; 329(1-2):139-153.
128. Olsen LI, Palmgren MG. Many rivers to cross: the journey of zinc from soil to seed. Front Plant Sci. 2014; 5:30.
129. Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A *et al*. Concentration and localization of zinc during seed development and germination in wheat. Physiologia Plantarum. 2006; 128(1):144-152.
130. Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon *et al*. A new version of Golden Rice with increased pro-vitamin A content. Nat Biotech. 2005; 23:482-487.
131. Palanisamy S. Genetic Analysis of Biofortification of Micronutrient Breeding in Rice (*Oryza sativa* L.). Rice Crop: Current Developments, 2018, 17.
132. Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK *et al*. Zinc biofortification of cereals: problems and solutions. Trends Plant Sci. 2008; 13:464-473.
133. Paltridge NG, Palmer LJ, Milham PJ, Guild GE, Stangoulis JC. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. Plant and soil. 2012; 361(1-2):251-260.
134. Pandey N, Gupta B, Pathak GC. Foliar application of Zn at flowering stage improves plant’s performance, yield and yield attributes of black gram. Indian J Exp Biol. 2013; 51:548-555.
135. Petry N, Egli I, Gahutu JB, Tugirimana PL, Boy E, Hurrell R. Stable iron isotope studies in Rwandese women indicate that the common bean has limited potential as a vehicle for iron biofortification. The Journal of nutrition. 2012; 142(3):492-497.
136. Pfeiffer WH, McClafferty B. Biofortification: Breeding Micronutrient-Dense Crops. Breeding Major Food Staples, 2008.
137. Phattarakul N, Rerkasem B, Li LJ, Wu LH, Zou CQ, Ram H *et al*. Biofortification of rice grain with zinc through zinc fertilization in different countries. Plant Soil. 2012; 361:131-141.
138. Pottier M, Maslaux_daubresse C, Yoshimoto K, Thomine S. Autophagy as a possible mechanism for micronutrient remobilization from leaves to seeds. Front. Plant Sci. 2014; 5:1-8.
139. Prasad AS. Zinc deficiency: its characterization and treatment. Met Ions Biol Syst. 2004; 41:103-37.
140. Prasad R, Shivay YS. Relative grain zinc loading ability of cereals (rice, wheat, maize) and a grain legume (chickpea). Indian Journal of Agricultural Sciences. 2018; 88(2):307-313.
141. Prom-u-Thai C, Dell B, Thomson G, Rerkasem B. Easy and rapid detection of iron in rice grain. Science Asia. 2003; 29:203-207.
142. Prom-u-Thai C, Fukai S, Godwin ID, Huang L. Genotypic variation of iron partitioning in rice grain. Journal of the Science of Food and Agriculture. 2007; 87(11):2049-2054.
143. Rada N. India's post-green-revolution agricultural performance: what is driving growth? Agricultural Economics. 2016; 47(3):341-350.
144. Rai KN, Govindaraj M, Rao AS. Genetic enhancement of grain iron and zinc content in pearl millet. Quality Assurance and Safety of Crops & Foods. 2012; 4(3):119-125.
145. Ram H, Sohu VS, Cakmak I, Singh K, Buttar GS, Sodhi GPS *et al*. Agronomic fortification of rice and wheat grains with zinc for nutritional security. Current Science. 2015, 1171-1176.
146. Rashid A. Secondary and micronutrients; soil science. Islamabad: National Book Foundation, 2001, 372-379.
147. Rattan RK. Whither micronutrient and metal research in twenty first century in the Indian context. Journal of the Indian Society of Soil Science. 2015; 63:S59-S77.
148. Rattan RK, Datta SP, Katyal JC. Micronutrient management-research achievements and future challenges. Indian J Fert. 2008; 4(12):93-118.
149. Rattan RK. *Quo vadis* zinc research: Golden march with green revolution. 44th RV Tamhane Memorial Lecture delivered on 12th December, 2017 during 82nd Annual Convention of the Indian Society of Soil Science held at Amity University, Kolkata, 2017.
150. Ray P, Datta SP, Rakshit R, Golui D. Agronomic biofortification of food crops with zinc and iron for ameliorating their deficiencies in humans: constraints and possibilities. Indian Journal of Fertilisers. 2016; 12(7):28-35.
151. Rehman H. N-Zn dynamics under different rice production systems [PhD dissertation]. Faisalabad: University of Agriculture, 2012.
