

Review on Effect of Zinc on Growth, Yield, Quality of Rice and Human Health

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Abstract: Over three billion people are currently micronutrient malnourished, resulting in egregious societal costs including learning disabilities among children, increased morbidity and mortality rates, lower worker productivity and high healthcare costs. All factors diminishing human potential, felicity and national economic development. Nutritional deficiencies (e.g. iron, zinc, vitamin A) account for almost two-thirds of the childhood death worldwide. Most of those afflicted are dependent on staple crops for their sustenance. Rice, maize and wheat are the staple foods for two thirds of the world's population, FAO. 1995^[23] and nearly half of the world's population consumes rice as a staple food. After green revolution people's are shifting from quantity to quality grains. Food security systems should begin with local communities who can develop and manage community gene, seed, grain and water banks. Implementation of the right to food will involve concurrent attention to policy, production, procurement, preservation and public distribution. Higher production in perpetuity should be achieved through an ever green revolution based on the principles of conservation and climate-resilient farming. Biodiversity loss and damage to ecosystem services is taking place at an alarming rate. This has serious implications in relation to our capacity to deal with the new challenges arising from climate change and trans boundary pests. Field gene bank through in situ on farm conservation of local land races, seed bank for ensuring the availability of seeds during times of drought and flood, grain bank involving storage of local food crops. Thus, curiosity, care, cooperation, conservation, cultivation, consumption and commerce can be linked into a food security as well as balance nutrition continuum. In most of the tribal area's people having several land races of rice. Similar situation present in bastar which is a mega biodiversity centre of world. Local people use to feed the red rice to the pregnant ladies by indigenous knowledge. In this regard's no systematic and scientific studies are done so far.

Keywords: zinc, rice, human health, micro nutrient

1. Introduction

Rice (*Oryza sativa* L.) is a "global grain" cultivated widely across the world and feeds millions of mankind. It is one of the most important staple food crops and is almost exclusively consumed by humans, with 90 % of rice grown and consumed in south and southeast Asia, where the normal consumption of rice range from 300 to around 800 g per day per person. Micronutrient malnutrition has affected lives of billions, with about 5 billion suffering from iron and 2.7 billion suffering from zinc deficiency all over the world (Anonymous 2004)^[8]. Hotz and Brown (2004)^[52] reported that Zn plays a key role in physical growth and development, functioning of immune system, reproductive health, sensory function and neuro behavioral development. More over recommended daily intakes range between 3 and 16 mg Zn day⁻¹, depending on age, gender, type of diet and other factors. It has been estimated that around 33 % of the world's human population has diets deficient in Zn, but this ranges between 4 and 73 % in different countries. On the basis of recommended daily allowances and bioavailability values, 22 µg g⁻¹ grain Zn contents in polished rice grains have been decided as reported by White and Broadley (2005)^[127] and Harvest Plus (2005)^[47] but current status of consumable rice shows that it has only around 20 % of this value. A striking figure of 27% of total population in India is affected by Zn deficiency related disorders such as poor immune system, diarrhea, poor physical and mental growth WHO (2007). The micronutrient zinc is essential for all organisms Andreini *et al.* (2006)^[7] and Broadley (2007)^[19]. Zinc has multiple roles in the human body including the efficient functioning of cellular metabolic activities and stimulation of the immune system. Zinc is also present in nearly 300 enzymes in the human body Barnett *et al.* (2010)^[12], is important for bone mineralization, the growth of body tissues and the fetus, sperm production and

fertility, smell, vision, taste and appetite, healthy growth of skin, hair and nails, as well as blood clotting and wound healing, functioning of the immune system and thyroid, cell division, protein and DNA synthesis. On an average, the grain comprises 80% starch, 7.5% protein, 0.5% ash and 12% water. The average adult in China and India ingests about 300 g of raw rice day⁻¹ and annual consumption is 62–190 kg year⁻¹. The daily Zn requirement is 15 mg for both adults and children that are 4 for older, but this cannot be achieved through a typical rice based vegetarian diet. Though rice is the predominant source of energy, protein and micronutrients for more than 50% of the world population, it does not provide enough essential mineral nutrients to match human requirements. The amount of mineral nutrients in rice grain is a key determinant of its nutritive value Anuradha *et al.* (2012)^[10]. Brown rice comprises 90% endosperm, 6–7% bran and 2–3% embryo on average by weight. Daily intake of Zn is important as the mammalian body has limited Zn stores and the daily requirement is influenced by gender and physiological stage. Zinc deficiency is recognized as one of the major nutrient disorders in humans and its effects are more profound in children Boonchuay *et al.* (2013)^[17]. Zinc deficiency is responsible for the development of a large number of illnesses and diseases including stunting of growth, compromised immune system function Barnett *et al.* (2010)^[12], cancer susceptibility to infectious diseases, iron deficiency anemia, and poor birth outcomes in pregnant women Graham *et al.* (2012)^[45], hair and memory loss, skin problems, weakening of body muscles, infertility in men, and pneumonia in children as reported by Das and Green (2013)^[25]. Virk and Barry (2009)^[122] reported that in some parts of the world rice consumption is as high as 990 g day⁻¹ person⁻¹. In 24 countries, rice provides at least one-third of the daily caloric intake (70 kg year⁻¹ or 200g day⁻¹) Beretta *et al.* (2012)^[15]. In Southeast Asian countries, the average per capita rice consumption is 130 kg year⁻¹ or 360

