

Review

Genetic bio-fortification of cereals from a plant breeding perspective

Tumbare Tererai and Maphosa Mcebisi*

Faculty of Agricultural Sciences, Department of Crop and Soil Science, Lupane State University Box 170, Lupane, Zimbabwe.

Received 15 July, 2022; Accepted 25 November, 2022

Genetic “biofortification” presents an opportunity to provide sustainable and cost effective solution to the perennial problem of ‘hidden hunger’ prevalent in most marginalized regions of the world. Diets in households across many developing countries typically consist of high calorie crops which are mostly cereals with limited diversified food. Production of cereal crops with elevated quantities of micronutrients needed by the human body is thus imperative. This review outlines “biofortification” of cereal crops with emphasis on existing genetic variability, genetic and molecular basis for essential amino acids, zinc and iron accumulation in crops. Key issues emerging are that in most crops there is scope to undertake conventional improvement given the sufficient variability influenced by additive genes or associated with polymorphic molecular markers. Availability of high potential non-commercialized genetically modified cereals points to prohibitive legislative frameworks and the need to adopt other tools such as gene editing, mutation breeding not subject to stiff restrictions. Furthermore, given the output nature of “biofortification” traits there is need to integrate this genetic enhancement in a pipeline breeding approach which integrates breeding objectives so that communities and processors can access these peculiar traits in every new improved variety.

Key words: Essential amino acids, micronutrients, nutrition-sensitive agriculture, malnutrition.

INTRODUCTION

Global incidence of micronutrient malnutrition across the world population is estimated to be above 2 billion, with a greater percentage of this statistic being resource poor households (Cashman, 2020). Children of pre-school age, adolescent women and reproductive women in general are at high risk of suffering from micronutrient deficiency health related conditions (Bouis et al., 2020). Inadequate essential micronutrients quantity, 'hidden hunger' can culminate into various physical, mental, social and economic ills such as increased morbidity, disability, stunted and physical growth and reduced

national socio-economic development (Ekholuenatale et al., 2020). High mortality amongst children of school going age and complications in pregnant women due to micronutrient deficiencies in low to medium income countries has also been reported (Ahsan et al., 2017; Castrogiovanni and Imbesi, 2017). The World Health Organization (WHO) and Food and Agricultural Organization (FAO) report of 2003, listed Zinc, Iron, Iodine and Vitamin A deficiencies as the most prevalent deficiencies in developing countries which lead to various notable health conditions. There is however growing

*Corresponding author. E-mail: mmaphosa@lsu.ac.zw.

concern in deficiency of folates, Vitamin D, Thiamine (B1), Lysine, Tryptophan, and Selenium (Strobbe et al., 2017; Holick, 2017; Cashman, 2020; Motukuri, 2019; Ligowe et al., 2020).

The prevalence of micronutrient deficiency in resource poor communities is largely attributed to dysfunctional food systems, depending heavily on inherently micronutrient poor staple cereal crops (maize, wheat, rice, sorghum, millets) with limited access to diverse foods which supply a variety of micronutrients (Conti et al., 2021). Global food systems have always been largely a direct function of the output from agricultural systems which largely focused more on increasing yield output with little regard to the quality of the produce. This has contributed significantly to the rise of unprecedented consequences such as micronutrient malnutrition amongst resource poor communities (Davis et al., 2019). Studies show that apart from food security, nutritional security is also equally important and as such, a robust and sustainable approach to agriculture production, which addresses both the energy as well as nutrition and health requirements, is thus required (Conti et al., 2021).

FOOD FORTIFICATION STRATEGIES TO COMBAT MICRONUTRIENT DEFICIENCIES

Developed economies have made significant strides in combating malnutrition through various interventions which include, fortifying processed foods, supplementation, and diversifying diets (Mannar et al., 2018). However, in low to middle income countries, these strategies have low feasibility due to the associated high cost factor. As such, remediation approaches that can reach low-income rural populations with limited access to commercially marketed fortified foods are a strategic solution. The following sections focus on highlighting the merits and demerits of the common food fortification strategies.

Industrial fortification

This is the enrichment of processed food products with specific essential nutrients during processing. It can be done either as a commercial product enhancement strategy by the food processors or as a policy influenced intervention stipulated by governments. Industrial fortification normally targets basic food commodities such as sugar, cooking oil, milk, butter, maize meal, table salt and wheat flour, due to their widespread and regular consumption in almost all households (Kumar et al., 2019). This malnutrition combating strategy is fairly effective owing to its wide reach of consumers; however it also notably has a number of downside factors. In cases of inefficient quality monitoring, systems, there is a huge risk of exposing consumers to mineral toxicity if

excessive doses of the micronutrients are added (Maqbool and Beshir, 2019). Another major concern also comes on the cost implication of the industrial fortification process which is factored into the fortified products thus transferring the cost burden to consumers (Lalani et al., 2019). Consequently, this makes industrially fortified food products more expensive than unfortified products. This has greater disadvantages for resource poor communities in developing countries who cannot bear such costs.

Dietary supplements

Dietary supplements are normally acquired through nutritional intervention programs by governments, non-Governmental Organizations (NGOs) or direct purchases from pharmacies (Marra and Bailey, 2018). Several supplements, supplying micronutrients such as Zinc, specific vitamins like Vitamin A and C as well as protein, creatine and other amino acid supplements are available on the markets for open purchase (Goredema-Matongera et al., 2021). The positive impact of government or NGO driven dietary supplementation programs in Africa, for instance, cannot be ignored but however an evaluation of dietary supplementation as a strategy to reduce micronutrient deficiencies suggests low sustainability. Like industrial “biofortification”, overdosing on dietary supplements can lead to toxicity in consumers (Dwyer et al., 2018). The intervention is also limited by the absence of critical supporting infrastructure or health services like pharmacies or clinics in malnutrition prone regions. The socio-economic environment in most of the malnutrition prone regions is also not favorable to enable regular supply of supplements more so pricing of the supplements is beyond the reach of target consumers (Garg et al., 2018).

Dietary diversification

Consuming diets based on a variety of food sources decreases the risk of suffering from micronutrient deficiency related illnesses. This strategy is one of the most efficient, long-term solutions to militate against malnutrition. Consumption of a balanced diet supplies the body with all the nutrients it requires in sufficient quantities. Some of the micronutrient rich foods include animal based food sources like eggs, milk, red meat, vegetables, fruits, fish and sea food (Aakre et al., 2020; Wallace et al., 2016; Manwaring et al., 2016). Despite the benefits, adequate dietary diversification is beyond the financial capacity of many households in low to medium income countries (Manwaring et al., 2016). A number of donor funded organizations have tried to bridge this gap by introducing programs that promote establishment of nutritional gardens in rural areas; however the extent of coverage is still not wholesome. Such programs would

also require the support of governments through their agricultural extension services to maintain the nutritional gardens if NGO funding pulls out of those areas (Singh et al., 2016).

Bio-fortification

Amongst the many available agricultural tools that can be implemented to increase micronutrient concentrations in food crops, “biofortification” is the first agricultural tool that has been implemented at a global scale with notable success (Bouis et al., 2013). “Biofortification” of cereal crops is the deliberate and targeted enrichment of grain micronutrient density, through conventional and molecular plant breeding; transgenic, micro-flora and fertilization techniques. “Biofortification” is considered cost effective, long term and sustainable approach to address malnutrition in resource poor communities (Sharma et al., 2016).

Agronomic “biofortification” involves the application of specific fertilizers to the soil or directly to foliage to increase the mineral concentration available for uptake by the plant. Various studies with different minerals have proven that increasing mineral concentration available for uptake by plants translate to increased levels of the micronutrient in edible portions of the crop (Zhang et al., 2018; Wang et al., 2016; Joy et al., 2017). Successful implementation of agronomic “biofortification” is however affected by several factors which include availability of the mineral in a form that can be available for uptake by the plant roots, physiologically related interferences to efficient translocation of the mineral in the crop plant, and remobilization of minerals from vegetative tissues into storage tissues (Rehman et al., 2021). The cost of fertilizers is also another huge obstacle to the successful implementation of agronomic “biofortification” as a mitigation measure against malnutrition (Bouis et al., 2020).

Recent novel discoveries on the role of certain bacteria strains that solubilize metals such as zinc, making them more available for uptake by plant roots have presented an additional approach towards “biofortification” (Mumtaz et al., 2017). Inoculating crop seeds with such microbial strains have the potential of not only increasing micronutrient densities in edible portions but also can significantly enhance soil fertility and crop yield (Hussein et al., 2018).

Genetic “biofortification” on the other hand involves the use of both conventional and transgenic breeding techniques to introgress genes that promote high micronutrient accumulation into elite genotypes or manipulate the crop genetic makeup using genome editing tools in order to express proteins that enhance micronutrient accumulation (Ludwig and Slamet-Loedin, 2019). Genetic “biofortification” of staple crops presents

a sustainable, low cost and durable solution to delivering micronutrients in the diets of malnourished populations.

ADVANTAGES OF GENETIC BIOFORTIFICATION

Cost effective and inexpensive

Developing bio-fortified staple crops is a one-time investment, with very low recurrent costs (Sharma et al., 2016). Upon release, bio-fortified germplasm can be shared across communities and considering the global extent of cultivation of staple crops such as maize, sorghum and wheat impacts are far reaching.

Bio-fortified varieties can be grown sustainably

Seed of released bio-fortified crop varieties, especially self-pollinating crops, can be retained and grown without losing the trait. HarvestPlus programs supported by partnerships with country Agricultural Research Institutions have supported the development and promotion of bio-fortified crops. The focus has mainly thrived to reach marginalized communities and seed supply to ensure access to bio-fortified germplasm by vulnerable communities (Sharma et al., 2016; Yadav et al., 2020).

Breeding for higher micronutrient density in some crops has no yield penalty

Several character association studies performed on some bio-fortified crops, have revealed absence or no correlation between micronutrient accumulation in edible portions and yield (Welch and Graham, 2004; Velu et al., 2012; Kumar et al., 2015). Thus breeding for high micronutrient density can be done successfully without reducing the yield output that is expected for a given genetic background. Where yield penalty exist, exploration of other crop improvement tools such as genetic engineering can be exploited with minimal linkage drag.

STRATEGY FOR SUCCESSFUL GENETIC BIOFORTIFICATION

Success in genetic “biofortification” depends on increasing the existence of sufficient genetic variability for the nutrient in question in the final edible portion. Where variability is non-existent within the crop gene pool techniques such as mutation breeding, gene editing and genetic engineering can be employed depending on the likelihood for successful commercialization of the final product. A pipeline approach of fitting “biofortification”

objectives within regular crop improvement programs can be a sustainable way of ensuring consumer acceptance. Integrating an additional “biofortification” trait to farmer preferred varieties can be useful for enhanced adoption. If the sensory attributes of the crop are not affected and preparation methods are the same bio-fortified crops can thrive.

