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**Srinivasa Reddy Yerva**

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

**SK Singh**

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

**DK Singh**

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

**Sonali Habde**

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

**Amrutlal Khaire**

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

**Correspondence****Srinivasa Reddy Yerva**

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

## Recent developments in rice genomics with special reference to zinc

**Srinivasa Reddy Yerva, SK Singh, DK Singh, Sonali Habde and Amrutlal Khaire**

### Abstract

Zinc is an essential nutritional element for both plants and humans. In human beings, Zinc deficiency has a detrimental impact on growth, neuronal development, and immunity, and in severe cases its consequences are lethal. To address the malnutrition of global population, we have better option is that enhancing of nutritional value of food crops especially rice, where rice is staple diet for more than 2 billion people in Asia and 400 million in Sub-Saharan Africa. Zinc concentration in rice can be accelerated by improving the efficacy of rice plant in zinc uptake from the soil, root to shoot translocation and loading into the grain. This review discusses about recent advances in rice genomic studies in mapping QTL(s), characterization of Zn and Iron regulated transporter like protein (ZIP) gene family and transgenic approaches associated with increased efficiency of metal uptake and transport and lowering the expression of phytic acid which is indirectly involved in obstructing Zinc accumulation in grain endosperm and how it can enhance its absorption and utilization in human metabolic functions.

**Keywords:** Recent developments, rice genomics, plants and human

### Introduction

Rice is the predominant dietary energy source for 17 countries in Asia and Pacific, 9 countries in North and South America and 8 countries in Africa, which accounts for 20% of the world's dietary energy supply (FAO 2016). Most rice grown is consumed directly by humans, and about one-third of the population depends on rice for more than 50% of caloric intake (Kush *et al.*, 1997). Even though rice is rich in starch with 80% of total grain, deficient in major micronutrients such as Zinc and Iron. These micronutrients, especially Zinc has prominent role in human health by involving as cofactor in more than 300 enzymes (Anderson *et al.*, 2001; Barnett *et al.*, 2010) [7, 3]. Epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems are the organs most affected due to zinc deficiency. As per the recommended daily allowance, the daily Zn requirement per capita should be ranged in between 3 and 14mg/d<sup>-1</sup>, Daily Zn requirement mainly determined by Age, gender and type of diet of that particular individual (Hotz and Brown 2004) [19]. The present day rice cultivars are able to supply only 20% of the total recommended dose. Availability of zinc in fish, oyster other meat products relatively higher than the staple food crops, unfortunately in developing countries economically weaker sections cannot afford these items because of their low income which is even less than 2 dollars a day. Harvest plus projected to increase the zinc content in unpolished grain from baseline 16ppm to 28ppm in 3 phases and they succeeded in overcoming the first phased target by developing zinc rich rice variety "BRR1 dhan 62" with 22-24ppm in Bangladesh, 2013. However, with limited variation in grain Zn content across the rice gene pool, conventional breeding efforts have fallen short of reaching 28 micrograms of Zn per gram of polished rice to fulfill 30% of the estimated average requirement (EAR) in humans. The genetically engineered rice has significantly increased levels of Zn (up to 45.7 micrograms) per gram of polished rice that human cells can potentially absorb. Increase in Zn levels was achieved because of recent advances in genomics through rigorous gene optimization, large-scale plant transformation and genetic engineering.

### Genetics of grain zinc

Achieving success only with conventional breeding may not render the solution to human Zn deficiency, because trait to be improved is largely influenced by changed environment and soil conditions.