Volume 9 Issue 2, February 2020

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g day⁻¹, providing nearly two-thirds of caloric intake, FAO2007^[34] report. The high consumption of rice reflects a lack of dietary diversity, which, when combined with the poor micronutrient content of polished rice, is a risk factor for low micronutrient intake. Rice, one of the world's most important cereal crops is affected by Zn deficiency. At least 70 % of the rice crop is produced in flooded conditions resulting in increment in phosphorus and bicarbonate concentration which reduces soil Zn availability to the crop. About 50 % paddy soils are Zn deficient with 35 % in Asia alone Cakmak (2008) ^[24]. In an Indian scenario around 49 % soils from all the main agricultural areas are deficient in Zn Naik and Das (2008) ^[83]. There are many factors that have led to the present state of Zn deficiency in agricultural soils. The main soil factors responsible for causing Zn deficiency in staple food crops, such as rice are low total contents of Zn, high pH, high contents of calcite, high concentrations of bicarbonate ions and salts and high levels of available phosphorus Alloway(2009)^[3]. Timmer (2010)^[117] worldwide, annual rice consumption is more than 440 million metric tons (USDA) ^[1]. Unprocessed paddy rice is a good source of thiamine (vitamin B1), niacin (vitamin B3), and vitamin B6 ,Kennedy(2002) ^[66]. However, due to dehulling, milling, washing, and cooking, polished rice is a poor source of micronutrients. The principles, which have been well described in the World Health Organization (WHO)/Food and Agriculture Organization (FAO) guidelines on food fortification with micronutrients, are similar to those of fortification of other foods Allen *et al.* (2006)^[1]. Eco-agriculture aims at mutually reinforcing relationships between agricultural productivity and conservation of nature. Innovative eco-agriculture approaches can draw together the most productive elements of modern agriculture, new ecological insights and the knowledge that local people have developed from thousands of years of living in harmony with nature. Eco-agriculture is defined as an approach that brings together agricultural development and conservation of biodiversity as explicit objectives in the same landscapes Kesavan and Swaminathan, (2006)^[65] and Ammann (2008 and 2009)^[5,6]. IRRI has undertaken research on enriching rice genetically with iron and other micronutrients. Fortification, promotion of balanced diets, new semi-processed foods involving an appropriate blend of rice and micro nutrient rich millets as well as genetic improvement, could all form part of an integrated strategy to combat the following major nutritional problems in predominantly rice eating family's dietary deficiencies of thiamin, riboflavin, fat, calcium, vitamin C and zinc. Availability of Zn to plant is hampered by its immobile nature and adverse soil conditions. Thus, Zn deficiency is observed even though high amount is available in soil. Root shoot barrier, a major controller of zinc transport in plant is highly affected by changes in the anatomical structure of conducting tissue and adverse soil conditions like pH, clay content, calcium carbonate content, etc. Zn deficiency results in severe yield losses and in acute cases plant death. Zn deficiency in edible plant parts results in micronutrient malnutrition leading to stunted growth and improper sexual development in humans. To overcome this problem several strategies have been used to enrich Zn availability in edible plant parts, including nutrient management, biotechnological tools, and classical and molecular breeding approaches. Polished rice grains contain only about 20 % of the daily

requirement of zinc and a small amount of iron. Not surprisingly, therefore, in countries where rice is a major staple, Fe and Zn deficiencies are most prevalent with almost over three billion people affected worldwide .Welch and Graham (2004)^[125] , most of this population residing in developing countries. In this review, critically analyzed the biodiversity, soil, physiological and environmental factors that determine Zn availability, uptake, transport and utilization in rice as well as importance of Zn for human being.

