Microbial-assisted and genomic-assisted breeding: a two way approach for the improvement of nutritional quality traits in agricultural crops

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Abstract

Both human and animals, for their nutritional requirements, mainly rely on the plant-based foods, which provide a wide range of nutrients. Minerals, proteins, vitamins are among the nutrients which are essential and need to be available in adequate amount in edible portion of the staple crops. Increasing nutritional content in staple crops either through agronomic biofortification or through conventional plant-breeding strategies continue to be a huge task for scientists around the globe. Although some success has been achieved in recent past, in most cases, we have fallen short of expected targets. To maximize the nutrient uptake and partitioning to different economic part of plants, scientists have employed and tailored several biofortification strategies. But in present agricultural and environmental concerns, these approaches are not much effective. Henceforth, we are highlighting the recent developments and promising aspects of microbial-assisted and genomic-assisted breeding as candidate biofortification approach, that have contributed significantly in increasing nutritional content in grains of different crops. The methods used to date to accomplish nutrient enrichment with recently emerging strategies that we believe could be the most promising and holistic approach for future biofortification program. Results are encouraging, but for future perspective, the existing knowledge about the strategies needs to be confined. Concerted scientific investment are required to widen up these biofortification strategies, so that it could play an important role in ensuring nutritional security of ever-growing population in growing agricultural and environmental constraints.

Keywords Nutritional security · PGPRs · Rhizoengineering · Biofortification · *Omics*’ technologies

Introduction

Despite the years of advancements in crop improvement programs across the world to cope up with the food and nutritional security issues, the progress is still at hostile level. Several hidden conflicts and climate alterations are trouncing the poorest people targeting several parts of the world. While the world is committed to accomplish “Zero Hidden Hunger” by 2030, more than 805 million people worldwide are currently “chronically undernourished” (Global Hunger Index 2018). This alarming situation shows how far we are from achieving this vision. Even though access to food has improved substantially over the past few decades, large section of the people worldwide is unable to receive enough nutrition from the food they eat. These foods are substantially poor in overall nutritional contents. Recently, it was noticed and documented that in the nearby future, several threatening causes such as changing climatic conditions, global population explosion, constricting agricultural land, rising food

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prices and environmental stressors will have significant and highly uncertain impacts on both food and nutritional security status of the world (Global Hunger Index 2018).

As we know, nutrient deficiencies retard the overall growth and development of both crops and animals by interfering biochemical, physiological and metabolic functions of the cells. Insufficient micronutrient availability in the soil not only limits crop productivity but also contributes poor nutritional quality of food and fodder, which together translates nutrition and health status of humans and cattle (Kumssa et al. 2015). Dietary deficiencies of bioavailable micronutrients cause rigorous but invisible health complications, which include impairments of physical growth, metabolic disorders such as diabetes and obesity, combined with increased risk of infections and in some cases, lead to death of individuals (FAO 2017). These dietary deficiencies can be alleviated by adopting nutrition-specific (direct) and/or nutrition-sensitive (indirect) interventions through dietary diversification, industrial fortification and nutraceutical supplementation (Ruel and Alderman 2013). But these nutrition-sensitive interventions are not much effective in terms of cost, time, availability and impact. Although strategy of dietary diversification, supplementation and food fortification work best in urban areas, however, biofortification is the most feasible means of reaching malnourished rural populations with limited access (Bouis and Welch 2010). Therefore, there is an urgent need to take U-turn from fortification to biofortification, which could be most feasible and persistent strategy to address the underlying nutritional insecurity concerns. As it is well known that nutrient concentration in the grains or edible portion of the plants can be augmented by adopting integrated agricultural practices and conventional breeding approaches assisted with high-throughput 'Omics' technologies.

Since adoptions of green revolution, the plant breeders mainly focused on yield improvement of crops to alleviate food security concerns, which was the first and foremost priority during the 1960s and even today also. The scenario has changed slowly and food availability is no more a concern for masses; however, quality of food became the priority, which is now considered as the frontier area of research in plant sciences. In fact, scientific advancements realized us that nutritional deficiencies in food grains are the major reasons for malnutrition. During the crop improvement process especially breeding for high per se performance, selections were focussed mainly for allelic variants responsible for high yield, while the other allelic forms responsible for quality parameters were knowingly or unknowingly ignored. Consequently, the production efficiency of plants was improved leaving quality in orphan state. This stimulated scientists to think and work on development of healthy crops which can support not only the overall nutritional and health status of humans, but also of the cattle. Such type of crop varieties can