Grain Zn has a moderate to high broad-sense heritability (Zhang *et al.* 2014) [36], while reports of narrow sense heritability clearly indicated significant additive and dominant genetic effects. Inherent description 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). These results show that there is a sufficient variation for grain Zn concentration with moderate to high heritability and genetic advance. Thus it is possible to improve the Zn concentration of popular rice varieties by exploiting high Zn gemplasm in the breeding programs. The combining ability analysis by diallel crosses involving seven specific rice varieties with different levels of Zn showed that additive genetic effects were more important for Zn, while the co-efficient of variation (CV) for Zn varied significantly among the entries over the years and locations, indicating significant genotype and environment interactions (G x E) (Sharifi 2013). In another study involving black pericarp indica rice, genetic and cytoplasmic effects influenced the final grain Zn content, but the genetic effect was stronger and it constituted the major portion of the seed genetic effects. The heritability of the seed genetic effect was highly significant and narrow-sense heritability was very high, suggesting single plant selection as an effective approach for improving Zn content. There is also a positive correlation between grain Zn and the grain characteristics such as grain weight, grain length and width, so during the selection process, some consideration should be given to grain traits (Zhang *et al.* 2004) [36]. However, in a RIL population platykurtic and skewed distributions were observed for grain Zn, indicating involvement of several minor genes with duplicate gene interactions indicating little improvement by direct selection (Banu and Jagadeesh 2014) [6]. Grain quality traits and grain Zn was also found to be correlated (Anandan *et al.* 2011) [2]. All the associations of grain Zn with different mineral elements and grain quality traits must be taken into consideration while breeding for high Zn rice. One of the most important aspects of high Zn rice development is the relationship between grain Zn concentration and grain yield. Several reports indicate a significant negative association between grain Zn concentration and yield in rice (Wissuwa *et al.* 2007) [34], but a positive relationship between grain yield and grain Zn concentration was observed under Zn-deficient soil and also in different panel of aromatic rice and land races under Zn sufficient conditions non significant correlations were observed between yield and grain Zn (Gangashetty *et al.* 2013) [13].

### ZIP Transporter Genes

Application of genomic tools to plant breeding efforts will give precise results. Genomic studies for understanding the basic knowledge of zinc absorption by the plant could augment in evolving staple food grain with high zinc. Zinc from the soil moves toward root in a mass flow method and absorbs it in Zn<sup>2+</sup> form through various transporter genes (yenoyama *et al.* 2015). Molecular characterization of Zinc and iron regulated Transporter like Proteins (ZIP) for Zinc uptake, root to shoot translocation, loading into grain have been characterized well in recent years. Absorbed Zn in roots of rice unites with small proteins or phytate and its movement driven by the transpiration stream (Sadeghzaden, 2013). In phloem Zn is complexed to Nicotine amine which is predominant ligand in rice phloem sap (Nishiyama *et al.*

2002). A portion of total zinc absorbed by plant will be stored at basal root, remaining will be transported to shoots and distributed to various plant parts except grain before flowering initiation (Sasaki *et al.* 2015) [28]. The ZIP family transporter genes of OsZIP4, OsZIP5, Oszip 8 were found to be involved in root to shoot Zn transport. Recently OsHMA2 was also found in Zn distribution to the growing tissues in association with oszip3. There are two more gene families which includes YSL (Yellow stripe like proteins), or Natural resistance associated macrophage protein (NRAMP) found their involvement in the Zn remobilization recently. The most common approach used by different research groups is to increase the expression of NAS genes. Rice lines expressing HvNAS1 under the control of CaMV35S or Actin1 promoter accumulate more Fe and Zn in seeds suggesting that NA overproduction have the potential to increase the Fe and Zn translocation to rice grains (Masuda *et al.* 2009). Increasing the expression of OsNAS2, through activation tagging, increased the expression of DMA biosynthetic pathway genes as well as genes related to Zn-uptake. Thus increasing the amount of NA increases DMA secretion from roots and stimulates Zn uptake and translocation to seed. Moreover, the enhanced NA and DMA in endosperm increased the content of bio-available Zn as revealed by feeding trials in mice. Increasing the expression of OsNAS3 through activation tagging also increases the Fe and Zn content of rice seeds (Lee *et al.* 2009). Similarly rice plants over expressing OsNAS1, OsNAS2 and OsNAS3 accumulated more Fe and Zn in the seed indicating that NA is important for Fe and Zn transport and could be successfully deployed for bio-fortification of Zn (Johnson *et al.* 2011). The overexpression of OsNAS1 not only increases the concentration of Zn in rice seeds but also increases its bio-availability (Zheng *et al.* 2010). Overexpression of barley dioxygenase gene, IDS3, involved in the biosynthesis of MAs also increased the rice seed Fe and Zn concentration (Masuda *et al.* 2008). On the other hand regulating the expression of ZIPs to increase the seed Zn content was not successful. Transgenic rice plants over-expressing OsZIP4 under the control of the CaMV35S promoter accumulated more Zn in roots while the shoot Zn contents decreased significantly (Ishimaru *et al.* 2007). Similarly OsZIP5 and OsZIP8 overexpression rice also accumulated more Zn in roots at the expense of shoot Zn. The OsZIP5 overexpression rice lines were sensitive to Zn excess, while the oszip5 knock out plants were tolerant to Zn excess. Rice sucrose promoter (OsSUT1), is highly expressed in the mature phloem of all the vegetative tissues involved in the long-distance assimilate transport Pathway during grain filling, in addition to the flag leaf blade and sheath prior to (Scofield *et al.* 2007). The expression of OsYSL2 when controlled by the sucrose transporter promoter increased the Fe concentration in polished rice up to 4.4-fold compared to WT (Ishimaru *et al.* 2010). These results suggest that the controlling the temporal and spatial expression of OsZIP genes could be effective to increase the Zn in rice seeds.