In India, zinc deficiency was first reported by Nene (1966)^[84] on paddy fields. Zinc deficiency was identified as khaira in India, Akagare type II in Japan and Hadda in Pakistan Yoshida and Tanaka(1969)^[133]. While, Taya and Apulapaya in Philippines and the suffocating disease in Taiwan Yoshida *et al.*(1973)^[134]. From then onwards, zinc deficiency was realized as plant nutritional problem throughout rice growing countries such as Japan, USA, Brazil, and Philippines Deb(1992)^[27]. Zinc is a major component and activator of several enzymes involved in metabolic activities Klug and Rhodes (1987)^[68] . Zn is the only metal to be involved in all six classes of enzymes: oxido-reductases, transferases, hydrolases, lyases, isomerases and ligases reported by Barak and Helmke(1993)^[13]. Among the micronutrients, Zn is the most limiting nutrient whose deficiency is a wide spread nutritional disorder of wetland rice Neue and Lantin(1994)^[85]. Besides these factors multiple cropping coupled with the use of high analysis Zn free fertilizers has depleted the soil zinc source Singh *et al.* (1999)^[106]. Zinc deficiency has increased with the introduction of modern varieties, crop intensification and increased Zn removal Slaton *et al.* (2001)^[109]. Rashid (2001)^[94] reported that diffusion is believed to be the dominant mechanism for Zn²⁺ transport to plant roots. The physiological stress caused by Zn deficiency results in development of abnormalities in plants. In case of severe acute Zn deficiency, visible symptoms include stunted growth, chlorosis of leaves, small leaves and spikelet sterility. The quality of plant products is also adversely affected and plants have increased susceptibility to injury by high light intensity and temperature and to infection of certain fungal diseases Cakmak (2000)^[23]. Zinc (Zn) ions have both beneficial and toxic effects on plant cells. It is inimitable in several plant metabolic processes such as enzyme activation like RNA polymerases, superoxide dismutase, alcohol dehydrogenase, carbonic anhydrase, protein synthesis and metabolism of carbohydrate, lipid and nucleic acid Cakmak (2000)^[23] and Palmer and Guerinot (2009)^[90]. Further, Lucca *et al.*(2002)^[73] reported that importance of rice for human food it is one of the most important crop plants on earth. According to a WHO(2002)^[130] report, Zn deficiency ranks fifth amongst the most important health risk factors in developing countries and eleventh worldwide. Alloway(2008)^[2] reported that the total Zn content, clay content, calcium carbonate content, redox conditions, and microbial activity in the rhizosphere, soil moisture status, concentration of other trace elements, concentration of macro nutrients, especially phosphorus and climate. With more or less contribution of all of these factors combined, results in reduced availability of Zn in soils for plant absorption which leads to deficiency of Zn in plants, and

when these plants and their parts are consumed by humans and animals Zn deficiency occurs in them, giving rise to severe problem of micronutrient malnutrition. To overcome this problem cultivars having better Zn uptake potential or improved partitioning of Zn to edible parts or a combination of these two approaches is necessary. On marginally Zn deficient soils, yields can be reduced and quality affected without the appearance of obvious symptoms this is called 'hidden' (or 'latent') deficiency Alloway(2009)^[3]. Swaine(1955)^[113] and White(1993)^[126] reported that the total Zn content in soil depends upon the parent rock, weathering, organic matter, texture and pH. The most quoted range of total Zn in normal soils in 10–300 mg kg⁻¹ with a mean value of 50 mg kg⁻¹ as reported by Vinogradov (1959)^[121]. Katyal and Sharma (1991)^[64] reported that total Zn (mg kg⁻¹) in some Indian soils was 47 in *Entisols*, 60 in *Inceptisols*, 61 in *Aridisols*, 63 in *Vertisols*, 44 in *Alfisols*, 43 in *Altisols*, 30 in *Mollisols* and 72 in *Oxisols*. While, in *bastar* soil Zn content ranged between 0.8 to 1.2 (mgkg⁻¹). Soils formed from basic rocks such as basalt are richer in Zn than those from acid rocks such as granite and gneisses as reported by Vinogradov (1959)^[121]. The total Zn content in soils is generally lower in lighter soils and higher in heavier soils Frank *et al.*(1976)^[43]. Zn is known to occur in soil in a number of discrete chemical forms differing in their solubility and availability to plants Deb(1992)^[27]. Zn exists as five distinct pools in soils viz., water soluble, exchangeable, adsorbed, chelated Zn. These pools differ in strength and therefore in their susceptibility to plant uptake, leaching and extractability. The equilibrium among different pools is influenced by pH, concentration of Zn and other cations, particularly iron and manganese Mandal *et al.*(1993)^[76]. The Zn that is available to plants is that present in the soil solution, or is adsorbed in a labile form. The soil factors affecting the availability of Zn to the plants are those which control the amount of Zn in the soil solution and its sorption and desorption from/into the soil solution. Dubey and Chauhan (2002)^[28] reported that the minimum Zn uptake was recorded at no crop residue (control) and maximum at 100% crop residue. Tripathi and Rawat (2002)^[118] reported that the increase in Zn content may be attributed to increased availability of Zn due to addition of crop residue. Integrated use of organics and zinc have been found to more effective in maintaining higher productivity and stability through correction of deficiency of zinc in the course of mineralization on one hand and favorable physical soil. The higher rice yield due to zinc is attributed to its involvement in many metallic enzymes system, regulatory function and auxin production enhanced synthesis of carbohydrates and their transport to the site of grain production. Slaton *et al.*(2001)^[109] observed 12 to 180% and Fageria *et al.*(2011)^[36] reported 97% increase in rice yield due to zinc fertilization. Application and could be ascribed to variation in the availability of applied zinc in the root zone and their role in the growth and development of plant Robson (1993)^[96]. Zn deficiency in humans is a serious threat not only to the health of individuals but also to the economy of developing nations. Zn deficiency claims about 4.4 % of the total child deaths in the world Black(2003)^[16]. Tapeiro and Tew(2003)^[115] reported that Zn is required in small but critical amounts by both plant and animals (including humans). In higher animals and humans it is estimated that approximately 3,000 proteins contain Zn prosthetic groups.