152. Rehman A, Farooq M, Naveed M, Nawaz A, Shahzad B. Seed priming of Zn with endophytic bacteria improves

the productivity and grain biofortification of bread wheat. European Journal of Agronomy. 2018; 94:98-107.

153. Rehman HU, Aziz T, Farooq M, Wakeel A, Rengel Z. Zinc nutrition in rice production systems: a review. Plant and soil. 2012; 361(1, 2):203-226.
154. Renault P, Cazevieille P, Verdier J, Lahlah J, Clara C, Favre F. Variations in the cation exchange capacity of a ferralsol supplied with vinasse, under changing aeration conditions: Comparison between CEC measuring methods. Geoderma. 2009; 154(1-2):101-110.
155. Robson AD. Zinc in soils and plants: proceedings of the international symposium on ‘zinc in soils and plants’ held at the University of Western Australia, 27–28 September, New York, NY: Springer Science & Business Media, 1993-2012, 55.
156. Römheld V, Marschner H, Function of micronutrients in plants. In: Mortvedt, J.J. (Ed.), Micronutrients in Agriculture. Soil Science Society of America, Madison, WI, 1991, 297-328.
157. Rose TJ, Impa SM, Rose MT, Pariasca-Tanaka J, Mori A, Heuer S *et al*. Enhancing phosphorus and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. Ann Bot. 2013; 112:331-345.
158. Sadeghzadeh B. A review of zinc nutrition and plant breeding. Journal of soil science and plant nutrition. 2013; 13(4):905-927.
159. Saha S, Chakraborty M, Padhan D, Saha B, Murmu S, Batabyal K *et al*. Agronomic biofortification of zinc in rice: Influence of cultivars and zinc application methods on grain yield and zinc bioavailability. Field Crops Research. 2017; 210:52-60.
160. Sala M, CR AK, Geetha S, Gnanamalar RP, Hemalatha G. Variability studies for iron and zinc content on segregating population of rice. Electronic J Plant Breed. 2013; 4:1201-1204.
161. Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y *et al*. Biofortification: progress toward a more nourishing future. Global food security. 2013; 2(1):9-17.
162. Samak A, Hittalamani H, Shashidhar HE, Biradar H. Studies on genetic variability and genetic control for protein and micronutrient content in F4 and F5 generation of rice (*Oryza sativa* L). Asian J Plant Sci. 2011; 10:376-379.
163. Sasaki A, Yamaji N, Ma JF. Over expression of OsHMA3 enhances Cd tolerance and expression of Zn transporter genes in rice. J Exp Bot. 2014; 65:6013-6021.
164. Sathisha TN. Genetic Variation among Traditional Landraces of Rics with Specific Reference to Nutritional Quality (Doctoral dissertation, UAS, Dharwad), 2013.
165. Seneweera SP, Blakeney AB, Milham P. Influence of rising atmospheric CO_2 and phosphorus nutrition on the grain yield and quality of rice (*Oryza sativa* cv. Jarrah). Cereal Chemistry. 1996; 73(2):239-243.
166. Seneweera S, Makino A, Hirotsu N, Norton R, Suzuki Y. New insight into photosynthetic acclimation to elevated CO2: the role of leaf nitrogen and ribulose-1, 5-bisphosphate carboxylase/oxygenase content in rice leaves. Environmental and Experimental Botany. 2011; 71(2):128-136.
167. Sengupta S, Dey S. Universal multi-nutrient extractants in soil analysis - Scope & Prospects. Agriculture & Food: e- Newsletter (ISSN: 2581-8317). 2019; 1(11)406-410.
168. Seth A, Sarkar D, Datta A, Mandal B, Chowdhury A, Masto RE *et al*. Suitability of complex extractants for assessment of available soil zinc for nutrition of rice (*Oryza sativa* L.) in subtropical India. Soil Science. 2017; 182(1):28-35.
169. Sharifi P. Genetic analysis of nutritional quality traits in milled kernels of hybrid rice based on the additive-dominance model. Journal of Tropical Agriculture and Food Science. 2013; 41(2):193-203.
170. Sharifianpour G, Zaharah AR, Ishak CF, Hanafi MM, Khayyambashi B, Alifar N *et al*. Effects of application of different sources of Zn and composts on Zn concentration and uptake by upland rice. J Agron. 2015; 14:23-29.
171. Shivay YS, Kumar D, Prasad R, Ahlawat LPS. Relative yield and zinc uptake by rice from zinc sulphate and zinc oxide coatings onto urea. Nutr Cycl Agroecosyst. 2008; 80:181-188.