### Over expression of ZIP transporters during flowering and grain development

The DMA biosynthesis genes are expressed during flowering and seed development and are expressed in anther, ovary, embryo and endosperm. In Ovary the expression of OsNAS1 decreased from 1 to 3 days after flowering (DAF) and then increased at 5 and 7 DAF. The expression pattern of OsNAS3, OsNAAT1 and OsDMAS1 increased from 1 to 5 DAF, and then the expression of OsNAS3 decreased, while the

expression of OsNAAT1 and OsDMAS1 did not change significantly. In embryo, the expression of OsNAS1 decreased from 7 to 28 DAF, while the expression of OsNAS2 and OsNAS3 remain unchanged. The expression of OsNAAT1 decreased slowly from 7 to 42 DAF, while expression of OsDMAS1 decreased from 7 to 10DAF and then increased from 14 to 28DAF. Slight changes for the expression of these genes were also observed in endosperm. The information about the expression of OsZIP genes during different stages of flowering and seed development is scarce. OsZIP1, OsZIP3, OsZIP4, OsZIP5 and OsZIP8 express in the anther. The expression of OsZIP3 varies significantly from 1 mm from base of anther to 1.2–1.5 mm from base. The expression of OsZIP5 is slightly higher at the bottom of anther compared to the top. In ovary, the expression of OsZIP1, OsZIP3, OsZIP4, OsZIP5 and OsZIP8 changes significantly. The expression of OsZIP3 varies significantly from 3 DAF to 5 DAF. The expression of OsZIP4 and OsZIP8 is higher compared to OsZIP1 and OsZIP3. The expression of OsZIP5 increases from 1 to 3 DAF and then decreases at 5 and 7 DAF. In embryo, the expression of OsZIP4 is significantly high compared to other OsZIPs. The expression of OsZIP1, OsZIP3, OsZIP4, and OsZIP5 increases slowly from 7 to 42 DAF. OsZIPs also express in endosperm. Again the expression of OsZIP4 is significantly high compared to other OsZIPs. The expression of OsZIP4 increases slowly from 7 to 28 DAF and then decreases slightly. The expression of OsZIP5 decrease slowly from 7 to 42 DAF. As OsZIPs are expressed during all the flowering and seed developmental stages, it is reasonable to conclude that these genes play an important role in Zn transport to developing seed. The role of OsZIP4 seems particularly important in transporting the Zn to the developing seed.