Bio-fortification of maize

Maize (*Zea mays*) remains one of the most targeted cereals for “biofortification” owing to its widespread cultivation as a staple crop and key source of diverse products in developing regions of sub Saharan Africa (SSA), South America and South Asia. The wide native genetic variability in micronutrient concentration enables elite breeding lines of the crop to be nutritionally enhanced through plant breeding approaches. Furthermore, the cultivation of maize is possible across a broad agro-ecosystem coverage which further justifies its continued inclusion in “biofortification” programs.

In SSA, 30% of the total calories in cereal based diets of 20 countries are provided by maize grain. High dependency on the cereal pushes its daily per capita consumption in the region up to 450 g/person/day (Ekpa et al., 2019). Consumption of maize in SSA is primarily as boiled, roasted green mealies or alternatively as a thick porridge prepared from ground maize meal and served with an accompanying relish. The dependency on consumption of maize in these poor and marginalized regions coupled with the inherent low essential micronutrient density characteristic of normal maize (Cakmak and Kutman, 2018), puts such consumers at greater risk of suffering various micronutrient deficiency related conditions.

Extensive work on “biofortification” of maize has been done by the International Maize and Wheat Improvement (CIMMYT) in partnership with HarvestPlus whose mandate is to improve nutrition and public health through development of bio-fortified food crops. Most of the interventions in bio-fortifying maize have targeted improvement of proteins (lysine and tryptophan), zinc and pro-vitamin A grain concentration (Goredema-Matongera et al., 2021).

Quality protein maize (High Lysine and Tryptophan Content)

Maize kernels have a large portion of zein proteins in the endosperm which is deficient of essential amino acids. As a result nutritional enhancement efforts for the crop commenced as early as the 1960s with development of Quality Protein Maize (QPM) by CIMMYT after the discovery of the mutant *opaque-2* (*o2*) gene (Listman et al., 2019).

Genetic variability for grain lysine and tryptophan content in maize germplasm

Any crop improvement program primarily depends on the amount of genetic variability present for the given trait of interest. The extend of diversity enables effective selection and generation of an improved population with a higher population mean as compared to the mean of the original population. Singh et al. (2020), in a study to evaluate the protein content, lysine and tryptophan content in maize inbred genotypes, reported existence of broad diversity in lysine and tryptophan content in QPM germplasm. The concentration of lysine in QPM ranges from 3.3 to 4.0 g/100 g of total endosperm protein compared to normal maize grain, QPM has lysine and tryptophan content that is 30 and 55% higher (Nuss and Tanumihardjo, 2011).

Genetic basis for high grain lysine and tryptophan production and breeding efforts in maize

The genetic basis for QPM breeding is based on homologous recessive alleles of the mutant *opaque-2* gene which is normally homozygous dominant or heterozygous (Tripathy et al., 2017). The homozygous recessive genotype confers production of increased levels of the amino acids tryptophan and lysine than conventional maize (Maibvisira et al., 2018). However, initial efforts in breeding QPM revealed that, expression of the *opaque-2* mutant produced a soft chalky endosperm which was undesirable among consumers. It was however discovered that breeding for the presence of the mutant gene together with its accompanying genes produced a hard endosperm preferred by consumers. The ultimate success of QPM breeding thus requires a multi-pronged approach that manipulates 3 different genetic systems which are: (i) replacing the normal gene with the mutant gene on the *opaque-2* locus, (ii) modifier genes that enhance the expression of the *opaque-2* gene relative to lysine and tryptophan content, and (iii) modifier genes that induce development of a hard endosperm (Prasanna et al., 2020).

BREEDING STRATEGIES FOR QPM

Different breeding strategies have been employed to manipulate and improve the performance of specific maize genotypes with regards to grain lysine and tryptophan content. The most utilized strategy is hybridization done with the objective of exploiting heterosis. Using this breeding method, open pollinated varieties (OPVs) or hybrids can be produced (Tripathy et al., 2017). The preponderance of additive gene action for Quality Protein trait enables development of QPM OPVs through recurrent selection. This can be accomplished by crossing a recurrent parent which must be an improved

OPV and a trait donor parent with high lysine and tryptophan content followed by successive backcrosses with the recurrent parent to retain most of its original genome (Goredema-Matongera, 2021). Another breeding approach for production of QPM is hybrid development. The success of hybrid development largely depends on heritability of the trait, gene action governing expression of the trait as well as combining ability of different genotypes upon crossing (Goredema-Matongera, 2021). A study by Machida et al. (2010) reported significant GCA and SCA for tryptophan/protein content (Quality Index) from a diallel cross of QPM inbred lines. This indicates the possibility of producing hybrid QPM through exploiting the non-additive gene effects that can contribute significantly to increasing heterosis for the quality protein trait.

There is also evidence of current research on use of recent breeding tools such as Marker Assisted Breeding (MAB) and genome editing for producing QPM. MAB significantly increases the efficiency of the selection process since it is based on the operational principle where presence of a specific trait linked marker confirms presence of a trait (Maqbool et al., 2021). Tandzi et al. (2017) reported 3 simple sequence repeats (SSR) markers and these are *phi057*, *phi112* and *umc1066*. As such introgression of the *o2* gene to non-QPM genetic backgrounds can be made possible with relative ease and efficiency using the highly polymorphic markers. According to Goredema-Matongera et al. (2021), to date a number of QPM varieties have been released across the world for instance HQPM-5 (India), Obatanpa (Ghana), ZS261 (Zimbabwe), BHQP542 (Ethiopia), Q623 (South Africa), and Yanrui-1 (China).

Zinc bio-fortified maize

Maize grain is rich in calories but deficient in some essential micronutrients that include zinc (Ignjatovic-Micic et al., 2015).

Consumers who subsist on maize as a staple crop are thus at risk of suffering from zinc deficiency if other sources of zinc are not available. The maize zinc “biofortification” drive has been largely driven by CIMMYT and International Institute of Tropical Agriculture (IITA) in partnership with other private and public sector organizations, for widespread alleviation of the micronutrient deficiency in communities that subsist on cereal based diets was initiated (Prasanna et al., 2020).

Genetic variability for grain zinc content in maize germplasm

HarvestPlus set 33 µg/g as the breeding target for maize grain zinc content that would address deficiencies assuming 90% zinc retention after processing and 25% bioavailability (Andersson et al., 2017). The baseline

grain zinc level in maize is 20 µg/g thus a target increment of 13 µg/g would achieve the breeding target (Akhtar et al., 2018). Several screening procedures have revealed sufficient grain zinc variability in maize that can suffice to attain the breeding target. Mageto (2020) synthesized data from 23 reports on grain zinc screening studies done since the year 2000 on over 3000 genotypes that included landraces, inbred lines, hybrids, core accessions, improved genotypes, and QPM inbreds from various agro-ecological regions across the world and found grain zinc variability spanning a range of 3.8 to 95.6 µg/g. Such wide variability affirms the practicality of breeding for high grain zinc in maize through selective breeding.

Genetic and molecular basis for grain zinc accumulation in maize

The ultimate goal of zinc breeding is to increase zinc uptake and transport efficiency, increase the amount of bioavailable zinc and reduce concentration of anti-nutritional factors or increase concentration of promoters (Muluaem, 2015). Zinc accumulation in maize grain is reported to be a polygenic trait (Gupta et al., 2015). Apart from the genetic effects, it is also significantly dependent upon the integrated action of several other factors such as environmental effects, genotype × environment interaction, micronutrient availability in the soil, translocation and partitioning to different plant parts and uptake by roots (Hussain et al., 2018). This implies differential zinc concentration among genotypes depending on where they are grown.

Combining ability studies have revealed that the inheritance of zinc accumulation in maize grain is largely controlled by additive than non-additive gene effects (Fan et al., 2014; Fasahat et al., 2016), with heritability of maize grain zinc reported to be in the range of 59 to 76% (Fan et al., 2014; Cheah et al., 2020; Mageto et al., 2020). The extent of these genetic parameters suggests significant influence of genetic factors in determining grain zinc content as well as the potential of realizing high genetic gains through selection.

Marker Assisted Breeding (MAB) for complex traits like zinc grain accumulation in maize realizes quicker results than using conventional breeding and phenotypic characterization methods. As such, several QTL mapping and Genome Wide Association (GWAS) studies for zinc accumulation have been conducted in maize. The results from these studies however, have not been consistent with regards to gene loci, total variance explained by identified QTL and confidence levels (Mageto, 2020). Most researchers however concur that genomic regions associated with zinc accumulation in maize are located on chromosomes 1, 2, 6, 7, 9 and 10 (Zhang et al., 2017; Hindu et al., 2018; Guo et al., 2020; Mallikarjuna et al., 2020). These studies have further identified some of the important gene families that influence maize zinc

accumulation such as Natural Resistance Associated Macrophage Protein (NRAMP), Ferritin, Cation Efflux (CE), Yellow Stripe (YS), and Zinc Regulated Transporter Protein) family (ZIP) (Goredema-Matongera, 2021). Prasanna et al. (2020) reported the presence of 3 high utility SNPs from a total of 20 SNPs found to be associated with grain zinc. These SNPs can be used for Marker Assisted Selection (MAS) and Genomic Selection to accelerate genetic gain for grain zinc in maize.

BREEDING STRATEGIES AND PROSPECTS FOR ZINC BIOFORTIFIED MAIZE

Successful breeding of zinc dense maize depends on genotype × environment interactions as well as genetic correlation between zinc accumulation with other attributes such as concentration of other micronutrients or yield. Plant breeders can exploit the prevalence of additive gene action for grain zinc accumulation to develop improved Open Pollinated Varieties (OPVs) through recurrent selection (Goredema-Matongera, 2021). Narrow sense heritability of 72% was reported for grain zinc in maize (Hindu et al., 2018) thus with such high heritability and non-additive gene action reported for specific genotypes suggests high zinc hybrids can be developed. Plant breeders can also safely expedite the exploitation of SNP markers already identified as being strongly associated with grain zinc accumulation in maize for marker assisted or genomic selection purposes thus accelerating the breeding progress (Prasanna et al., 2020).

Pro-vitamin A maize

Yellow and orange maize contains carotenoids such as α -carotenoids, β -carotenoids and β -cryptoxanthin, which are precursors of the physiologically active Vitamin A or retinol by humans upon consumption (Kondwakwenda et al., 2019). Elevation of the carotenoids in maize, a crop that constitutes a significant percentage in the diet of communities in Sub-Saharan Africa is of paramount importance given the scarcity of cheap vitamin A sources in many developing countries.