172. Shukla AK, Tiwari PK, Pakhare A, Prakash C. Zinc and iron in soil, plant, animal and human health. Indian Journal of Fertilisers. 2016; 12(11):133-149.
173. Shukla K, Anshumali. Soil micronutrient pools and their transfer to paddy-crops in semi-arid agro-ecosystems, Central India. Soil and Tillage Research. 2018; 180:164-174.
174. Shuman LM. Chemical forms of micronutrients in soils. Micronutrients in agriculture, (micronutrientsi2), 1991, 113-144
175. Singh MK, Prasad SK. Agronomic aspects of zinc biofortification in rice (*Oryza sativa* L.). Proceedings of the national academy of sciences, India section B: biological Sciences. 2014; 84(3):613-623.
176. Singh MV. Micronutrient deficiencies in crops and soils in India. In Micronutrient deficiencies in global crop production Springer, Dordrecht. 2008, 93-125.
177. Singh N, Jayaswal PK, Panda K, Mandal P, Kumar V, Singh B *et al*. Single-copy gene based 50 K SNP chip for genetic studies and molecular breeding in rice. Scientific reports. 2015; 5:11600.
178. Slaton NA, Wilson CE, Ntamatungiro S, Norman RJ, Boothe DL. Evaluation of zinc seed treatments for rice. Agronomy Journal. 2001; 93(1):152-157.
179. Snowball K, Robson AD. Symptoms of nutrient deficiencies: subterranean clover and wheat. (University of Western Australia Press: Perth), 1983.
180. Snowball K, Robson AD. Symptoms of nutrient deficiencies: lupins. Department of Soil Science and Plant Nutrition, Institute of Agriculture, University of Western Australia, 1986.
181. Solymosi K, Bertrand M. Soil metals, chloroplasts, and secure crop production: a review. Agron Sus-tain Dev. 2012; 32(1):245-272.
182. Sommer AL, Lipman CB. Evidence of the indispensable nature of boron and zinc for higher green plants. Plant Physiology. 1926; 1:231-249.
183. Sperotto RA, Ricachenevsky FK, Waldow V, Müller ALH, Dressler VL, Fett JP Rice grain Fe, Mn and Zn accumulation: How important are flag leaves and seed number? Plant Soil Environ. 2013; 59:262-266.
184. Sposito G. The chemical forms of trace metals in soils. Applied environmental geochemistry, 1983, 123-170.
185. Stangoulis JC, Huynh BL, Welch RM, Choi EY, Graham RD. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. Euphytica. 2007; 154(3):289-294.
186. Stein AJ. Rethinking the measurement of under nutrition in a broader health context. Global Food Security. 2014; 3:193-199.

187. Stomph TJ, Hoebe N, Spaans E, Van der Putten PEL. The relative contribution of post-flowering uptake of Zn to rice grain Zn density. 3rd International Zn Symposium, Hyderabad, India, 2011.
188. Su C, Jiang L, Zhang W. A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. Environmental Skeptics and Critics. 2014; 3(2):24.
189. Sun W, Zhang X. Estimating soil zinc concentrations using reflectance spectroscopy. International journal of applied earth observation and geoinformation. 2017; 58:126-133.
190. Sunusi M, Lurwanu Y, Sulaiman AS, Zamfara MI. ZINC biofortification of rice (*Oryza sativa*. L.) Breeding and agronomic approaches: status and challenges for its adoption in sub-saharan African countries. Fudma Journal of Sciences. ISSN: 2616-1370, 2019; 3(1):291-298.
191. Susanto U. Mapping of quantitative trait loci for high iron and zinc content in polished rice (*Oryza sativa* L) grain and some agronomic traits using simple sequence repeats markers, Phd Thesis Submitted to Bogor Agricultural Univesrity Bogor Indonesia, 2008.
192. Suzuki M, Takahashi T, Tsukamoto T, Watanabe S, Matsuhashi S, Yazaki J *et al*. Biosynthesis and secretion of mugineic acid family phytosiderophores in Zn-deficient barley. Agron J. 2006; 48:85-97.
193. Suzuki M, Tsukamato T, Inoue H, Watanabe S, Matsuhashi S, Takahashi M *et al*. Deoxymugineic acid increases Zn translocation in Zn–deficienct rice plants. Plant Molecular Biology. 2008a; 66:609-617.
194. Swamy BM, Kumar A. Genomics-based precision breeding approaches to improve drought tolerance in rice. Biotechnology Advances. 2013; 31(8):1308-1318.