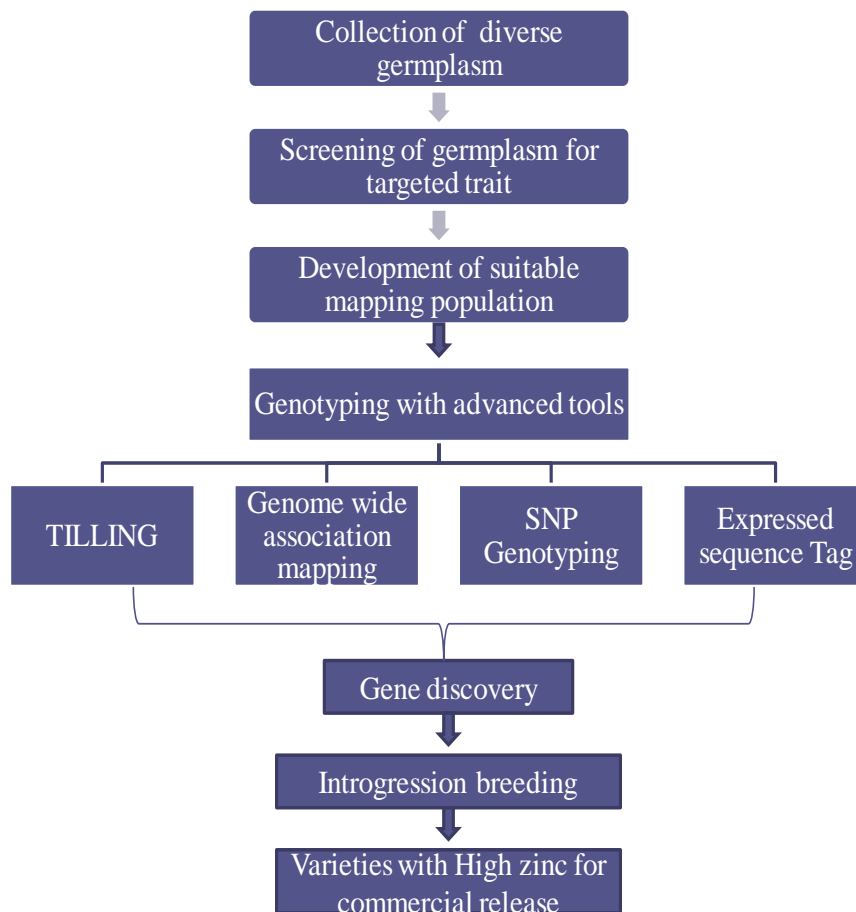
#### Advanced genomic efforts to enrich grain Zn

The primary objective of plant breeding has been to enhance farm productivity, usually by developing crops with higher yields. In contrast, improving micronutrient efficiencies and increasing nutrient content in plants has rarely been a breeding objective. In fact, crop nutritional problems have mostly been ignored in breeding. Some nutritional problems cannot be easily resolved by altering soil fertility or chemistry, and the application of modern breeding techniques to breed crops that are adapted to soils with a poor nutritional status is required. In the case of micronutrient deficiencies induced by high pH (i.e., Zn, Fe and Mn deficiencies), agronomic solutions (i.e., fertilizers) are not always successful, and a genetic solution is necessary (Cakmak and Braun, 2001) <sup>[10]</sup>. The results of such screening experiments can be variable because the severity of the nutrient deficiency varies between sites and years due to the effects of other growth-limiting factors, such as drought and diseases. Hence, reliable alternative methods are required. The use of controlled environments for screening is a common practice. Due to funding and time constraints, the screening of large populations for the development of molecular markers requires a pot culture screening system. Soil-based pot assays under controlled conditions allow for the relative efficiency. Genotypic variation for grain Zn concentration in rice can be exploited through breeding. For the past few years, breeding efforts to increase grain micronutrients have resulted in the development of biofortified crops including rice (Harvest Plus 2014) <sup>[18]</sup>. 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 donors have been crossed with popular high-yielding but low-Zn rice varieties and selection was carried out for agronomic traits in the segregating generations, with final fixed lines tested for grain Zn and yield in replicated large scale plots. This method was time consuming and resulted in modest increase in the Zn concentration, while the lines developed had moderate yield potential. However, a modified breeding program using high-Zn donors with acceptable yield potential crossed with popular high yielding, highly adapted, but low-Zn rice varieties, coupled with Zn testing in early segregating lines from the F<sub>4</sub> generation onwards along with the selections for acceptable agronomic traits, can hasten the process of high-Zn variety development and simultaneous maintenance of yield potential (Fig. 1). Multiple crosses involving several donors and recipient parents such as three-way, four-way crosses etc., reciprocal crosses with the donor parent, high Zn × high Zn crosses involving advanced Zn lines will enhance the Zn levels and yield potential. 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) <sup>[8]</sup> and these are a good resource for selecting high Zn lines and also provides an opportunity to select transgressive segregants for high Zn. Exploitation of heterosis of grain Zn and yield potential is also an important approach for developing high Zn rice hybrids. Reports have shown that there is very good heterosis for grain Zn in rice (Nagesh *et al.* 2012) <sup>[5]</sup>. Wild relatives of rice such as *O. nivara*, *O. rufipogon*, *O. barthii*, and *O. longistaminata*, 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 (Sarla *et al.* 2012). Advanced backcross breeding method can be used to exploit these wild resources to combine high Zn and high yield potential, and this will also help to broaden the genetic base of the popular rice varieties. Mutation breeding is also gaining importance as a strategy to improve Zn concentration in rice. Physical and chemical mutagens have been used in mutation breeding and mutants with high Zn have been identified. A number of IR64 mutants produced by the treatment with Sodium azide were reported to have high Zn (Jeng *et al.* 2012) <sup>[20]</sup>. There is a huge potential to use these markers in marker assisted breeding to improve grain Zn concentration in rice. Since there are many QTLs/ genes responsible for grain Zn concentration located on different chromosomes, QTL pyramiding, Marker Assisted Recurrent Selection (MARS) and Genomics Assisted Breeding approaches are worth trying to develop high-Zn rice. SNPs are becoming markers of choice for many breeding programs. Several diverse SNP chips such as 1536 SNPs diversity panel, 44 K, 50 K, 1 M SNP chips are available for rice. The cheaper, faster and high throughput SNP assays made it possible the routine use of markers in the breeding programs (Swamy and Kumar 2013b) <sup>[29]</sup>. The recent advances in sequencing technologies such as Next Generation/Second Generation Sequencing (NGS/SGS) and Third Generation Sequencing technologies (TGS) have revolutionized the breeding unprecedentedly (Varshney *et al.* 2009) <sup>[31]</sup>. Sequencing and resequencing of several thousands of accessions and breeding lines for use in sequence based mapping, genome wide association mapping,