Rice is grown in diverse soil and water regimes, consequently depletion and toxicity of micronutrients is encountered in many parts of India. Zinc deficiency is prevalent worldwide in temperate and tropical climate Fageria *et al.*(2003)^[39]. Singh and Tripathi (2005)^[108] reported that solubilisation of native as well as applied zinc at higher levels by crop residues which produces complexion agents. Zn ions are also neurotransmitters and cells in the salivary glands, prostate, immune system and intestine use Zn signaling Herschfinkel *et al.*(2007)^[50]. Zinc deficiency continues to be one of the key factors in determining rice production in several parts of the country Chaudhary *et al.* (2007)^[22]. Zinc deficiency is usually corrected by application of zinc sulfaten and response of rice to zinc under flooded condition has been reported by many workers Naik and Das (2007)^[82] and Fageria *et al.*(2011)^[36]. It is most common in flooded rice soils and has become increasingly important during the past decades. Zinc deficiency in rice appears right from seedling stage in nursery and three weeks after transplanting in transplanted rice plots. It was reported that zinc supply in form of fertilizer enhances rice yield Sudhalakshmi *et al.*(2007)^[112] and Jiang *et al.* (2008)^[59] but movement of zinc from plant parts to grains under Zn fertilizer application was not observed Jiang *et al.* (2008)^[59]. Meena *et al* (2008)^[78] noticed that the minimum Zn uptake was recorded at no crop residue (control) and maximum at 100% crop residue. In humans and higher animals Zn is a 'Type 2' nutrient, which means that its concentration in blood does not decrease in proportion to the degree of deficiency. As a result, physical growth slows down and excretion is reduced to conserve Zn. The Zn deficiency in human body reduces serum testosterone level, is linked to oligospermia, a severe immune dysfunction mainly affecting T helper cells, hyperammonemia, neu-rosensory disorders and decrease in lean body mass Prasad (2008)^[91]. To overcome this situation past decade has witnessed growing interest in developing varieties of staple grain crops with enhanced concentrations of Zn to improve the nutritional quality of grain for human consumption McDonald *et al.*(2008)^[77] and Wissuwa *et al.*(2008)^[129]. Unfortunately, populations residing both in developed and developing countries consume cereals as primary food components. Cereals are inherently low in Zn contents with lesser bioavailability. Zn enriched cereal grains would potentially generate major health benefits. Moreover, adequate Zn content is known to enhance crop productivity Cakmak (2008)^[24]. Virk and Barry 2009^[122] noticed that micronutrient malnutrition has been recognized as a gigantic and rapidly growing public health problem not only amongst the poor but also across the whole spectrum of people living on an unbalanced diet dominated by a single staple grain such as rice. However, as with many other staple food crops rice contains low levels of important micronutrients especially Fe and Zn Virk and Barry(2009)^[122] and Bouis and Welch(2010)^[18]. Rice is an indispensable staple food for half of the world's population providing 50–85 % of daily energy source and is consumed in large amounts. Therefore, even a small increase in the nutritive value of rice can be highly significant for human nutrition Zeng *et al.* (2010)^[136] and Chandel *et al.* (2010)^[20]. Poor grain nutritive value of cereals is an important reason for widespread micronutrient malnutrition among populations eating rice as staple food Chandel *et al.*