195. Swamy BM, Rahman MA, Inabangan-Asilo MA, Amparado A, Manito C, Chadha-Mohanty P *et al*. Advances in breeding for high grain zinc in rice. Rice, 2016; 9(1):49.
196. Thomine S, Lelièvre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H. AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. Plant J. 2003; 34:685-695.
197. Thongbam PD, Tarentoshi, Raychaudhury M, Durai A, Das SP, Ramesh T *et al*. Studies on grain and food quality traits of some indigenous rice cultivars of North-eastern Hill Region of India. J Agric Sci. 2012; 4:3.
198. Trijatmiko KR, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C *et al*. Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. Scientific reports. 2016; 6:19792.
199. Tyagi R, Sharma A, Srivastava PC, Shankhdhar D, Shankhdhar SC. Modulation of phytic acid and phytic acid-zinc molar ratio by different modes of zinc application in rice. Indian Journal of Plant Physiology, 2018; 23(3):529-535.
200. Varshney RK, Nayak SN, May GD, Jackson SA. Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends in biotechnology. 2009; 27(9):522-530.
201. Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RP. Biofortification strategies to increase grain zinc and iron concentrations in wheat. J Cereal Sci. 2014; 59:365-372.
202. Velu G, Bhattacharjee R, Rai KN, Sahrawat KL, Longvah T. A simple and rapid screening method for grain zinc content in pearl millet. Journal of SAT Agricultural Research, 2008; 6:1-4.
203. Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RP. Biofortification strategies to increase grain zinc and iron concentrations in wheat. Journal of Cereal Science. 2014; 59(3):365-372.
204. Velu G, Singh RP, Huerta-Espino J, Peña RJ, Arun B, Mahendru-Singh A *et al*. Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. Field Crops Research. 2012; 137:261-267.
205. Von Wirén N, Klair S, Bansal S, Briat JF, Khodr H, Shioiri T *et al*. Nicotianamine chelates both FeIII and FeII. Implications for metal transport in plants. Plant Physiol. 1999; 119:1107-1114.
206. Wang LC, Busbey S. Images in clinical medicine acquired Acrodermatitis enteropathica. N Engl J Med. 2005; 352:1121
207. Wang S, Zhang X, Liu K, Fei P, Chen J, Li X *et al*. Improving zinc concentration and bioavailability of wheat grain through combined foliar applications of zinc and pesticides. Agronomy Journal. 2019; 111(3):1478-1487.
208. Wang YH, Zou CQ, Mirza Z, Li H, Zhang ZZ, Li DP, *et al*. Cost of agronomic biofortification of wheat with zinc in China. Agronomy for Sustainable Development. 2016; 36(3):44.
209. Wani SH, Sah SK, Sági L, Solymosi K. Transplastomic plants for innovations in agriculture. A review. Agronomy for sustainable development. 2015; 35(4):1391-1430.
210. Waters BM, Uauy C, Dubcovsky J, Grusak MA. Wheat (Triticum aestivum) proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. Journal of Experimental Botany. 2009; 60:4263-4274.
211. Weber FA, Voegelin A, Kretzschmar R. Multi-metal contaminant dynamics in temporarily flooded soil under sulfate limitation. Geochimica et Cosmochimica Acta. 2009; 73(19):5513-5527.
212. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of experimental botany. 2004; 55(396):353-364.
213. Welch RM, Norvell WA. Growth and nutrient uptake by barley (*Hordeum vulgare* L. cv Herta): studies using an N-(2-Hydroxyethyl) ethylenedinitrilotriacetic acid-buffered nutrient solution technique (II. Role of zinc in the uptake and root leakage of mineral nutrients). Plant Physiology. 1993; 101(2):627-631.
214. Welch RM, Graham RD, Cakmak I. Linking agricultural production practices to improving human nutrition and health. Expert Paper Written for ICN2 Second International Conference on Nutrition Preparatory Technical Meeting, Rome, Italy, 2013.
215. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS ONE. 2012; 7:e50568.
216. WHO. Zinc. Trace elements in human nutrition and health. World Health Organization, Geneva, 1996, 72-104.
217. Wisawapipat W, Janlaksana Y, Christi I. Zinc solubility in tropical paddy soils: A multi-chemical extraction technique study. Geoderma. 2017; 301:1-10.

218. Wissuwa M, Ismail AM, Graham RD. Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. Plant and Soil. 2008; 306(1-2):37-48.
219. Wissuwa M, Ismail AM, Yanagihara S. Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. Plant Physiology. 2006; 142(2):731-741.