Genetic variability for PVA content in maize germplasm

Maize grain normally contains 0.5 to 1.5 $\mu\text{g/g}$ Pro-vitamin A (PVA). This amount weigh below the minimum amount required to address vitamin A deficiency (Mengesha et al., 2019). The breeding target for maize PVA was set at 15 $\mu\text{g/g}$ in order to provide 50% of the mean daily vitamin A requirement through normal consumption habits (Bouis et al., 2020). Initial screening studies of more than 1500 genotypes revealed considerable variability for carotenoid profiles in temperate, tropical and subtropical maize

germplasm, with results indicating PVA content between 0 and 19 $\mu\text{g/g}$ (HarvestPlus, 2014; Muthusamy et al., 2015; Muzhingi et al., 2017). Great polymorphism was also observed in alleles of genes encoding enzymes that affect concentration of grain PVA carotenoids (Kondwakwenda et al., 2019). This ultimately enables the stacking of these promoter genes into one genotype using Marker Assisted Breeding.

Genetic and molecular basis for PVA carotenoids accumulation in maize

Accumulation of PVA in maize has been largely attributed to the activity of three key enzymes in the carotenoid biosynthesis pathway, namely *phytoene synthase* (*PSY1*), *lycopene epsilon cyclase* (*lycE*), and *carotene hydroxylase 1* (*CrtRB1*) (Owens et al., 2014). However, the contribution of each of these towards PVA accumulation varies.

Phytoene synthase catalyses the condensation of 2 genanylgeranyl molecules (GGPP) into 1 molecule of phytoene. This reaction is responsible for development of yellow color in yellow maize (Babu et al., 2013). Variability in alleles of the *PSY1* gene (*Y1* gene) accounts for the differences in grain color in maize. A homozygous dominant (*Y1/Y1*) or heterozygous (*Y1/y1*) allelic combination results in production of high levels of carotenoids which accumulate in the endosperm of yellow maize. Conversely, a recessive combination (*y1/y1*) is typical of white maize (Maqbool et al., 2018).

Lycopene epsilon cyclase (*lycE*) catalyses the conversion of lycopene into α -carotene or β -carotene, however down-regulating the *lycE* gene favours progression of the β -carotene branch over α -carotene branch in the carotenoid biosynthesis pathway (Zunjare et al., 2017). Mapping analysis and allele mining studies revealed that there are 4 *lycE* polymorphic sites located on chromosome 8. These polymorphisms explain 58% of phenotypic variance in maize PVA accumulation (Azmach et al., 2013).

β -carotene hydroxylase (*crtRB1*) facilitates the conversion of β -carotene to β -cryptoxanthin (Maqbool et al., 2018). The *crtRB1* locus has been shown to be the major QTL linked with β -carotene accumulation and other downstream non PVA carotenoids. Mapping analysis and allele mining studies have shown the *crtRB1* locus on chromosome 10 and also existence of 3 polymorphic sites (Maqbool et al., 2018).

Various studies to evaluate combining ability and heritability of high PVA content in maize concur that PVA carotenoid content is controlled by both additive and non-additive gene action with preponderance of non-additive gene action (Babu, 2014; Halilu et al., 2016). Furthermore, Ewool and Akromah (2017) and Menkir et al. (2017), observed medium heritability for both PVA and non PVA carotenoids. However, Halilu et al. (2016) reported low broad and narrow sense heritability for β -

cryptoxanthin and α -carotene but high broad sense heritability for β -carotene. These results are suggestive of response to selection using available diverse material for PVA breeding.

BREEDING STRATEGIES FOR PVA MAIZE BREEDING

The available information on PVA genetics allows for both conventional and molecular breeding strategies and selection methods to be used for developing high PVA maize cultivars. Azmach et al. (2013), recommended backcross breeding use in the early stages of bio-fortification efforts in tropical and subtropical regions. This is because most tropical maize genotypes have low PVA carotenoids thus introgressing has to be from donor high PVA temperate parents (Menkir et al., 2017).

Recurrent selection is also another viable strategy, especially for development of OPVs, which are the most economical and sustainable option for low income regions since seed can be retained for 2 or 3 generations. This exploits additive gene effects and medium to high heritability (Suwarno et al., 2014). Recurrent selection techniques such as intra-population recurrent selection and pedigree selection have been evaluated by different researchers for improvement of PVA in maize with some significant levels of success. A study by Dhliwayo et al. (2014), found that maize PVA carotenoid levels were significantly improved after 3 cycles of S_1 recurrent selection on 3 genetically diverse maize populations.

Hybrid development is also a key alternative in breeding for PVA in maize as it exploits heterosis, that can manifest in a number of other important traits such as yield and resistance or tolerance to biotic and abiotic stresses. To unlock the heterotic potential of PVA associated genes, stable high yielding and high PVA inbred lines are developed and these are subsequently crossed into single, 2-way or 3-way cross hybrids based on high SCA effects for PVA and other desirable agronomic traits (Kondwakwenda et al., 2019). Maqbool et al. (2018), however points out that, 3-way crosses are more ideal for PVA improved in maize due to practical implications, being economical in seed production and the relative ease of obtaining uniformity for the hybrid plants.

One of the major hurdles in the process of developing PVA bio-fortified maize is analysis, specifically partitioning and quantification of the different carotenoids present. This is because (1) maize contains different types of carotenoids, some of which are non-PVA associated (2) carotenoids form complex interactions with other biological molecules such as proteins or starch (3) like other biological molecules, carotenoids are prone to degradation (Guild et al., 2017). The practical potential for MAS in PVA breeding was exploited for identifying high PVA parental lines, tracking PVA-linked alleles during backcrossing as well as proteome quantification of

PVA genes (Azmach et al., 2013; Muthusamy et al., 2014; Suwarno et al., 2014).

To date, significant progress in PVA Marker Assisted Breeding (MAB), a pedigree breeding scheme using MAS to target selection of the 2 superior alleles was used to develop donor germplasm with $>20 \mu\text{g/g}$. This was done by selecting seeds which were homozygous for the favorable alleles at the *CrtRB1-3'TE* and *LcyE3'Indel* polymorphisms from segregating F_2 and F_3 populations (Zunjare et al., 2018; Ortiz-Covarrubias et al., 2019). CIMMYT and IITA developed Single Nucleotide Polymorphism (SNP) markers associated with the favorable alleles and this has increased the efficacy of using MAS for PVA breeding (Prasanna et al., 2020).

Bio-fortification of rice

Rice (*Oryza* species) is a major staple food for many Asian countries and feeds more than half of the world's population. Like the majority of cereals rice is deficient in micronutrients iron and zinc, which causes various disorders that are prevalent in many developing countries (Bashir et al., 2013). In rough rice, the micronutrients are present but reduce significantly during postharvest processing which is crucial to suit consumer needs and allow for long term storage of grain without deterioration. Dehulling and debranning reduces the micronutrients that are confined in the aleurone layer, pericarp and embryonic tip which predisposes communities that are dependent on rice as a major food to iron and zinc deficiency. Accordingly, any "biofortification" efforts in rice have greater chances of success if they target the endosperm that remains after processing.

The drive to biofortify rice to address zinc and iron deficiencies commenced following the HarvestPlus prepared "Biofortification" Index which identified a number of Asian countries that were critically zinc deficient (Bouis et al., 2020).

HarvestPlus in partnership with International Rice Research Institute (IRRI) have made significant strides in developing zinc biofortified rice varieties for target countries like Bangladesh, Philippines, India, and Indonesia. Significant research and breeding efforts to improve rice micronutrient quantities have also been done to improve iron and carotenoid concentration with a number of varieties released to date. Target values for improved grain iron and zinc in rice that can reach 30% of human Estimated Average Requirement (EAR) were set at 13 and 28 $\mu\text{g/g}$, respectively (Bouis et al., 2011).

Genetic variability of zinc and iron concentration in rice

Unlike most staple cereals with elevated zinc and iron traits available within the species gene pool, there is limited variation for these two in rice. Some wild rice relatives such as *O. latifolia*, *O. nivara*, *O. officinalis*, *O.*

barthii, *O. longistaminata*, and *O. rufipogon* have significant iron and zinc with potential use in pre-breeding. The major impediment is linkage drag during introgression that compromises yield (Majumder et al., 2019). Despite the existence of synergism between iron and zinc in rice grain, only zinc “biofortification” has gained more traction and several varieties are already released in Asia (Calayugan et al., 2021). The success of zinc “biofortification” came from the use of *aus* germplasm in conventional breeding programs. However, there is need for multi-environment evaluation of breeding materials given the significant genotype-by-environment interaction for zinc. On the contrary, there is no high iron germplasm within the rice germplasm which means any improvement in this micronutrient will require use of recombinant DNA technology, gene editing or mutation breeding (Matres et al., 2021). In rice micronutrient biofortification several mutants and products of genetic engineering show great potential but commercialization has been limited due to prohibitive GM legislative frameworks in the targeted developing countries.

Genetic and molecular basis for zinc and iron uptake and accumulation in rice grain

Numerous studies have shown that zinc and iron uptake and accumulation in grain is controlled by several genes. Characterization of gene families in zinc homeostasis have identified gene families such as *OsNAS*, *OsTOM1*, *OsDMAS*, *OsSAMS* and *OsNAAT* which increase metal uptake by roots through their involvement in biosynthesis, transport and secretion of phytosiderophores (Kavakani et al., 2018; Raza et al., 2019; Dixti et al., 2019; Yang et al., 2020). Equally important are the ZIP family genes responsible for transport of zinc and ferrous iron as well as the *OsYSL* family proteins which are actively involved in long distance transport of metals such as zinc and iron in the phloem and their subsequent influx into the seed endosperm (Swamy et al., 2016).

Genetic characterization of several rice genes for grain zinc have revealed significant phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), genetic advance as well as broad and narrow sense heritability (Sala et al., 2015; Ajmeera et al., 2017; Umarani et al., 2017; Dhakal et al., 2020; Prasannakumari et al., 2020; Rathod et al., 2020). Studies on combining ability have also shown significant additive gene effects for grain zinc (Zhang et al., 1996; Sharifi, 2013). In other studies, heterosis and transgressive segregants have also been identified in rice breeding populations by Babu et al. (2012) and Stangoulis et al. (2017).