(2010)^[20]. Zn deficiency in humans is widespread and is estimated to affect more than 25 % of the world's population Maret and Sandstead (2006)^[75]. Overcoming malnutrition related disorders has been identified as a top priority by a panel of distinguished economists Chandel *et al.* (2010)^[20]. Also Zn ions are integral parts of Zn finger family of transcription factors controlling cell proliferation and differentiation Lin *et al.* (2005)^[71] and Palmer and Guerinot (2009)^[90]. Besides these, Zn plays major role in chloroplast development and function, of which most important are the Zn-dependent activity of SPP peptidase and the repair process of photo system II by turning over photodamaged D1 protein Hansch and Mendel (2009)^[46]. Thus cells need mechanisms for maintaining zinc homeostasis when available supplies decreases Eide (2009)^[30]. In plants, Zn deficiency reduces growth, tolerance to stress and chlorophyll synthesis Kawachi *et al.* (2009)^[63], Lee *et al.* (2010)^[70] and Widodo *et al.* (2010)^[128]. Further, Singh *et al.* (2005)^[107] stated one of the widest ranging abiotic stresses in world agriculture arises from low zinc availability in calcareous soils, particularly in cereals. Among all the micronutrients, Zn deficiency is the most widespread micronutrient disorder among different crops Naik and Das (2008)^[83]. Though rice is the predominant source of energy and micronutrients for more than half of the world population, it does not provide enough zinc (Zn) to match human nutritional requirements. Moreover, climate change, particularly rising atmospheric carbon dioxide concentration, reduces the grain Zn concentration. Ehsanullah *et al.* (2011)^[29] evaluated the effect of different methods and timing of zinc application on growth and yield of rice at Faisalabad. Zinc application methods and timing had significantly pronounced effect on paddy yield. Maximum paddy yield (5.21 t ha⁻¹) was achieved in treatment Zn (Basal application at the rate of 25 kg ha⁻¹ 21 % ZnSO₄) and minimum paddy yield (4.17 t ha⁻¹) was noted in Zn (foliar application at 75 DAT @ 0.5% Zn solution). Zinc application increases the crop growth rate of rice. Jha *et al.* (2013)^[58] noted that green manure had the greatest contribution to total N, total P, zinc, iron, and manganese (Mn). Yadav *et al.* (2013)^[132] observed that Zn uptake obviously increased with the application of Zn. Zn and crop residue treatment exerted a favorable influence on zinc uptake by rice crop. Tripathi and Kumar (2013)^[119] noticed that minimum Zn uptake was recorded at no crop residue (control) and maximum at 100% crop residue. Chandel *et al.* (2013)^[21] showed that crop residue increased the grain and straw yield of rice and iron content and ultimately iron uptake by the crop. Yadav *et al.* (2013)^[132] studied the response of rice to zinc and organic matter application and noted that rice grown in flooded conditions has higher requirement of zinc because the availability of other nutrients in submerged condition increases while decreases zinc availability to crop. Dietary deficiency of zinc (Zn) is a substantial global public health and nutritional problem Myers *et al.* (2014)^[81]. One third of the world population is at risk due to low dietary intake of Zn Myers *et al.* (2015)^[80], including 2 billion people in Asia and 400 million in sub-Saharan Africa. Most of those at risk depend on C₃ grains and legumes as their primary dietary source of Zn and have a high reliance on cereals, especially rice that has a low Zn concentration with poor bioavailability compared to other cereals Myers *et al.* (2014)^[81]. Therefore, Zn deficiency is a

chronic problem among human populations that have rice based diets Johnson *et al.* (2011)^[60]. Modern high yielding varieties remove large quantities of soil Zn at every harvest, lowering the residual concentration of soil Zn and contributing to lower future grain Zn concentration Desteur *et al.* (2014)^[26]. Further, the availability of Zn for plant uptake from the soil is affected by the concentrations of macro and micro nutrients, the physico-chemical and biological properties of a soil Fageria *et al.* (2012)^[37] and Hafeez *et al.* (2013)^[48], as well as temperature and water availability Fernando *et al.* (2014a)^[41]. Elevated atmospheric carbon dioxide concentration CO₂ also reduces the grain micronutrient concentration including Zn Fernando *et al.* (2014b)^[42]. Any genetic and environmental interactions resulting in lower grain Zn concentration in cereals have potentially large negative implications for human health. The aim of Zn biofortification of human food grains is to increase Zn concentration and its bioavailability in food, and this appears to be the most feasible, sustainable and economical approach to address Zn deficiency in the human diet Salunke *et al.* (2011)^[98] and Atiqueur *et al.* (2014)^[11]. Biofortification could be accomplished genetically through plant breeding and agronomically through Zn fertilization. Identification of the amount of genetic variability for Zn concentration in the germplasm is the initial step, then improving rice Zn concentration Anuradha *et al.* (2012)^[10]. Further, a sound understanding of Zn uptake, root to shoot translocation, distribution and grain loading is essential to achieve the biofortification target. Limited progress has been made to increase the Zn concentration in rice grain through biofortification despite a large effort, an outcome that may be a consequence of incomplete understanding of the physiological and molecular mechanisms of Zn uptake, utilization and its environmental interactions Shehu and Jamala (2010)^[104], Gao *et al.* (2011)^[44] and Ishimaru *et al.* (2011)^[57]. In general, internal Zn levels of plants are controlled by a number of mechanisms in which Zn transporters play an important role. However, there is limited information on the long distance Zn transport in the plants. On the other hand, transporters of divalent metal cations also play an important role in Zn uptake, but those transporters show broad substrate specificity, so that deficiency in calcium (Ca), iron (Fe), copper (Cu), manganese (Mn), or magnesium (Mg) may result in enhanced uptake of Zn, which could lead to higher grain Zn concentration Hafeez *et al.* (2013)^[48]. Zinc can be supplemented through dietary sources such as seafood, meat, green leafy vegetables and grains. However, maintaining a sufficient Zn concentration in rice grain is important for more than half of the world population for whom rice is the staple diet. Rice is one of the most important global staple food crops with a very long history of cultivation. Bran is the major repository for lipids, proteins, vitamins, minerals, and dietary fiber compared to the endosperm Sun *et al.* (2010)^[11], Hoekenga (2014)^[51] and Shahzad *et al.* (2014)^[102]. Recent X-ray micro fluorescence investigations demonstrated that the concentrations of Zn, Fe and potassium (K) decrease in the order: bran > hulls > whole grain > brown rice and polished rice Johnson (2013)^[61] and Lu *et al.* (2013)^[72]. Zinc is distributed throughout the endosperm of polished rice Takahashi *et al.* (2012)^[114] and Johnson (2013)^[61], which because of its relatively large mass accounts for 75% of grain Zn Wang *et al.* (2011)^[123]. Since