genome wide predictions and genomic selections have become possible with the help of these technologies (Deschamps *et al.* 2012) <sup>[12]</sup>. In rice, 3000 accessions have been sequenced and efforts are ongoing to sequence 10,000 accessions. More than 20 M SNPs have been identified from 3 K panel (Alexandrov *et al.* 2015) <sup>[1]</sup>. High quality and high throughput sequencing coupled with Rapid Generation Advance (RGA) and high throughput phenotyping can hasten the breeding process especially for complex traits such as grain micronutrients. Genome Wide Association Studies (GWAS) and Genomic Selections (GS) approach have not been explored much for improving grain micronutrients but they hold great promise for improving the grain Zn concentration of several popular rice varieties and highly useful in main streaming of the Zn breeding. While breeding for high Zn rice, special attention should be given to the amount of anti-nutrients as they significantly influence Zn bioavailability. Phytate is the major anti-nutrient inhibiting the quantity of Zn absorbed. In rice, Zn is preferentially stored together with phytate, which is a strong chelator of divalent cations (Petry *et al.* 2012) <sup>[25]</sup>. Hence, selections should be made for low phytate content. The use of high Zn donors with low phytate, selection of segregating lines and advanced fixed lines with low phytate, and integrating phytate phenotyping along with grain Zn in the breeding program will help in developing high Zn lines with 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) <sup>[21]</sup>. Precision phenotyping of grain Zn concentration is vital for breeding high Zn rice variety. Since breeding programs handle huge amount of materials, fast, accurate, and inexpensive methods of phenotyping grain Zn are essential for

making timely and effective selection decisions when advancing the material. Seed sampling, hulling, and milling procedures without any metal contaminations have already been standardized for rice (Stangoulis and Sison 2008). Traditionally, Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) are being used in elemental analysis (Zarcinas *et al.* 1987) <sup>[35]</sup>, while these methods are highly accurate, they require expensive equipment, highly trained analysts, contamination free reagents, and extensive sample preparation (Velu *et al.* 2013) <sup>[32]</sup>. Alternatively, colorimetric approaches have been developed for Zn and Fe analysis in different cereal crops; however, these approaches are only semi-quantitative and laborious when applied in large scale (Ozturk *et al.* 2006) <sup>[1]</sup>. X-Ray Fluorescence (XRF) Spectrometry is very useful in non-destructive determination of relative Zn and Fe concentration in rice breeding lines to discard low Zn lines, and the resulting high Zn lines selected on the basis of XRF can be validated with ICP. Most of the biofortification programs are using XRF for metal analysis. The Synchrotron based X-ray micro fluorescence imaging and isotope discrimination techniques are helpful in understanding the pathways of metal uptake, translocation and re translocation, portioning and distributions among different tissues and organs. Takahashi *et al.* (2009) <sup>[30]</sup> and Lu *et al.* (2013) <sup>[22]</sup> characterized the dynamic changes in the pattern and distribution of different metals in germinating rice seedlings using X-ray imaging and concluded that metals have different patterns and preferences in their movement and accumulations. This novel Isotope fractionation technique is highly useful in better understating the physiological mechanisms, and the genotype and environment interactions involved in the Zn accumulation in grains.