BREEDING STRATEGIES FOR HIGH GRAIN IRON AND ZINC IN RICE

There is substantial and useful genetic variation in zinc

content sufficient to be exploited in a breeding program to develop biofortified rice. On the contrary, genetic variability for iron is limited thus most of the available literature on conventional breeding of these essential metals focuses on zinc breeding. The narrow genetic variability coupled with significant losses of the micronutrient realised during polishing disqualifies selective breeding as a viable option for iron content improvement in rice (Boy et al., 2017). However, with regard to zinc content improvement in rice, Balakrishnan et al. (2020) suggested use of the advanced backcross method for genetic dissection of wild rice, and for developing high zinc introgression lines, as an attractive approach for efficient use of wild rice accessions. Narrow sense heritability high, single plant selection can be utilized as an effective approach for improving nutrient content (Calayagan et al., 2020). Furthermore, use of multiple crosses involving several donors and recipient parents such as 3-way or 4-way crosses or reciprocal crosses to enhance zinc levels and yield potential is also a viable alternative. Meng et al. (2016) highlighted the use of Multi-parent Advanced Generation Inter-cross (MAGIC) as a viable method to pool genes for high zinc as well as providing a good resource for selecting high zinc lines and transgressive segregants. Heterosis can also be exploited to develop high grain zinc and high yielding hybrids since reports show that there is satisfactory heterosis in rice (Naik et al., 2021).

Every breeding effort must ensure that essential agronomic attributes such as high yield are not lost as other traits are introgressed. Calayugan et al. (2020), reported a positive correlation between zinc content and grain weight, thus implore that yield related traits must be considered during selection. Despite contrasting results on the relationship between yield and zinc in rice, most of the recent studies have found no significant relationship between the 2 traits thus ensuring the possibility of developing zinc rich and high yielding varieties (Trijatmiko et al., 2016; Jaksomsak et al., 2017; Anusha et al., 2021).

Iron biofortified pearl millet

Pearl millet (*Pennisetum glaucum*) is a crop of economic significance in the semi-arid and arid regions of Africa and India where it is consumed as a staple crop by some communities (Govindaraj et al., 2020). Its grain zinc content is comparatively higher than in other cereals such as rice and wheat, however major concern is on the grain iron content produced by most pearl millet genotypes. This prompted the need to undertake crop improvement efforts focused on raising iron content in OPVs and hybrids for Africa and India, respectively (Boy et al., 2017).

Genetic variability of iron content in pearl millet

A target increment of +30 ppm iron was set by

HarvestPlus to achieve a target level of 70 ppm iron in pearl millet grain from a baseline level of 47 ppm (Govindaraj et al., 2020). Germplasm screening by different institutions and researchers have revealed wide variability for iron across diverse populations that include landraces, inbred parents, population progenies and hybrids. To date, ICRISAT screening studies report of grain iron concentration spanning a range of 18 to 135 µg/g across the aforementioned populations (Govindaraj et al., 2019).

Genetic and molecular basis for iron accumulation in pearl millet grain

Iron concentration in pearl millet is largely controlled by additive gene effects. Studies done to evaluate the combining ability of numerous and genetically diverse breeding lines and early generations progenies have confirmed preponderance of additive gene action controlling iron content in pearl millet (Kanatti et al., 2016; Gavali, 2018; Warriar et al., 2020). The trait is also associated with high heritability thus it has the capacity to respond well to selection thereby realising significant genetic gain in improved populations (Kumar et al., 2020; Anuradha et al., 2017). Gene mapping and molecular analysis studies have revealed that grain iron accumulation in pearl millet is controlled by several gene families which include *PgZIP*, *PgINRAMP* and *PgIFER* (Pujar et al., 2020; Kumar et al., 2016; Mahendrakar et al., 2020). These genes play an important role in iron metabolism at different stages of the crop development, and they provide a foundation for exploitation of iron metabolism genes in pearl millet molecular breeding programs.

BREEDING STRATEGIES FOR HIGH IRON PEARL MILLET

Iron content breeding in pearl millet can be done via the pedigree method where progeny populations can be developed from bi-parental crosses (Srivastava et al., 2022). In order to advance genetic gains in yield, composites can also be used as a base population to develop diverse hybrid parents. Development of hybrids for exploitation of heterosis is another viable breeding strategy for pearl millet (Srivastava et al., 2020). This is done by developing high iron inbred lines and later evaluating their combining ability. Currently, most pearl millet hybrids are produced through utilizing the various cytoplasmic male sterility systems available in pearl millet (Pujar et al., 2019). Pearl millet has no maternal effects for iron accumulation in grain thus high iron inbreds can be used as either males or females without affecting the outcome of the crosses (Kanatti et al., 2016).

Key to biofortification of any crop is the relationship

between the target micronutrient and yield. Govindraj et al. (2020) reported that iron is negatively and non-significantly associated with grain yield thus higher productivity does not necessarily lower iron levels in pearl millet grain. However, other agronomic traits of significance such as grain weight have shown positive correlation with grain iron concentration, implying the need for careful selection in the initial breeding stages.

Zinc biofortified wheat

Wheat (*Triticum aestivum*) has over the years played a very crucial role in the global food systems accounting for approximately 20% of the world's dietary needs (Mohammadi-joo et al., 2015). It is a fairly good adaptive crop that can thrive in various agro-ecosystems of the world thus making it one of the widely cultivated food crops (Muslim et al., 2015). Although substantially enriched with calories, wheat grain is however notably deficient in essential micronutrients such as iron and zinc and this is exacerbated by the milling process (Sharma et al., 2020).

Genetic variability for grain zinc content in wheat

A target increment of +12 µg/g was set to increase the grain zinc concentration from a baseline level of 25 to 37 µg/g in improved wheat varieties (HarvestPlus, 2014). Large scale germplasm screening for grain zinc content across wheat landraces and wild relatives by CIMMYT identified significant variability that could suffice to achieve target increments for the metal in biofortified varieties (<http://www.cimmyt.org/germplasm-bank/>). More than 3000 germplasm accessions were screened and zinc content was found to be in the range of 20 to 115 µg/g (HarvestPlus, 2014). A report by the Bangladesh Agricultural Research Institute noted average zinc concentration in wheat grain spanning a range of 20 to 35 µg/g (Das et al., 2019). Furthermore, Gupta et al. (2021), reported the presence of higher Zn and Fe levels in landraces relative to cultivars. Zinc and iron levels reported by Gupta et al. (2021), reached as high as 87.29 mg/kg Zn (up to 53.3 mg/kg in wheat cultivars) and up to 122.20 mg/kg Fe (up 56.5mg/kg in wheat cultivars).

Various high zinc genotypes with potential to be used as parental lines in zinc breeding programs were identified and most are progenitors of modern high zinc hexaploid wheat (Sharma et al., 2020).

Genetic and molecular basis for grain zinc accumulation in wheat

Grain zinc accumulation in wheat is under quantitative genetic control and to date several mapping studies have

identified a number of QTLs (Velu et al., 2014; Velu et al., 2017; Shariatipour et al., 2021). While the *Gpc-B1* gene was identified as being highly significant in zinc homeostasis and subsequent zinc accumulation in wheat grain, a recent study by Velu et al. (2018), further asserts the significance of the zinc-finger motif transcription factor and phosphatase as playing a major role in zinc loading in wheat grain. The same study also highlights group 2 and 7 chromosomes as holding genes for nutrient uptake, translocation and sequestration of mineral elements in wheat. It must be noted however that although many genes that regulate zinc homeostasis have been characterised, their role in genotypic variation is still largely unclear (Garcia-Oliviera et al., 2018). Several studies reported zinc accumulation in wheat having high heritability thus suggesting high potential for responding to selective breeding (Velu et al., 2014, 2015; Alomari et al., 2018; Zhao et al., 2022).

BREEDING STRATEGIES FOR HIGH GRAIN ZINC IN WHEAT

It is recommended that zinc breeding be location or environment specific since the ultimate zinc concentration in grain is largely affected by zinc concentration in soils as shown by high genetic correlations between locations in some studies (Velu et al., 2015). The presence of additive QTLs allows for high zinc wheat varieties to be bred through conventional breeding. With the ever-increasing information on QTLs and molecular markers for zinc homeostasis in wheat, the use of Marker Assisted Selection to increase the ease and efficacy of selection for high zinc expression is another practical approach (Saini et al., 2020).

Iron and zinc biofortified sorghum

Sorghum (*Sorghum bicolor* L. Moench) is an important food crop in arid and semi-arid regions of Asia and Africa and is ranked the 4th most consumed cereal. Its grain is known to be rich in starch, protein, crude fibre and other micronutrients (Abah et al., 2020). However, significant amounts of iron and zinc are lost during the decorticating process which removes them from the aleurone layer and scutellum (Proietti et al., 2015). Furthermore, bioavailability of iron and zinc from sorghum is also low ~5 and ~20%, respectively (Zhao et al., 2019) largely due to the inhibitory effect of anti-nutrients such as phytates that form insoluble complexes with these micronutrients.

Genetic variability for iron and zinc in sorghum

Under the HarvestPlus Program secondary investments, a target increment of +30 µg/g iron and +12 µg/g zinc

was set to increase the concentration of sorghum grain iron and zinc to 60 ppm iron and 20 ppm zinc (HarvestPlus, 2014). Several screening studies for grain iron and zinc in diverse sorghum genotypes including, parental lines, cultivars, advanced breeding lines, germplasm accessions, yellow sorghum and elite lines, have revealed that the crop has wide variability for the micronutrients spanning a range of 8 to 133 µg/g and 13 to 91 µg/g, respectively (Kumar et al., 2010, 2018; Hariprasanna et al., 2014; Upadhyaya et al., 2016; Satish et al., 2016; Sen et al., 2019; Guild and Stangoulis, 2021). These results indicate the wide and significant variability that can allow for identification of genotypes that can be used as parents for high iron and zinc breeding in sorghum.

Genetic basis for grain iron and zinc accumulation in sorghum

Iron and zinc density in sorghum is controlled by both additive and non-additive gene effects and was also found to have high heritability (Phuke et al., 2017; Gaddameedi et al., 2020). Trait association studies have also revealed positive correlation between grain iron and zinc content which suggest the possibility of simultaneous improvement of the two using conventional plant breeding methods. Furthermore, no significant correlation between iron and zinc accumulation in sorghum grain and other agronomic traits including yield were observed (Kumar et al., 2015).

BREEDING STRATEGIES FOR IRON AND ZINC IN SORGHUM

Breeding for high iron and zinc concentration in sorghum can be done simultaneously, due to their highly significant positive correlation (Motlhaodi et al., 2018; Phuke et al., 2017). Iron and zinc accumulation is however under the control of different genetic effects which determine the most appropriate breeding strategy to undertake. Grain zinc concentration is largely controlled by additive gene effects while iron concentration is predominantly governed by non-additive gene effects (Kumar et al., 2015; Gaddameedi et al., 2020). This implies that high zinc sorghum lines can be developed using progeny selection in pedigree or population breeding. Conversely, iron concentration can be improved through heterosis breeding as well progeny selection. At least one parent in heterosis breeding for grain iron must possess high iron concentration whilst for zinc, both parents must be having high grain concentrations (Kumar et al., 2015). Large genotype × environment effects have been observed for iron and zinc content in sorghum thus prompting the need for multiple environmental tests for improved genotypes to identify the highly stable lines.