the Zn concentration in bran is 3 times greater than that in the hulls and endosperm Lu *et al.*(2013)^[72] and dehulling and polishing of rice removes bran from the grain, polishing rice depletes the very element that is deficient in the diets of many of its consumers. Polished rice grains supply only one fifth of daily Zn requirements Promuthai *et al.* (2010)^[92] and Sharma *et al.*(2013)^[103]. Therefore, it is important to increase the Zn concentration in rice endosperm, and this can only be achieved by understanding of the genetics of Zn uptake, remobilization, transport in the plant and environmental interactions on these processes. Rice grain Zn concentration is affected by a large number of plant and environmental factors. Plant factors affect the uptake, transport and remobilization of Zn to developing grains. The uptake and storage of nutrients are influenced by tissue demand, plant age and the root system, but all depend on the genetic makeup Fageria (2013)^[35]. Environmental variables that influence the Zn concentration of rice grains include soil Zn status, temperature and atmospheric [CO₂] Fernando (et al., 2012, 2014b)^[40,42]. There is limited understanding of how these plant and environmental factors influence and interact to affect Zn uptake, transport and loading into the grain. Lowland rice is grown under continuously submerged conditions where low availability of Zn has been widely reported Fageria (2013)^[35] and Meng *et al.*(2014)^[79]. Zinc in the soil solution is transported toward the roots by mass flow and the amount intercepted is increased by diffusion and root extension Yoneyama *et al.* (2015)^[135]. Rice roots absorb Zn Yoneyama *et al.* (2015)^[135] as the divalent cation, Zn²⁺, or as its complexes with organic ligands, via different transporter systems. Most Zn is taken up by active transport and the energy demand is largely supported through the light reactions of photosynthesis Yoneyama *et al.* (2015)^[135]. The ZIP (Zinc-regulated transporters, Iron-regulated transporter-like Protein) family of transporter genes *OsZIP1* and *OsZIP3* are involved in Zn uptake in rice Bashir *et al.* (2012)^[14] and Humayan *et al.* (2014)^[53]. The molecular aspects of this response are now partly elucidated, but genetic and environmental impacts on ZIP family genes have not been explored. Zinc taken up by roots is transported to vascular tissues through the epidermis, cortex and endodermis Yoneyama *et al.* (2015)^[135] and both symplastic and apoplastic pathways play an important role. Olsen and Palmgren (2014)^[89] during symplast to symplast movement, Zn enters the apoplast before it is acquired by the new symplast. The fundamental role of membrane bound proteins in Zn translocation across tonoplast, chloroplast and plasma membranes is well recognized. The negative plasma membrane potential energetically favors the import of Zn over its export. Zinc in the phloem is coupled with nicotianamine (NA), which is the predominant ligand in rice phloem sap Nishiyama *et al.* (2012)^[87]. Despite the contribution of ZIP in Zn homeostasis, homolog *OsHMA2* (P-type heavy metal ATPase) is also engaged in root to shoot transport of Zn in rice Satoh *et al.*(2012)^[99] and Takahashi *et al.*(2012)^[114]. Zinc allocation among different plant parts appears to be largely influenced by physiological growth stages and the nutrient status of the rice plant Seneweera (2011)^[100], Impa *et al.*(2013a)^[54], Shivay *et al.*(2015)^[105] and Yoneyama *et al.*(2015)^[135]. At the later stage of development, Zn partitioning between the grain, leaf blade, and shoots also varied Seneweera (2011)^[100]. At lower Zn concentrations, grain yield decreases sharply, thus it is