be developed by manipulating the soil physical and chemical properties as well as by identifying suitable genotypes as donors in breeding programs aimed at the development of nutrient-rich high-yielding genotypes. Further amendments can be done by exploiting and manipulating genes and proteins underplaying significant role in nutrient homeostasis. For the success of such programs, the knowledge of molecular insights into relation to minerals and other nutrient regulatory pathways is required. However, the molecular understanding till date is only available for few minerals (Iron and Zinc in wheat, rice, barley and maize; Selenium in maize and Calcium in finger millet) (Bauer and Hell 2006; Malagoli et al. 2015; Sharma et al. 2017). Moreover, the available information is also not explained well in context to other nutrient bioavailability pathway. Thus, there is a huge knowledge gap in understanding the molecular insights into nutrient acquisition and partitioning in crops/plants, and their ultimate bioavailability in animals. By understanding molecular aspects of nutrient homeostasis pathways, one can target the candidate genes to improve the cereals for elevated levels of nutrients as well as their bioavailability. Since, the soil is the main source of nutrients for all plants, the final nutrient status of food grains depends directly upon the available nutrients in the soil. But efficiency of crop plant or genotype to uptake and partition to various plant parts especially in edible portions are variable. It is mediated through a complex physio-biochemical interaction between soil associated with plant, microflora and plants or genotypes of a particular plant species (crop). Hence, with this holistic vision, the present review elaborates the progress and prospects of microbial-assisted and genomic-assisted breeding as candidate approach(es) for improving nutrient content in crops, which is likely to play an important role in ensuring comprehensive nutritional security for future.

Biofortification: approaches and hidden challenges

Increased environmental concerns, rapid urbanization and industrialization have resulted in drastic shrinking of our productive lands day-by-day. It is believed that global food production may soon be insufficient if the growth rate of population will not be checked. Ensuring adequate food for the growing population in the coming years shall not be an easy task. To feed all, it is enormously essential that agricultural productivity must be extensively amplified within the next few decades. For this, conventional breeding should acquire intensive agricultural practices, but it needs more agricultural land, additional farm mechanization, and extensive use of agrochemicals, for getting better crop productivity. Therefore, this strategy is not much effective and does not provide long-term solutions. It might happen because of several reasons, such as continuous crop production in same land with extensive external inputs result in soil saturation

after sometime (Dimpka and Bindran 2016) and further uncontrolled supply of agrochemicals completely deteriorates the sustainability of soil ecosystem. Moreover, the intensive cropping system although provides more food, the nutritional quality is compromised and the nutrient availability remains minimal resulting in malnutrition in both humans and animals. To secure nutrient enriched food for every mouth, crop scientists have to devise a strategy through which they can get better productivity of nutrient-enriched crops without distressing soil health perspectives. Therefore, in this context, biofortification through improved agricultural strategies which includes agronomic (microbial-assisted breeding) and genetic manipulations (genomic-assisted breeding) could be the possible interventions to address the underlying nutritional security concerns. These strategies have been discussed as follows:

Microbial-assisted breeding

Soil is a very rich and dynamic living matrix of nutrients, which not only support plant growth and development, but also harbor various microorganisms and maintain the ecological balance. Since inception of green revolution, because of continuous supply of agrochemicals, fertility as well as stability of the soil is severely affected, this ultimately affects the nutrient use efficiency (NUE) of crops or plants. Thus, to cope up this situation, it needs alternatives so that NUE of crops can be enhanced in sustainable manner. The recent emerging technologies such as “Rhizoengineering” could be an alternative approach to alleviate this problem.

Rhizobacteria and endophytes as a potent biological mediator

Microbial-assisted breeding is mainly governed by a set of biological mediators so called plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting endophytic bacteria (PGPE). Bacterial communities associated with either plant roots in rhizospheric soil or the leaves (phyllosphere) or may inhabit the internal sections of the plant tissues as endophytes (Walker et al. 2011; Bhattacharyya and Jha 2012; Ma et al. 2016). These rhizobacteria as a central biological mediator, known to facilitate various plant growth-promoting activities either directly through assisting nutrient assimilation (nitrogen, phosphorus, potassium and other essential minerals) or by modulating phytohormones level in crops, and indirectly by reducing deleterious effects of various pathogens and their toxins on the plant growth and development. Like PGPRs, plant growth-promoting endophytic bacteria (PGPE) directly facilitate the growth promotory activities of their host plant by forming root nodules (root colonizers), production of phytohormones, solubilization of minerals, degrading xenobiotic compounds and