220. World Health Organization. The world health report 2002: reducing risks, promoting healthy life. World Health Organization, 2002.
221. Wu CY, Lu LL, Yang XE, Geng Y, Wei YY, Hao HL *et al*. Uptake translocation and remobilization Zinc absorbed at different growth stages by rice genotypes of different Zn densities. J Agric Food Chem. 2010; 58:6767-6773.
222. Yamaguchi N, Ishikawa S, Abe T, Baba K, Arao T, Terada Y. Role of the node in controlling traffic of cadmium zinc and manganese in rice. J Exp Bot. 2012; 63:2729-2737.
223. Yamaji N, Xia J, Mitani-Ueno N, Yokosho K, Feng Ma J. Preferential delivery of zinc to developing tissues in rice is mediated by P-type heavy metal ATPase OsHMA2. Plant Physiol. 2013; 162:927-939.
224. Yang X, Ye ZQ, Shi CH, Zhu ML, Graham RD. Genotypic differences in concentrations of iron, manganese, copper, and zinc in polished rice grains. Journal of Plant Nutrition. 1998; 21(7):1453-1462.
225. Yilmaz A, Ekiz H, Torun B, Gultekin I, Karanlik S, Bagci SA *et al*. Effect of different zinc application methods on grain yield and zinc concentration in wheat grown on zinc-deficient calcareous soils in Central Anatolia. J Plant Nutr. 1997; 20:461-471.
226. Yin HJ, Gao XP, Stomph T, Li L, Zhang F, Zou CQ. Zinc concentration in rice (*Oryza sativa* L.) grains and allocation in plants as affected by different zinc fertilization strategies. Commun Soil Sci. Plant Anal. 2016; 47:761-768.
227. Yoshida S, Tanaka A. Zinc deficiency of the rice plant in calcareous soils. Soil Science and Plant Nutrition. 1969; 15(2):75-80.
228. Yoshida S, Ahn JS, Forno DA. Occurrence, diagnosis, and correction of zinc deficiency of lowland rice. Soil science and plant nutrition. 1973; 19(2):83-93.
229. Yuan L, Wu LH, Yang CL, Lv Q. Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. J Sci Food Agric. 2013; 93(2):254-261.
230. Zaman QU, Aslam Z, Yaseen M, Ihsan MZ, Khaliq A, Fahad S *et al*. Zinc biofortification in rice: leveraging agriculture to moderate hidden hunger in developing countries. Archives of Agronomy and Soil Science. 2018; 64(2):147-161.
231. Zarcinas BA, Cartwright B, Spouncer LR. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. Communications in Soil Science and Plant Analysis. 1987; 18(1):131-146.
232. Zhang CM, Zhao WY, Gao AX, Su TT, Wang YK, Zhang YQ *et al*. How could agronomic biofortification of rice be an alternative strategy with higher cost-effectiveness for human iron and zinc deficiency in China?. Food and nutrition bulletin. 2018; 39(2):246-259.
233. Zhang MW, Guo BJ, Peng ZM. Genetic effects on Fe, Zn, Mn and P contents in Indica black pericarp rice and their genetic correlations with grain characteristics. Euphytica. 2004; 135(3):315-323.
234. Zhang M, Peng Z, Du Y. Combining ability and stability analysis of the content of trace elements Fe, Zn and Mn in special rice grains. Zhongguo shuidao kexue. 1996; 10(4):201-206.
235. Zhang M, Pinson SR, Tarpley L, Huang XY, Lahner B, Yakubova E *et al*. Mapping and validation of quantitative trait loci associated with concentrations of 16 elements in unmilled rice grain. Theoretical and Applied Genetics. 2014; 127(1):137-165.
236. Zhao FJ, McGrath SP. Biofortification and phytoremediation. Curr Opin Plant Biol. 2009; 12(3):373-380.
237. Zhao FJ, Shewry PR. Recent developments in modifying crops and agronomic practice to improve human health. Food Policy. 2011; 36:S94-S101.
238. Zhou CQ, Zhang YQ, Rashid A, Ram H, Savasli E, Arisoy RZ *et al*. Biofortification of wheat with zinc through zinc fertilization in seven countries. Plant and Soil. 2012; 361:119-130.
239. Zou CQ, Zhang YQ, Rashid A, Ram H, Savasli E, Arisoy RZ *et al*. Biofortification of wheat with zinc through zinc fertilization in seven countries. Plant and soil. 2012; 361(1-2):119-130.