There is currently limited literature on success stories of the use of Marker Aided Breeding for iron and zinc in sorghum; therefore, there is an opportunity to develop molecular markers to expedite the breeding process for these two nutrients in sorghum.

FUTURE PERSPECTIVES

As global food prices escalate due to Covid19 induced lockdowns, climate change and variability manifestation and conflict, alternative nutritious food will continue to be beyond the reach of many particularly in developing countries. Accordingly, there is need to enhance the nutritional value of adapted cereals to curb the nutritional deficiencies. Genetic “biofortification” will inarguably continue to present an opportunity to sustainably and economically address global malnutrition issues due to its cost effectiveness in comparison to other food fortification approaches. Use of plant breeding will be at fore given the low rate of commercialisation of products of biotechnology in many developing countries. To complement conventional plant breeding there is need for mainstreaming of Marker Assisted Breeding (MAB) in “biofortification” programs to expedite the crop improvement processes. Moreover given the complexity in analysing micronutrients such as zinc and iron there is need to integrate cost effective, sensitive, high throughput phenotyping tools in the breeding process. Crops targeted for “biofortification” should ideally have farmer preferred traits to enhance adoption. Alternatively, “biofortification” can be integrated into the pipeline breeding approach so that all new varieties possess the key micronutrients. Overall, the process should involve the different end users so that the importance of these outputs traits are apparent and maximum benefit is derived from the crops in this era of nutrition sensitive agriculture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Aakre I, Bøkevoll A, Chaira J, Bouthir FZ, Frantzen S, Kausland A, Kjelleve M (2020). Variation in nutrient composition of seafood from North West Africa: Implications for food and nutrition security. *Foods* 9(10):1516.
- Abah CR, Ishiwu CN, Obiegbuna JE, Oladejo AA (2020). Sorghum Grains: Nutritional composition, functional properties and its food applications. *European Journal of Nutrition and Food Safety* 12(5):101-111.
- Ahsan S, Mansoori N, Mohiudin SM, Mubeen SM, Saleem R, Irfanulla M (2017). Frequency and determinants of malnutrition in children aged between 6-59 months in district Tharparkar, a rural area of Sindh. *Journal of Pakistan Medical Association* 67(9):1369-1373.
- Ajmeera S, Sudheer KS, Ravindrababu V (2017). Evaluation of genetic variability, heritability and genetic advance for yield and yield components in rice genotypes. *International Journal of Current Microbiology and Applied Sciences* 6(10):1657-1664.
- Akhtar S, Osthoff G, Mashingaidze K, Labuschagne M (2018). Iron and zinc in maize in the developing world: Deficiency, availability and breeding. *Crop Science* 58(6):2200-2213.
- Alomari DZ, Eggert K, Von Wiren N, Alqudah AM, Polley A, Plieske J, Ganai MW, Pillen K, Röder MS (2018). Identifying candidate genes for enhancing grain Zn concentration in wheat. *Frontiers in Plant Sciences* 9:1313.
- Andersson MS, Saltzman A, Virk PS, Pfeiffer WH (2017). Progress update: Crop development of biofortified staple food crops under Harvestplus. *African Journal of Food, Agriculture, Nutrition and Development* 17(2):11905-11935.
- Anuradha N, Satyavathi CT, Bharadwaj C, Nepolean T, Sankar M, Singh SP, Meena MC, Singhal T, Srivastava RK (2017). Deciphering genomic regions for high grain iron and zinc using association mapping in pearl millet. *Frontiers in Plant Science* 8:412. doi.org/10.3389/fpls.2017.00412.
- Anusha GD, Rao DS, Jaldhani V, Beulah P, Neeraja CN, Gireesh C, Anantha MS, Suneetha K, Santhosha R, Hari Prasad AS, Sundaram RM, Sheshu Madhav M, Fiyaz A, Brajendra P, Tuti M D, Bhawe MHV, Radha Krishna KV, Ali J, Subrahmanyam D, Senguttuvel P (2021). Grain iron and zinc content, heterosis, combining ability and its association with grain yield in irrigated and aerobic rice. *Scientific Reports* 11:1-12. doi.org/10.1038/s41598-021-90038-4
- Azmach G, Gedil M, Menkir A, Spillane C (2013). Marker-trait association analysis of functional gene markers for provitamin A levels across diverse tropical yellow maize inbred lines. *BMC Plant Biology* 13:227. doi.org/10.1186/1471-2229-13-227
- Babu R, Rojas NP, Gao S, Yan J, Pixley K (2013). Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on pro-vitamin A concentrations for 26 tropical maize populations. *Theoretical and Applied Genetics* 126(2):389-399. doi.org/10.1007/s00122-012-1987-3
- Babu M, Arnold R, Bundalovic-Torma C, Gagarinova A, Wong KS, Kumar A (2014). Quantitative genome-wide genetic interaction screens reveal global epistatic relationships of protein complexes in *Escherichia coli*. *PLoS Genetics* 10:e1004120. doi.org/10.1371/journal.pgen.1004120
- Balakrishnan D, Surapaneni M, Yadavalli VR, Addanki KR, Mesapogu S, Beerelli K, Neelamraju S (2020). Detecting CSSLs and yield QTLs with additive, epistatic and QTL x environment interaction effects from *Oryza sativa* x *O. nivara* IRGC81832 cross. *Scientific Reports* 10(1):1-18. doi.org/10.1038/s41598-020-64300-0
- Bashir K, Takahashi R, Nakanishi H, Nishizawa NK (2013). The road to micronutrient biofortification of rice: progress and prospects. *Frontiers in Plant Science* 4:1-7.
- Bouis H, Birol E, Boy E, Gannon BM, Haas JD, Low J, Metha S, Michaux K, Mudyahoto B, Pfeiffer W, Qaim M, Reinberg C, Rocheford T, Stein AJ, Strobbe S, Van Der Straeten D, Verbeeck V, Welch R (2020). Food biofortification: reaping the benefits of science to overcome hidden hunger. In October webinar on The Need for Agricultural Innovation to Sustainability Feed the World by 2050 (No. 69) Council for Agricultural Science and Technology (CAST). Available at: <https://cast-science.org>
- Bouis H, Low J, McEwan M, Tanumihardjo S (2013). Biofortification: Evidence and lessons learned linking agriculture and nutrition. The Food and Agriculture Organisation (FAO) and The World Health Organisation (WHO). www.fao.org/publications
- Boy E, Haas J, Petry N, Cercamondi CI, Gahutu J, Mehta S, Finkelstein JL, Hurrell RF (2017). Efficacy of iron biofortified crops. *African Journal of Food, Agriculture, Nutrition and Development* 17(2):11879-11892.
- Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011). Biofortification: A new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin* 32(Supplement 1):S31-S40.
- Cakmak I, Kutman UB (2018). Agronomic biofortification of cereals with zinc: A review. *European Journal of Soil Science* 69(1):172-180.
- Calayagun MIC, Formantes AK, Amparado A, Descalsota-Empleo GI, Nha CT, Inabangan-Asilo, AN, Swe ZM, Hernandez JE, Borromeo TH, Lalusin AG, Mendiolo MS, Diaz MGQ, Viña CB, Reinke R,