**Fig. 1.** Developing Zn rich Rice varieties with recently evolved genomic tools

## Conclusions

Biofortification of rice with Zn is a cost-effective and sustainable solution to mitigate Zn deficiency problems in the rice consuming malnourished Asian populations. There is a significant genetic variation for grain Zn in rice germplasm resources which can be exploited by breeding to develop high Zn rice varieties. The recent advances in understanding the physiological, genetic and molecular basis of Zn uptake, Zn transport and loading into grains has allowed effective breeding for increased Zn, however the physiological barriers for loading Zn into grains are still a significant obstacle for attaining the targeted level of Zn. A complete understanding of Zn allocation, reallocation, and remobilization within and between vegetative and reproductive tissues is lacking. Agronomic interventions to improve the grain Zn have inconsistent results, but a combination of agronomic and genetic interventions is likely to prove a more effective approach. Several major effect and consistent QTLs for grain Zn have been identified; there is great potential to use them in MAS. Two high Zn rice lines have been released in Bangladesh, and several high Zn lines are in the advanced stages of evaluation for release in other partner countries.

## References

- Alexandrov N, Tai S, Wang W, Mansueto L, Palis K, Fuentes RR, *et al.* SNP-Seek database of SNPs derived from 3000 rice genomes. *Nucleic Acid Res.* 2015; 43:1023–1027
- 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. *J Food Sci.* 2011; 76:122–130.
- Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. *J Am. Coll. Nutr.* 2001; 20:212–218. 10.1080/07315724.2001.10719034
- Arnold T, Markovic T, Kirk GJD, Schonbachler M, Rehkamper M, Zhao FJ, Weiss DJ. Iron and zinc isotope fractionation during uptake and translocation in rice (*Oryza sativa*) grown in oxic and anoxic soils. *Geoscience*, 2015.
- Babu VR, Shreya K, Dangi KS, Usharani G and Nagesh P. Genetic variability studies for qualitative and quantitative traits in popular rice (*Oryza sativa* L) hybrids of India. *Int J Scientific and Res Publ.* 2012; 2:2250–3153
- Banu H, Jagadeesh BN. Genetic analysis of skewness and kurtosis for yield and its parameters, total grain protein, macro and micro nutrients in F<sub>7</sub> generation of rice (*Oryza Sativa* L). *Environ Ecol.* 2014; 32:381–385
- Barnett JB, Hamer DH, Meydani SN. Low zinc status: a new risk factor for pneumonia in the elderly? *Nutr. Rev.* 2010; 68:30–37. 10.1111/j.1753-4887.2009.00253
- Bandillo N, Raghava 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; 6:11.
- Bouis H. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. Administrative Committee on Coordination-Subcommittee on Nutrition of the United Nations. ACC/SCN c/o WHO, Geneva, Switzerland. *SCN News.* 1995; 12:15-19.
- Cakmak I, Braun HJ. Genotypic variation for zinc efficiency. In: Reynolds MP, Ortiz-Monasterio JI, McNab A. (Eds.), *Application of Physiology in Wheat Breeding* DF. CIMMYT, Mexico. 2001, 183-199.
- Cakmak I, Torun B, Erenoglu B, Ozturk L, Marschner H, Kalayci M, Ekiz H, *et al.* Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica.* 1998; 100:349-357
- Deschamps S, Llaca V, May GD. Genotyping-by-Sequencing in Plants. *Biology.* 2012; 1:460-483
- Gangashetty P, Salimath PM, Hanamaratti NG. Association analysis in genetically diverse non-basmati local aromatic genotypes of rice (*Oryza sativa* L). *Mol Plant Breed.* 2013; 4:31–37