alleviate heavy metal-induced phytotoxicity when plants grown in metal-tainted soils (Pereira and Castro 2014; Liu et al. 2015; Ma et al. 2016; Mattarozzi et al. 2017; Bavandpour et al. 2018). Basically, PGPRs and PGPEs are a group of important and agronomically useful soil microbiota, which may classified based on their functional activities into (1) Biofertilizers (Nitrogen fixer: *Azospirillum* sp., *Rhizobium* sp., *Azotobacter* sp., *Clostridium* sp.; Phosphate solubilizer: *Pseudomonas striata*, *Bacillus megaterium*, *Aspergillus awamori*; Potassium solubilizer: *Bacillus mucilaginosus*), (2) Phytostimulators (*Bacillus amyloliquefaciens*, *Bacillus subtilis*), (3) Rhizoremediators (*Nitrosomonas* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Actinomyces* sp.) and (4) Biopesticides (*Bacillus thuringiensis*, *Bacillus subtilis*, *Trichoderma* sp.) (Singh 2013; Kong et al. 2015; Sobari et al. 2016; Kong and Glick, 2017). Even though the role of PGPRs and PGPEs on plant growth promotion and their phytoremediation potential in metal-polluted soils has been comprehensively studied, but little is known on how the phyto-endophytic bacterial interaction occur in metalliferous soils for phytoremediation (Babu et al. 2015; Kong et al. 2017; DalCorso et al. 2019).

Why have to engineer the rhizosphere?

The term ‘rhizosphere’ can be defined as a narrow zone of soil directly influenced by the root hairs, soil, and the associated microbes (Dessaux et al. 2016; DalCorso et al. 2019). These three separate but interacting components have been studied separately but in current scientific perspectives, understanding the precision behind the rhizosphere remains a “black boxes” for scientific community. Similar to this, to fill the knowledge gap concerning plant–microbe interactions, nature and abundance of soil microbiome, complexity lies within the biology and chemistry of rhizosphere remain unresolved (Walker et al. 2011; Szymanska et al. 2016). Henceforth, it is an urgent prerequisite to solve the mystery resides within the rhizosphere, so that a safe, sound and sustainable agricultural practice can be employed to produce healthy food to feed ever-growing population in nutritional insecure environment. Thus, to cope up this situation, the “Rhizoengineering”, an emerging technology could be practiced as an alternative approach to alleviate this problem.

The effects of PGPRs and endophytic bacteria on soil health and crop productivity have been studied extensively under both field and greenhouse conditions. It was found in recent experiments that bio-inoculation of diverse endophytic (symbiotic and non-symbiotic) bacteria stimulate mineral acquisition, biomass growth and accelerated phytoremediation process by enhancing the soil fertility, cell elongation, and alters the phytoavailability of soil minerals in metal-stressed condition (Babu et al. 2015; Ma et al. 2016). Microbial genera in each soil microbiota are highly

selective in activity for growth promotory responses in their host plants in diverse agro-ecosystems. For example, rhizobiaceae family exerts growth promotory responses through fixing nitrogen by forming symbiosis with leguminous (rhizobia) and non-leguminous plants (Frankia). However, free living endophytes (*Azospirillum* sp., *Pseudomonas* sp. and *Azotobacter* sp.) fix nitrogen by non-symbiotic mode in their host plants (Kong and Glick 2017). Furthermore, in most studied cases, it was found that the PGPRs especially root-colonizing rhizobacteria have significant role in increasing plant growth, plant biomass, grain yield, grain protein content and nutrient uptake in barley (Mustafa et al. 2006), tomato (Gravel et al. 2007; Omar et al. 2013), rice (Rodrigues et al. 2008), garden pea (Egamberdieva et al. 2008), chickpea (Wani et al. 2008; Sathya et al. 2016), Indian mustard (Ma et al. 2009), mungbean (Zahir et al. 2010), soybean (Sharma et al. 2011), wheat (Sharma et al. 2011; Sahin and Turan 2014; Abaid-Ullah et al. 2015; Shaikh and Saraf, 2017), and maize (Singh, 2013; Rojas-Tapias et al. 2014; Vejan et al. 2016; Hussain et al. 2017). Likewise, several successful efforts have been made, which are summarized in Table 1. Based on the existing knowledge, it is now well known that microorganisms present in the rhizosphere plays pivotal role in providing the essential nutrients in solubilized form, by performing a series of biochemical reactions (Sahin and Turan 2014; Szymanska et al. 2016). Rhizosphere engineering may reduce our reliance on agrochemicals for better productivity by replacing it with beneficial PGPRs, endophytes, biostimulants or transgenic plants. Henceforth, it is of immense importance to identify or engineer crop (or genotype)-specific consortia of microorganisms which not only serve as biostimulants for bioaccumulation of ample amount of essential macronutrients, but can also harbor micronutrients in the edible portion of staple crops. Omics approaches for studying the plant–microbe interaction within rhizosphere in this system can be explored to develop biofertilizer which will not only increase the quantity and quality of crop yield but also return nutrients into the soil for the use of other crops. Thus, in this context the rhizosphere engineering could be a candidate approach for development of newer genotypes responsive to specific nutrients.