important to maintain at least the critical internal Zn concentration. Therefore, assessment of critical nutrient requirements of Zn efficient and inefficient cultivars will provide new insights into Zn homeostasis mechanisms. The distribution of Zn within the rice plant is associated with several step wise processes that involve both the xylem and phloem Impa and Johnson (2012)^[56] and Impa *et al.*(2013a,b)^[54,55]. Phloem mobility of Zn from leaves to rice grain is considered to vary depending on genotype Impa *et al.* (2013a)^[54]. However, there has been little exploration of the complex genetic traits associated with Zn uptake and transport, particularly the role of remobilization in Zn loading into the grain. Grain Zn is derived either through root uptake (subject to soil Zn availability and root activity during the grain-filling phase) or by internal remobilization, where the rate of leaf senescence plays an important role Arnold *et al.* (2010)^[4] and Impa and Johnson (2012)^[56]. These factors may explain part of the genetic variation in grain Zn concentration Sperotto(2013)^[110], which can be large Meng *et al.* (2014)^[79], but they are not fully investigated. Under ample soil Zn conditions, direct root uptake contributes the major portion of grain Zn for most of rice genotypes Sperotto (2013)^[110]. When Zn uptake is dominant, Zn concentrations in leaves, sheaths and roots remain unchanged or continue to increase during senescence Impa *et al.*(2013b)^[55]. Large amounts of grain micronutrients may remain in the outer aleurone layers of the grain Wang *et al.* (2011)^[123] and Myers *et al.*(2014)^[81] and the reasons why this Zn is not loaded into the endosperm are not well understood. If the major target is biofortification, the mechanism(s) that determine the allocation of Zn between the aleurone layer and inner endosperm need to be resolved. There is evidence that genotypic variation in Zn partitioning between the endosperm and the aleurone layers may be due partly to differences in Zn loading into the inner endosperm Impa *et al.*(2013b)^[55]. It has been suggested that Zn readily transports from aleurone to the inner part of the endosperm, particularly during early grain growth, indicating that there is no particular restriction at that time Wang *et al.*(2011)^[124]. However, later during grain filling, Zn transport is inhibited, particularly once starch is laid down Wang *et al.*(2011)^[124]. By this time large amounts of phytic acid (PA) have accumulated in the outer aleurone layer. The role of PA in Zn transfer to the endosperm is not well understood. When the soil Zn is deficient and its uptake is low, Zn remobilized from the leaves, stems, and roots is the main source of grain Zn Impa *et al.*(2013a)^[54] and Sperotto (2013)^[110]. Transporter genes such as *OsZIP4* and *OsZIP8* play key roles in grain Zn loading. These genes are likely to be influenced by factors such as temperature, pH and other micro-nutrients Ning *et al.*(2015)^[86] and Yoneyama *et al.*(2015)^[135]. These complex organic ligands have been identified for their ability to improve the uptake of metals from the rhizosphere and further, facilitate internal metal transport Impa and Johnson (2012)^[56]. Recent isotope fractionation studies and mathematical modeling support the concept that PS release is the major mechanism explaining the differences in Zn uptake by rice genotypes with varying uptake efficiency Arnold *et al.* (2010)^[4]. However, natural genetic variation in PS release kinetics and Zn absorption by rice roots are not well understood, and alterations in the quantity of and the efficiency of PS release exhibits both

intra and inter specific variation.

Ishimaru *et al.* (2011)^[57] reported that soil available Zn is recognized as one of the key factors contributing to grain Zn concentration and this can be further increased by supplementation with Zn fertilizers. About 30% of the cultivable soils Anjos *et al.* (2012)^[9] and about 50% of the cereal growing soils in the world are low in Zn, which has serious consequences on crop growth, and consequently on human and animal health. The diagnosis of Zn deficiency depends on plant tissue tests because soil tests for bio available Zn are generally unreliable Rayment and Lyons, (2010)^[95]. Other macro and micro nutrients, temperature and the biological properties of the soil may also be involved in Zn bioavailability as reported by Fageria *et al.* (2012)^[37], Hafeez *et al.* (2013)^[48] and Atiqueur *et al.*, (2014)^[11]. Aerobic conditions reverse these effects and the oxidation of soil organic matter can release Zn²⁺, but high levels of organic matter and clay particles could lead to Zn deficiency because Zn²⁺ binds with humic substances Hafeez *et al.* (2013)^[48]. However, there is limited understanding of the interactive effect of soil organic matter and clay on Zn availability. Higher dietary Cd is a concern because many populations for which rice is the staple carbohydrate are already over exposed to Cd. The atmospheric concentration of CO₂ is rising rapidly and the current level of 400 mmol mol⁻¹ (NOAA, 2013)^[88]. Effects of elevated CO₂ on the climate and on food production have become a major concern for global food and nutrient security. Importantly, CO₂ is likely to have a profoundly affect on plant growth, yield and grain quality as reported by many researchers such as Seneweera (2011)^[100], Seneweera and Norton (2011)^[101], Fernando *et al.* (2014b)^[42], Myers *et al.* (2014)^[81] and Thilakarathne *et al.* (2014)^[116] including rice Seneweera (2011)^[100], Seneweera and Norton (2011)^[101] and Myers *et al.* (2014)^[81]. Without nutrient and water limitations CO₂ increases yield by enhancing photosynthesis and reducing crop water use Hasegawa *et al.* (2013)^[49]. In addition, substantial reductions in grain quality of a number of species including rice and wheat have been reported under CO₂. Kant *et al.* (2012)^[62] suggested that CO₂ alters the balance between carbon metabolism and nutrient uptake and utilization. In wheat, decreased grain protein, and changes in protein quality, starch properties and in micronutrient densities become more prominent under this scenario. Fernando *et al.* (2012)^[40] and Myers *et al.* (2014)^[81] reported that overall concentrations of most of the macro and all micro nutrients declines as a consequence of CO₂. Despite the well documented physiological effect of CO₂ on growth and biomass of rice, information is scant regarding the mechanisms that mediate the effects of CO₂ on mineral concentrations, especially of Zn. A recent meta-analysis showed that CO₂ reduces the concentration of N, P, Ca, Mg, Zn, and Cu in the grain of most important cereal crops including rice. Others have reported 2–20% declines in concentrations of Mg, Zn, and Fe in crop species including rice, wheat and barley under CO₂. In free air CO₂ enrichment study using wheat under low rainfall, there was an overall reduction in the concentration of grain Zn (22%) and Fe (10%), and there was a strong positive correlation between protein and these three elements Fernando *et al.* (2014b)^[42]. In another experiment with wheat, grain Zn and Fe declined by 13–23 and 18.3%, respectively Erbs *et al.* (2010)^[31].