- Graham RD, Knez M, Welch RM. How much nutritional iron deficiency in humans globally is due to an underlying zinc deficiency? *Adv. Agron.* 2012.
- Graham RD, Ascher JS, Hynes SC. Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant and Soil.* 1992; 146:241-250.
- Graham RD, Rengel Z. Genotypic variation in Zn uptake and utilization by plants. In: Robson, A.D. (Ed.), *Zinc in Soils and Plants.* Kluwer Academic Publishers, pp. 107-114, Dordrecht, The Netherlands, 1993P, 107-114
- Gregorio GB. Progress in breeding for trace minerals in staple crops. *J Nutr.* 2002; 132:500–502
- Harvest Plus. Biofortification progress briefs, 2014. [http://www.harvestplus.org/sites/default/files/Biofortification\\_Progress\\_Briefs\\_August2014\\_WEB\\_0.pdf](http://www.harvestplus.org/sites/default/files/Biofortification_Progress_Briefs_August2014_WEB_0.pdf)
- Hotz C, Brown KH. Assessment of the risk of Zn deficiency in populations and options for its control. *Food Nutr. Bull.* 2004; 25:S91-S204.
- 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. *J Food Compos Anal.* 2012; 28:149-154.
- 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). *Theor Appl Genet.* 2007; 114:803-814.
- Lu L, Tian S, Liao H, Zhang J, Yang X, Labavitch JM, Chen W. 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:e57360
- Nagesh P, Babu VR, Usharani G, Reddy TD. Heterosis studies for grain iron and zinc content in rice (*Oryza sativa* L). *Annals of Biological Res.* 2012; 3:179-184.
- 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. *Physiol Plant.* 2006; 128:144-152.
- 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. *J Nutr.* 2012; 142:492-497.
- Sadeghzadeh B. A review of zinc nutrition and plant breeding. *J Soil Sci. Plant Nutr.* 2003; 13:905-927.
- Sadeghzadeh, B. Mapping of chromosome regions associated with seed Zn accumulation in barley, PhD thesis. Faculty of Natural and Agricultural Sciences. The University of Western Australia, Perth, 2008.

28. Sasaki A, Yamaji N, Mitani-Ueno N, Kashino M, Ma JF. A node-localized transporter OsZIP3 is responsible for the preferential distribution of Zn to developing tissues in rice. *Plant J.* 2015; 84:374-384.
29. Swamy BPM, Kumar A. Genomics-based precision breeding approaches to improve drought tolerance in rice. *Biotechnol Adv.* 2013b; 31:1308-1318.
30. Takahashi M, Nozoye T, Kitajima N, Fukuda N, Hokura A, Terada Y, *et al.* In vivo analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X-ray Fluorescence Imaging of Fe, Zn, Mn, and Cu. *Plant Soil.* 2009; 325:39-51.
31. Varshney RK, Nayak SN, May GD, Jackson SA. Next generation sequencing technologies and their implications for crop genetics and breeding. *Trends Biotechnol.* 2009; 27:522-530.
32. Velu G, Bhattacharjee R, Rai KN, Sahrawat KL and Longvah T. A simple and rapid screening method for grain zinc content in pearl millet. *J SAT Agric Res*, 2008.
33. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp Bot.* 2004; 55:353-364.
34. Wissuwa M, Ismail AM, Graham RD. Rice grain zinc concentrations as affected by genotype native soil-zinc and zinc fertilization. *Plant Soil.* 2007; 30:637-48.
35. Zarcinas BA, Cartwright B, Spouncer LR. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun Soil Sci Plant Anal.* 1987; 18:131-146.
36. Zhang M, Pinson SRM, Tarpley L, Huang X and Lahner B. Mapping and validation of quantitative trait loci associated with concentration of 16 elements in un milled rice grain. *Theor Appl Genet.* 2014; 127:137-165.
37. Zhao FJ, McGrath SP. Biofortification and phytoremediation. *Curr. Opin. Plant Biol.* 2009; 12:373-380.