- Swamy BP (2020). Genetic analysis of agronomic traits and grain iron and zinc concentrations in a doubled haploid population of rice (*Oryza sativa* L.). *Scientific Reports* 10(1):1-14.
- Calayagan MIC, Swamy BPM, Nha CT, Palanog AD, Biswas BP, Descalsota-Empelo GI, Min YMM, Inabangan-Asilo AN (2021). Zinc-biofortified rice: A sustainable food based product for fighting zinc malnutrition. In Ali, J and Wani, S.H. (Eds). *Rice improvement: Physiological, molecular breeding and genetic perspectives*. Springer, IRRRI, Los Banos, Philippines. pp. 471-486.
- Cashman KD (2020). Vitamin D deficiency: Defining, prevalence, causes and strategies of addressing. *Calcified Tissue International* 106:14-29. doi.org/10.1007/s00223-019-00559-4
- Castrogiovanni R, Imbesi R (2017). The role of malnutrition during pregnancy and its effects on brain and skeletal muscle postnatal development. *Journal of Functional Morphology and Kinesiology* 2(3):30. doi.org/10.3390/jfkm2030030
- Conti C, Zanello G, Hall A (2021). Why are agri-food systems resistant to new directions of change? A systematic review. *Global Food Security* 31:100576. doi.org/10.1016/j.gfs.2021.100576
- Das S, Chaki AK, Hossain A (2019). Breeding and agronomic practices approaches for the biofortification of zinc in wheat (*Triticum aestivum* L.) to combat zinc deficiency in millions of a population: A Bangladesh perspective. *ActaAgrobotanica* 72(2). doi.org/10.5586/aa.1770
- Davis KF, Chhatre A, Rao ND, Singh D, Ghosh-Jerath S, Mridul A, Poblete-Cazenave M, Pradhan N, DeFries R (2019). Assessing the sustainability of post-Green Revolution cereals in India. *Proceedings of the National Academy of Sciences* 116(50):25034-25041.
- Dhakal A, Sharma S, Pokhrol A, Poudel A (2020). Variability and heritability estimate of 30 rice landraces of Lamjung and Tanahun districts, Nepal Indonesia. *Journal of Agricultural Sciences* 21(1):1-10.
- Dhliwayo T, Palacios-Rojas N, Crossa J, Pixley KV (2014). Effects of S1 recurrent selection for provitamin A carotenoid content for three open-pollinated maize cultivars. *Crop Science* 54(6):2449-2460. doi.org/10.2135/cropsci2013.11.0764.
- Dixit S, Singh UM, Abbai R, Ram T, Singh VK, Paul A, Virk PS, Kumar A (2019). Identification of genomic region(s) responsible for high iron and zinc content in rice. *Scientific Reports* 9(1):8136. doi: 10.1038/s41598-019-43888-y. Dwyer JT, Coates PM, Smith MJ (2018). Dietary supplements: regulatory, challenges and research resources. *Nutrients* 10(1):41. doi.org/10.3390/nu10010041
- Ekhloenetale M, Tuderne G, Onikan A, Ekholuenetale CE (2020). Socio-economic inequalities in hidden hunger, undernutrition and overweight among under five children in 35 sub-Saharan African countries. *Journal of the Egyptian Public Health Association* 95(1):1-15.
- Ekpa O, Palacios-Rojas N, Kruseman G, Fogliano V, Linnemann A (2019). Sub-Saharan African maize-based foods: Technological perspectives to increase the food and nutrition security impacts of maize breeding programs. *Food Reviews International* 35(7): 609-639. doi.org/10.1080/87559129.2019.1588290
- Ewool MB, Akromah R (2017). Genetic variability, coefficient of variance, heritability and genetic advance of pro-Vitamin A maize hybrids. *International Journal of Agriculture Innovation and Research* 6(1):2319-1473.
- Fan XM, Zhang YD, Yao WH, Bi YQ, Liu L, Chen HM, Kang MS (2014). Reciprocal diallel crosses impact combining ability, variance estimation and heterotic group classification. *Journal of Crop Science* 54(1):89-97.
- Fasahat P, Rajabi A, Rad JM, Derera J (2016). Principles and utilization of combining ability in plant breeding. *Biometrics and Biostatistics International Journal* 4(1):1-24. doi.org/10.15406/bbij.2016.04.00085
- Gaddameedi A, Phuke RM, Polavarapu KKB, Gorthy S, Subhasini V, Jagannathan J, Are AK (2020). Heterosis and combining ability for grain Fe and Zn concentration and agronomic traits in sorghum [*Sorghum bicolor* (L.) Moench]. *Journal of King Saud University-Science* 32(7):2989-2994.
- Garcia-Oliveira AL, Chander S, Ortiz R, Menkir A, Gendil M (2018). Genetic basis and breeding perspectives of grain zinc and iron enrichment in cereals. *Frontiers in Plant Science* 9:937. doi.org/10.3389/fpls.2018.00937
- Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition* 5:12. doi.org/10.3389/fnut.2018.00012
- Gavali RK (2018). Combining ability analysis and gene action studies in pearl millet [*Pennisetum glaucum* (L.) R Br.] *Electronic Journal of Plant Breeding* 9(3):908-915.
- Goredema-Matongera N, Ndhlela T, Magorokosho C, Kamutando CN, Van Biljon A, Labuschagne M (2021). Multinutrient biofortification of maize (*Zea mays* L.) in Africa: Current Status, opportunities and limitations. *Nutrients*, 13(3):1039. doi.org/10.3390/nu13031039
- Govindaraj M, Virk PS, Kannatti A, Cherian B, Rai KN, Anderson MS, Pfeiffer WH (2020). Biofortified pearl millet cultivars offer potential selection to tackle malnutrition in India. *Quantitative Genetics, Genomics and Plant Breeding*, CAB International pp. 385-396.
- Guo R, Dhliwayo T, Mageto EK, Palacios-Rojas N, Lee M, Yu D, Ruan Y, Zhang A, Vicente F, Olsen M, Crossa J, Prasanna BM, Zhang L, Zhang X (2020). Genomic prediction of kernel zinc concentration in multiple maize population using genotyping-by-sequencing and repeat amplification sequencing markers. *Frontiers in Plant Science* 11:534. doi.org/10.3389/fpls.2020.00534.
- Govindaraj M, Rai KN, Kanatti A, Rao AS, Shivade H (2019). Nutritional security in drylands: Fast track intra-population genetic improvement for grain iron and zinc densities in pearl millet. *Frontiers in Nutrition* 6: 74. doi.org/10.3389/fnut.2019.00074.
- Gupta HS, Hossain F, Nepolean T, Vignesh M, Mallirkajuna MG (2015). Understanding genetic and molecular basis of Fe and Zn accumulation towards development of micro-nutrient enriched maize. In: Rakshit, A., Singh, H.B., Sen, A (eds) *Nutrient use efficiency: from basics to advances*. Springer, New Delhi. doi.org/10.1007/978-81-322-2169-2_17
- Gupta PK, Balyan HS, Sharma S, Kumar R (2021). Biofortification and bioavailability of Zn, Fe and Se in wheat: Present status and future prospects. *Theoretical and Applied Genetics* 134(1):1-35. doi.org/10.1007/s00122-020-03709-7
- Guild GE, Stangoulis JCR (2021). EDXFR for screening micronutrients in lentil and sorghum biofortification breeding programs. *Plant and Soil Science* 463:461-469.
- Gore MA, Rocheford T (2014). A foundation for pro-vitamin A biofortification of maize: Genome-wide association and genomic prediction models of carotenoid levels. *Genetics Society of America* 198(4):1699-1716.
- Halilu AD, Ado SG, Aba DA, Usman IS (2016). Genetics of carotenoids for pro-vitamin A biofortification in tropical-adapted maize. *The Crop Journal* 4(4):313-322. doi.org/10.1016/j.cj.2016.05.002.
- Hariprasanna K, Agte V, Elongovan M, Patil JV (2014). Genetic variability for grain iron and zinc content in cultivars, breeding lines and selected germplasm accessions of sorghum [*Sorghum bicolor* (L.) Moench]. *Indian Journal of Genetics* 74(1):42-49.
- HarvestPlus (2014). Biofortification progress briefs. Retrieved from https://www.harvestplus.org/wp-content/uploads/2014/09/Biofortification_Progress_Briefs_August2014_WEB_2_0.pdf
- Hindu V, Palacios-Rojas N, Babu R, Suwarno WB, Rashid Z, Usha R, Saykhedkar GR, Nair SK (2018). Identification and validation of genomic regions influencing kernel zinc and iron in maize. *Theoretical and Applied Genetics* 131(7):1443-1457.
- Holick MF (2017). The Vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Reviews in Endocrine Metabolic Disorders* 18(2):153-165.
- Hussein A, Zahir ZA, Asghar HN, Ahmad M, Jamil M, Naveed M, Akhtar MFUZ (2018). Zinc solubilizing bacteria for zinc biofortification in cereals: A step toward sustainable nutritional security. Springer Nature Singapore Pvt. Ltd. doi.org/10.1007/978-981-13-0044-8_7
- Ignjatovic-Micic D, Vancetovic J, Trbovic D, Dumanovic, Z, Kostadinovic M, Bozinovic S (2015). Grain nutrient composition of maize (*Zea mays* L.) drought tolerant populations. *Journal of Agricultural and Food Chemistry* 63(4):1251-1260.
- Jena J, Sethy P, Jena T, Misra S, Sahoo S, Dash G, Palai J (2018). Rice biofortification: A brief review. *Journal of Pharmacognosy and Phytochemistry* 7(1):2644-2647.