Micronutrient dilution due to higher soluble carbohydrate and starch at CO₂ do not fully explain lower Zn concentrations in wheat grain, because decreases in Zn concentration were not always associated with yield increases Fernando *et al.* (2014b)^[42]. Shehu and Jamala (2010)^[104] and Fageria *et al.* (2011)^[38] reported that there is increasing evidence that improved growth, yield, and grain Zn concentration could be achieved through Zn fertilization of many crops, including rice. Thus, it is important to ensure that there is adequate Zn supply, either by soil Zn fertilization or foliar Zn application at critical growth stages such as heading and early grain filling as reported by Boonchuay *et al.* (2013)^[17] and Mabesa *et al.* (2013)^[74]. Nitrogen and P applications could also considerably influence grain Zn concentration of rice because N application during grain filling promotes Zn uptake and remobilization Erenoglu *et al.* (2011)^[32] and Khan *et al.* (2015)^[67]. Although, high rates of P application may improve shoot growth and grain yield of rice Fageria (2013)^[35]. Recently, it has been reported that nanoparticles of titanium dioxide and ZnO boost nutrient concentration and growth of tomato plants Raliya *et al.* (2015)^[93]. The mechanisms and physiological impact of nano particle uptake and translocation should be unraveled. Irrespective of the genotypes used and any differences in Zn efficiency, removal of Zn in grain depletes soil Zn, which must be replaced. As described in section Rising CO₂ and Grain Zn, CO₂ reduces both Zn and Fe concentrations in rice grains relative to other micronutrients, and that the negative effect on Zn may be greater if P is in higher supply. These findings emphasize the importance of maintaining soil fertility to improve, or at least to maintain, existing levels of grain micronutrients, especially Zn and Fe, under CO₂. Boonchuay *et al.* (2013)^[17] reported that germplasm screening is the initial step for a breeding program to raise grain Zn concentration and to achieve breeding objectives there should be a wide genetic variation in grain Zn concentration. In addition, substantial genetic variation of Zn concentration in brown rice (13.5–58.4 mg kg⁻¹) has been reported for a large collection of rice germplasm at the International Rice Research Institute (IRRI), with an average of 25.4 mg Zn kg⁻¹. Shahzad *et al.* (2014)^[102] reported that 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 harvest plus program. Kumar *et al.* (2017)^[69] reported that the land races of red rice having high in zinc content under ecological condition of Bastar plateau. Further, significantly highest zinc content (ppm) was registered under the treatment of N₁₂₀:P₈₀:K₆₀ during both the years and on mean basis, but it was at par with the treatments N₁₂₀P₈₀K₆₀ FYM_{5t}, N₈₀P₆₀K₄₀+Zn, N₈₀P₆₀K₄₀+S and N₈₀P₆₀K₄₀+B during 2014 and treatment of N₈₀P₆₀K₄₀ + B (T₇) on mean basis.

2. Conclusions

The combination of genetic and agronomic strategies is required to raise grain Zn concentration. The genetic approaches can be advanced through germplasm screening of old landraces, traditional varieties and wild species to create novel genetic tools to increase Zn concentration in

rice grain. Zinc concentration in rice grains is influenced by plant related factors (genetic factors) and environmental factors and crop management strategies (agronomic factors). Greater understanding of how these factors interact to influence grain Zn accumulation is vital for enriching Zn concentration in rice grain. Improved Zn uptake and efficient remobilization are identified as key bottleneck for Zn contents. These bottlenecks should be addressed by exploiting the wide genetic diversity of rice germplasm. The rising atmospheric CO₂ is likely to reduce grain Zn concentrations and the underlying mechanism is not fully understood. Zn fertilization will also play important role, especially where soils are inherently low in bio available Zn. Consequently, emphasis should be given for biodiversity conservation and identification of land races high in micronutrients of red rice and management strategies need to be developed to minimize Zn deficiency for most of Indian in general and eastern region in particular whose staple food is rice.

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