- Joy EJ, Ahmad MW, Zia MH, Kumssa DB, Young SD, Ander EL, Watts MJ, Stein AJ, Broadley MR (2017). Valuing increased Zinc (Zn) fertilizer-use in Pakistan. *Journal of Plant and Soil Science* 411(1):139-150. doi.org/10.1007/s11104-016-2961-7
- Jaksomsak P, Rerkasem B, Prom-u-Thai C (2017). Responses of grain zinc and nitrogen concentration to nitrogen fertilizer application in rice varieties with high-yielding low grain zinc and low-yielding high grain zinc concentration. *Plant and Soil* 411(1):101-109. doi.org/10.1007/s11104-016-3056-1
- Kanatti A, Rai KN, Radhika K, Govindaraj M (2016). Tester effect on combining ability and its relationship with line performance per se for grain iron and zinc densities in pearl millet. *Crop Science* 56(2):01-08. doi.org/10.2135/cropsci2015.08.0486
- Kavakani Y, Bhullar NK (2018). Molecular processes in iron and zinc homeostasis and their modulation for biofortification in rice. *Journal of Integrative Plant Biology* 60(12):1181-1198.
- Kondwakwenda A, Sibiya J, Zengeni R, Musvosvi C, Tesfay S (2019). Screening of pro-vitamin A maize inbred lines for drought tolerance: Beta-carotene content and secondary traits. *Agronomy* 9(11):692. doi.org/10.3390/agronomy9110692
- Kumar AA, Reddy BV, Sahrawat KL, Ramaiah B (2010). Combating micronutrients malnutrition: Identification of commercial sorghum cultivars with high grain iron and zinc. *Journal of SAT Agricultural Research* 8:1-5.
- Kumar A, Anuradha K, Ramaiah B, Frederick H, Rattunde W, Virk P, Pfeiffer WH, Grando S (2015). Recent advances in sorghum biofortification research. *Plant Breeding Reviews* 39:89-124.
- Kumar S, Hash CT, Thirunavukkarasu N, Singh G, Rajaram V, Rathore A, Senapathy S, Mahendrakar MD, Yadav RS, Srivastava RK (2016). Mapping quantitative trait loci controlling high iron and zinc content in self and open pollinated grains of pearl millet [*Pennisetum glaucum* (L.) R Br.] *Frontiers in Plant Science* 7:1636. doi.org/10.3389/fpls.2016.01636
- Kumar AA, Mehtre S, Anuradha K, Jaganathan J, Prasanna H, Gorthy S, Gadakh S, Chavan U, Kalpande H, Tonapi V (2018). Delivering bioavailable micronutrients through biofortifying sorghum and seed chain innovations *Science Forum 2018, Stellenbosch; South Africa*. October 10-12.
- Kumar S, Palve A, Joshi C, Srivastava RK, Rukhsar L (2019). Crop biofortification for Iron (Fe), Zinc (Zn) and Vitamin A with transgenic approaches. *Heliyon* 5(6) doi.org/10.1016/j.heliyon.2019.e01914
- Kumar M, Rani K, Ajay BC, Patel MS, Mungra KD, Patel MP (2020). Study of genetic variability, heritability and path analysis for grain micronutrients concentration, yield and component traits in pearl millet [*Pennisetum glaucum* (L.) R Br.]. *Journal of Pharmacognosy and Phytochemistry* 9(2):1402-1409.
- Lalani B, Beechoff A, Bennett B (2019). Which choice of delivery model (s) works best to deliver fortified foods? *Nutrients* 11(7):1594. doi.org/10.3390/nu11071594
- Ligowe IS, Phiri FP, Ander EL, Bailey EH, Chilimba ADC, Gashu D, Joy EJM, Lark RM, Kabambe V, Kalimbira AA, Kumssa DB, Nalivata PC, Young SD, Broadley MR (2020). Selenium deficiency risks in sub-Saharan African food systems and their geospatial linkages. *Proceedings of the Nutrition Society* 79(4):457-467. doi.org/10.1017/s0029665120006904
- Ludwig Y, Slamet-Loedin IH (2019). Genetic biofortification to enrich rice and wheat grain iron: From genes to product. *Frontiers in Plant Science* 10:6833. doi.org/10.3389/fpls.2019.00833
- Listman GM, Guzman C, Palacios-Rojas N, Pfeiffer WH, San Vicente F, Govindan V (2019). Improving nutrition through biofortification: Preharvest and postharvest technologies. *Cereal Foods World* 64(3):1-7. doi.org/10.1094/CFW-64-3-0025
- Machida L, Derera J, Tongoona P, MacRobert J (2010). Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in subtropical environments. *Crop Science* 50(5):1708-1717. doi.org/10.2135/cropsci2009.09.0538
- Mageto EK, Lee M, Dhiliwayo T, Palacios-Rojas N, San Vicente F, Burgueno J, Hallauer AR (2020). An evaluation of kernel zinc in hybrids of elite quality protein maize (QPM) and non-QPM inbred lines adapted to the tropics based on a mating design. *Agronomy*, 10(5):695. doi.org/10.3390/agronomy10050695
- Maibvisira NA, Gasura E, Maphosa M, Nyakurwa CS (2018). Genetic basis and the current breeding efforts for quality protein maize in Southern Africa. *African Crop Science Journal* 26(4):529-541. doi.org/10.4314/acjs.v26i4.7
- Mahendrakar MD, Parveda M, Kishor PB, Srivastava RK (2020). Discovery and validation of candidate genes for grain iron and zinc metabolism in pearl millet [*Pennisetum glaucum*(L.) R Br.] *Scientific Reports*, 10(1): 1-16. doi.org/10.1038/s41598-020-73241-7
- Majmuder S, Datta K, Datta SK (2019). Rice biofortification: High iron, zinc and vitamin A to fight against "hidden hunger". *Agronomy* 9(12):803. doi.org/10.3390/agronomy9120803
- Mallikarjuna MG, Thirunavukkarasu N, Sharma R, Shiriga K, Hossain F, Bhat JS, Mithra ACR, Maria SS, Manjaiah KM, Rao AR, Gupta HS (2020). Comparative transcriptome analysis of iron and zinc deficiency in maize (*Zea mays* L.). *Plants* 9(12):1812. doi.org/10.3390/plants9121812
- Mannahr MV, Garret GS, Hurrell RF (2018). Future trends and strategies in food fortification. *Food Fortification in a Globalized World*, Academic Press pp. 375-381 doi.org/10.1016/B978-0-12-802861-2-00040-7
- Manwaring HR, Bligh HFJ, Yadav R (2016). The challenges and opportunities associated with biofortification of pearl millet (*Pennisetum glaucum*) with elevated levels of grain iron and zinc. *Frontiers in Plant Science* 7:1944. doi.org/10.3389/fpls.2016.01944
- Matres JM, Arcillas E, Cueto-Reano MF, Sallan-Gonzales R, Trijatmiko KR, Slamet-Loedin I(2021). Biofortification of rice grains for increased iron content. In: Ali, J and Wani, S.H. (Eds). *Rice improvement: Physiological, molecular breeding and genetic perspectives*. Springer, IRRRI, Los Banos, Philippines pp. 471-486.
- Maqbool MA, Aslam M, Beshir A, Khan MS (2018). Breeding for provitamin A biofortification of maize (*Zea mays* L.). *Plant Breeding* 137(4):451-469. doi.org/10.1111/pbr.12618
- Maqbool MA, Beshir A (2019). Zinc biofortification of maize (*Zea mays* L.): Status and challenges. *Plant Breeding*, 138(1):1-28. doi.org/10.1111/pbr.12658
- Maqbool MA, Issa ABB, Khokhar ES (2021). Quality protein maize: Importance, genetics, timelines of different events, breeding strategies and varietal adoption. *Plant Breeding* 140(3):375-399.
- Marra MV, Bailey RL (2018). Position of the Academy of Nutrition and Dietetics: micronutrient supplementation. *Journal of the Academy of Nutrition and Dietetics* 118(11):2162-2173. doi.org/10.1016/j.jand.2018.07.022
- Meng L, Zhao X, Ponce K, Ye G, Leung H (2016). QTL mapping for agronomic traits using multi-parent advanced generation inter-cross (MAGIC) populations derived from diverse elite indica rice lines. *Field Crops Research* 189:19-42. doi.org/10.1016/j.fcr.2016.02.004
- Menkir, A, Maziya-Dixon B, Mengesha W, Rocheford T, Alamu O (2017). Accruing genetic gain in pro-vitamin A enrichment from harnessing diverse maize germplasm *Euphytica* 213(5):105. doi.org/10.1007/s10681-017-1890-8
- Mengesha W, Menkir A, Meseka S, Bossey B, Afolabi A, Burgueno J, Crossa J (2019). Factor analysis to investigate genotype and genotype x environment interaction effects on pro-vitamin A content and yield in maize synthetics. *Euphytica* 215(11):1-15. doi.org/10.1007/s10681-019-2505-3
- Mulualem T(2015). Application of bio-fortification through plant breeding to improve the value of staple crops. *Biomedicine and Biotechnology* 3(1):11-19. doi.org/10.12691/bb-3-1-3.
- Motukuri KSR (2019). Quality Protein Maize: An alternative food to mitigate protein deficiency in developing countries. KLEF University, Guntur, Andhra Pradesh, India, doi.org/10.5772/intechopen/89038
- Muthusamy V, Hossain F, Thirunavukkarasu N, Saha S, Agrawal PK, Guleria SK, Gupta HS (2015). Genetic variability and inter-relationship of kernel carotenoids among indigenous and exotic maize (*Zea mays* L.) inbreds. *Cereal Research Communications* 43(4):567-578. doi.org/10.1556/0806.43.4
- Mohammadi-joo S, Mirasi A, Saeidi-Aboeshaghi R, Amiri M (2015). Evaluation of bread wheat (*Triticum aestivum* L.) genotypes based on resistance indices under field conditions. *International Journal of Biosciences* 6(2):331-337.
- Muslim M, Romshoo SA, Rather AQ (2015). Paddy crop yield estimation in Kashmir Himalayan rice bowl using remote sensing and simulation model. *Environmental Monitoring and Assessment*,

- 186(6):1-12. doi.org/10.1007/s10661-015-4564-9
- Motlhaodi T, Bryngellson T, Chite S, Fatih M, Ortiz R, Geleta M (2018). Nutritional variation in sorghum [*Sorghum bicolor* (L.) Moench] accessions from Southern Africa revealed by protein and mineral composition. *Journal of Cereal Science* 83:123-129. doi.org/10.1016/j.jcs.2018.08.010
- Mumtaz MZ, Ahmad M, Jamil M, Hussain T (2017). Zinc solubilizing *Bacillus spp.* potential candidates for biofortification in maize. *Microbiological Research* 202:51-60. doi.org/10.1017/j.micres.2017.06.001
- Muthusamy V, Hossain F, Thirunavukkarasu N, Choudhary MM, Saha S, Bhat JS (2014). Development of beta-carotene rich maize hybrids through marker assisted integration of beta-carotene hydroxylase allele. *PLoS One*, 9: e113583. doi.org/10.1371/journal.pone.0113583
- Naik SR, Singh SK, Singh, DK, Khaire AR, Korada M, Habde S, Majhi PK (2021). Estimation of heterosis for yield related traits and grain zinc in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding* 12(4):1227-1235.
- Nuss ET, Tanumihardjo SA (2011). Quality protein maize for Africa: Closing the protein inadequacy gap in vulnerable populations. *Advances in Nutrition* 2(3): 217-224. doi.org/10.3945/an.110.000182
- Owens BF, Lipka AE, Magallanes-Lundback M, Tiede T, Diepenbrock CH, Kandianis CB, Kim E, Cepela J, Mateos-Hernandez M, Buell CR, Buckler ES, DellaPenna D, Zunjare RU, Hossain, F, Muthusamy V, Baveja A, Thirunavukkarasu N, Chauhan HS, Saha S, Gupta HS (2017). Influence of rare alleles of β -carotene hydroxylase and lycopene epsilon cyclase genes on accumulation of pro-vitamin A carotenoids in maize kernels. *Plant Breeding* 136(6):872-880. doi.org/10.1111/pbr.12548
- Ortiz-Covarrubias Y, Dhliwayo T, Palcios-Rojas N, Ndhlela T, Magorokosho C, Anguilar-Rincon VH., Cruz-Morales AS, Trachsel, S (2019). Effects of drought and low nitrogen stress on provitamin A carotenoid content of biofortified maize hybrids. *Crop Science* 59:2521-2532. doi.org/10.2135/cropsci2019.02.0100
- Phuke RM, Anuradha K, Radhika K, Jabeen F, Anuradha G, Ramesh T, Hariprasanna K, Mehtre SP, Deshpande SP, Anil G, Das RR, Rathore A, Hash T, Reddy BVS, Kumar AA(2017). Genetic variability, genotype x environment interaction, correlation and GGE biplot analysis for grain iron and zinc concentration and other agronomic traits in RIL population of sorghum [*Sorghum bicolor* (L.) Moench]. *Frontiers in Plant Science* 8: 712. doi.org/10.3389/fpls.2017.00712
- Prasanna BM, Palacios-Rojas N, Hossain F, Muthusamy V, Menkir A, Dhliwayo T, Ndhlela T, San Vicente F, Nair SK, Vivek BS, Zhang X, Olsen M, Fan X(2020). Molecular breeding for nutritionally enriched maize: Status and prospects. *Frontiers in Genetics* 10:1392. doi.org/10.3389/fgene.2019.01392
- Prasannakumari M, Akilan M, Kalaselvan A, Subramanian P, Janaki P, Jeyaprakash P (2020). Studies on genetic parameters, correlation and path analysis for yield attributes and iron content in a backcross population of rice (*Oryza sativa* L.) *Electronic Journal of Plant Breeding* 11(3):881-886.
- Pujar M, Gangaprasad S, Govindaraj M, Gangurde SS, Kanatti A, Kudapa H (2020). Genome-wide association study uncovers genomic regions associated with grain iron, zinc and protein content in pearl millet. *Scientific Reports* 10(1):1-15. doi.org/10.1038/s41598-020-76230-y
- Pujar M, Govindaraj M, Gangaprasad S, Kanatti A (2019). Effect of isonuclear-alloplasmic cytoplasmic male sterility system on grain yield traits in pearl millet. *Indian Journal of Genetics and Plant Breeding* 79(1):141-149.
- Proietti I, Frazzoli C, Mantovani A (2015). Exploiting nutritional value of staple foods in the world's semi-arid areas: Risks, benefits, challenges and opportunities of sorghum. *Healthcare* 3(2):172-193.
- Rathod R, Suman K, Madhu Babu P, Neeraja CN, Eswari KB, Srinivasa Chary D, Subbarao LV (2020). Genetic studies for yield and nutritional traits in unpolished rice RILs of MTU1010xBR2655. *Indian Journal of Genetics and Plant Breeding* 80(3):324-328. doi.org/10.31742/IJGPB.80.3.12
- Raza Q, Riaz A, Muhammad Sabar M, Muhammad Atif R, Bashir K(2019). Meta-analysis of grain iron and zinc associated QTLs, identified hotspot chromosomal regions and positional candidate genes for breeding biofortified rice. *Plant Science*, 288:110214. doi.org/10.1016/j.plantsci.2019.110214
- Rehman A, Farooq M, Ullah A, Nadeen F, Im SY, Park SK, Lee DJ (2020). Agronomic biofortification of zinc in Pakistan: Status, benefits and constraints. *Frontiers in Sustainable Food Systems* 22. doi.org/10.3389/fsufs.2020.59172
- Saini DK, Devi P, Kaushiki P (2020). Advances in genomic interventions for wheat biofortification: A review. *Agronomy* 10(1):62. doi.org/10.3390/agronomy10010062
- Satish L, Shilpha J, Pandian S, Rency AS, Rathinapriya P, Ceasar SA, Largia MJV, Kumar AA, Ramesh M (2016). Analysis of genetic variation in sorghum [*Sorghum bicolor* (L.) Moench] genotypes with various agronomical traits using SPAR methods. *Gene* 576(1):581-585.
- Sala M, Ananda Kumar CR, Geetha S (2015). Variability studies for quality traits in rice with high iron and zinc content in segregating population. *Rice Genomics and Genetics* 6(4):1-5. doi.org/10.5376/rgg.2015.06.0004.
- Sen RAM, Saini RK, Singh SK, Kumar A (2019). Study of genetic variability of fodder yield and its components in forage sorghum [*Sorghum bicolor* (L.) Moench]. *Forage Resources* 45(2):156-158.
- Shariatipour N, Heidari B, Richards CM (2021). Meta-analysis of QTLome for grain zinc and iron contents in wheat (*Triticum aestivum* L.). *Euphytica*, 217(5):1-14.
- Sharma P, Aggarwal P, Kaur A (2016). Biofortification: A new approach to eradicate hidden hunger. *Food Reviews International* 33(1):1-21. doi.org/10.1080/87559129.2015.1137309
- Sharma A, Grag S, Sheikh I, Vyas P, Dhaliwal HS (2020). Effect of wheat grain protein composition on end-use quality. *Journal of Food Science and Technology* 57(8):2771-2785.
- Sharma S, Kaur G, Kumar A, Meena V, Ram H, Kaur J, Pandey AK (2020). Gene expression pattern of vacuolar-iron transporter like (VTL) genes in hexaploid wheat during metal stress. *Plants* 9(2):9. doi.org/10.3390/plants9020229
- Sharifi P (2013). Genotype plus environment by environment interaction (GGE) biplot analysis of nutrient quality traits in rice (*Oryza sativa* L.). *Philippine Journal of Crop Science* 38: 9-20. Singh M, Dotaniya ML, Mishra A, Dotaniya CK, Regar KL, Lata M (2016). Role of biofertilizers in conservation agriculture. In: Bisht JK, Meena VS, Mishra PK, Pattanayak A. (eds) *Conservation agriculture: an approach to combat climate change in Indian Himalaya*. Springer, Singapore pp. 113-134. doi.org/10.1007/978-981-10-2558-7_4
- Singh D, Kumar A, Kumar R, Kushwaha N, Mohanty TA, Kumar P(2020). Genetic variability analysis of QPM inbreds using morphological characters. *International Journal Current Microbiology Applied Science* 9(2):328-338. doi.org/10.20546/ijcmas.2020.902.042
- Srivastava K, Satyavathi CT, Singh RB, MukeshSankar S, Singh SP, Soumya SL, Kapoor C(2022). Pearl millet biofortification approaches in a micronutrient dense, climate-resilient nutri-cereal. In *Biofortification of Staple Crops*. Springer, Singapore pp. 175-193. doi.org/10.1007/978-981-16-3280-8_7.
- Strobbé S, Van der Straeten D(2017). Folate biofortification in food crops. *Current Opinion in Biotechnology* 44:202-211. doi.org/10.1016/j.copbio.2016.12.003
- Suwarno WB, Pixley KV, Palacios-Rojas N, Kaeppler SM, Babu R (2014). Formation of heterotic groups and understanding genetic effects in a pro-vitamin A biofortified maize breeding program. *Crop Science* 54(1):14-24. doi.org/10.2135/cropsci2013.02.0096.
- Swamy BPM, Rahman MA, Inabangan-Asilo MA, Amprado A, Manito C, Chada-Mohanty P, Reinike R, Slamet-Loedin IH (2016). Advances in breeding for high grain zinc in rice. *Rice* 9(1):49. doi.org/10.1186/s12284-016-0122.
- Tandzi LN, Mutengwa, CS, Ngonkeu ELM, Woin N, Gracen V(2017). Breeding for quality protein maize (QPM) varieties: A Review. *Agronomy* 7(4):80. doi.org/10.3390/agronomy7040080
- Trijatmiko KR, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C, Balindong J, Oliva N, Sapasap MV, Borrero J, Rey J, Francisco P, Nelson A, Nakanishi H, Lombi E, Tako E, Glahn RP, Stangoulis J, Chadha-Mohanty P, Johnson AAT, Tohme J, Barry G, Slamet-Loedin IH(2016). Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Scientific Report* 6:19792. doi.org/10.1038/srep19792

- Tripathy SK, Ithape DM, Maharana M, Prusty AM (2017). Quality protein maize (QPM): Genetic basis and breeding perspective. *Tropical Plant Research* 4(1): 145-152. doi.org/10.22271/tpr.2017.v4.il.021
- Umarani E, Radhika K, Padma V, Subbarao LV (2017). Variability, heritability and genetic advance for agro-morphological and grain quality parameters in landraces of rice (*Oryza sativa* L.). *Journal of Environment and Technology* 35(3):1682-1687.
- Upadhyaya HD, Dwivedi SL, Singh S, Sahrawat KL, Singh SK (2016). Genetic variation and post-flowering drought effects on seed iron and zinc in ICRISAT sorghum mini core collection. *Crop Science* 56(1):374-383.
- Velu G, Singh RP, Huerta-Espino J, Peña RJ, Arun B, Mahendru-Singh A, Mujahid MY, Sohu VS, Mavi GS, Crossa J, Alvarado G, Joshi AK, Pfeiffer WH (2012). Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crops Research* 137:261-267. doi.org/10.1016/j.fcr.2012.07.018
- Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RA (2014). Biofortification strategies to increase grain Zn and Fe concentrations in wheat. *Journal of Cereal Sciences* 59(3):365-372. doi.org/10.1016/j.jcs.2013.09.001
- Velu G, Singh, R, Balasubramanian A, Kumar-Misha V, Chand R, Tiwari C, Joshi A, Joshi, A, Virk P, Cherian B, Pfeiffer WH (2015). Reaching out to farmers with high zinc wheat varieties through public-private partnerships: An experience from Eastern-Gangetic plains of India. *Advances in Food Technology and Nutritional Sciences* 1(3):73-75. doi.org/10.1740/aftnsoj-1-112
- Velu G, Tutus Y, Gomez-Becerra HF, Hao Y, Demir L, Kara R, Crespo-Herrera LA, Orhan S, Yazici A, Singh RP, Cakmak I (2017). QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. *Plant and Soil* 411(1):81-99. doi.org/10.1007/s11104-016-3025-8
- Velu G, Singh RP, Crespo-Herrera L, Juliana P, Dreisigacker S, Valluru R, Stangoulis J, Sohu VS, Mavi GS, Mishra VK, Balasubramanian A, Chatrath R, Singh GP, Joshi AK (2018). Genetic dissection of grain Zn concentration in spring wheat for mainstream biofortification in CIMMYT wheat breeding. *Scientific Reports* 8(1):1-10. doi.org/10.1038/s41598-018-31951-z
- Wallace TC, Murray R, Zelman KM (2016). The nutritional value and health benefits of chickpeas and hummus. *Nutrients* 8(12):766. doi.org/10.3390/nu8120766
- Wang YH, Zou CQ, Mirza Z, Li H, Zhang ZZ, Li DP, Xu CL, Zhou XB, Shi XJ, Xie DT, He XH, Zhang YQ (2016). Cost of agronomic biofortification of wheat with zinc in China. *Agronomy for Sustainable Development* 36(3):1-7. doi.org/10.1007/s13593-016-0382-x
- Welch RM, Graham RD (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* 55(396):353-364.
- Warrier SR, Patel BC, Kumar S (2020). Combining ability and heterosis for grain minerals, grain weight and yield in pearl millet and SSR markers based diversity of lines and testers. *Journal of King Saud University-Science* 32(2):1536-1543.
- WHO and FAO. (2003). Joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases. World Health Organ. Geneva, Switzerland.
- Yadav DN, Sharma K, Bansal S, Tushir S, Kaur J (2020). Advantage of biofortification over fortification technologies. In: Gupta OP, Pandey V, Narwal S, Ram S, Singh GP (Eds) *Wheat and Barley Grain Fortification*. Woodhead Publishing pp. 257-273.
- Zhang MW, Peng ZM, Du YQ (1996). Combining ability and stability analysis of the content of trace elements Fe, Zn and Mn in special rice grains. *Chinese Journal of Rice Science* 10:201-206.
- Yang M, Li Y, Liu Z, Tian J, Liang L, Qiu Y, Wang G, Du Q, Cheng D, Cai H, Shi L, Xu F, Lian X (2020). A high activity zinc transporter OsZIP9 mediates zinc uptake in rice. *The Plant Journal* 103(5):1695-1709.
- Zhang H, Liu J, Jin T, Huang Y, Chen J, Zhu L, Zhao Y, Guo J (2017). Identification of quantitative trait locus and prediction of candidate genes for grain mineral concentration in maize across multiple environments. *Euphytica* 213(4):1-16. doi.org/10.1007/s10681-017-1875-7
- Zhang CM, Zhao WY, Gao AX, Su TT, Wang YK, Zhang YQ, Zhou XB, He XH (2018). How could agronomic biofortification of rice be an alternative strategy with higher cost-effectiveness? *Food and Nutrition Bulletin* 39(2):246-59. doi.org/10.1177/0379572117745661
- Zhao DY, Zhang ZW, Yuan YR, Zhang XL, Zhao WF, Li XP, Wang J, Siddique KHM (2022). Accumulation of zinc, iron and selenium in wheat as affected by phosphorus supply in salinised conditions. *Crop and Pasture Science* 73(5):537-545.
- Zhao ZY, Che P, Glassman K, Albertsen M (2019). Nutritionally enhanced sorghum for the arid and semi-arid regions of Africa. In: Zhao, Z.Y., Dahlberg, J (eds) *Sorghum. Methods in Molecular Biology, 1931*, Humana Press, New York, NY, doi.org/10.1007/978-1-4939-9039-9_14