How to engineer the rhizosphere?

Generally, PGPRs are the vital component of the rhizosphere. The composition and biodiversity of microbial populations within the rhizosphere markedly affects plant–microbe interaction (Kong and Glick 2017). Since the complexity and nature of the rhizosphere is unraveled, scientific community can attempt to create or design favorable conditions most suited to the plant growth and productivity by making either soil amendment, breeding or designing “super cultivars”, manipulating physico-chemical properties

of rhizospheric soil, and modification of plant–microbe interactions (Fig. 1) (Walker et al. 2011; Dessaux et al. 2016; Ahkami et al. 2017). To date, research has shown that there are several approaches under study which can be considered as candidate approach for engineering rhizospheric soil as given below:

- (a) Manipulation of physico-chemical properties of rhizospheric soil by fertilizer amendments.
- (b) Identification of new potential PGPRs or rhizosphere microbial community.
- (c) Engineering environment for plant–microbe interactions.
- (d) Engineering metabolic pathways for greater ion flux across the roots.
- (e) Engineering transport proteins/channels for greater ion flux across the roots.
- (f) Breeding or designing “super cultivars” responsive for better productivity under nutrient limiting condition.

Genomic-assisted breeding

Over the last decade, the science of nutritional biology has developed extensively and progressed through the development of food-based nutrigenomics. In the era of growing nutritional crisis, nutritious properties of cereals must be harnessed to develop a crop with novel nutritional values. Considering the objective, conventional breeding has developed large number of cultivars potentially rich with various nutritional traits. But, due to the growing agro-ecological concerns, this approach falls short to supply nutritious food to ever-growing mouths across the globe. Thus, to cope up this situation, ‘Omic’ technologies could open a new avenue for future nutritional biology research.

How it is different from conventional and molecular breeding?

Plant breeding has made outstanding progress in increasing crop productivity in combination with developments in agricultural technologies. To achieve high-yield response, breeding efforts mainly focused upon development of high yielding input-responsive genotypes (Shivay and Prasad 2012; Ramzani et al. 2016). By adopting innovative technologies and realizing potential yields of crop plants to attain complete food security, crop breeding is now at verge of transformation from food security to nutritional security (Ehsanullah et al. 2015). To achieve this unprecedented challenge, breeding needs some extraordinary efforts. In the current scenario, several strategies have been developed but the most suggested approach is by identifying or designing crop/genotype responsive to specific nutrient bioaccumulation. Though it was observed that the relative responsiveness of

Table 1 Plant growth-promoting rhizobacteria tested on various crop types for their nutritional content and growth promotory activity

S. No.	Plant growth-promoting rhizobacteria (PGPR)	Plant	Results of addition of PGPR to plant as Biofertilizer	References
1.	<i>A. chroococcum</i> , <i>B. megaterium</i> , <i>B. mucilaginous</i> , <i>G. mosseae</i>	<i>Z. mays</i>	Significant increase in total N, P and K content in different plant parts	Wu et al. (2005)
2.	<i>Bacillus RC01</i> , <i>Bacillus RC02</i> , <i>Bacillus RC03</i> , <i>Bacillus M-13</i>	<i>T. aestivum</i> , <i>H. vulgare</i>	Increased concentration of in plants, Enhanced Fe, Mn, Zn, N, P and Cu contents in seedling	Mustafa et al. (2006)
3.	<i>Bacillus OSU-142</i> , <i>Bacillus M3</i>	<i>R. crataegifolius</i>	Increased available P, K, Ca, Mg, Fe, Mn, Zn contents and overall growth	Orhan et al. (2006)
4.	<i>Bradyrhizobium</i> sp. (vigna) RM8	<i>V. radiata</i>	Enhances the protein contents by 13% in grain, and N by 41% & 37% in root and shoot, respectively	Wani et al. (2007)
5.	<i>P. Denitrificans psd6</i> , <i>P. alcaligenes psd15</i> , <i>B. polymyxa bcp26</i>	<i>G. barbadense</i> , <i>P. sativum</i>	Significant increase in total N, P and K uptake in both root and shoot parts	Egamberdieva et al. (2008)
6.	<i>Mesorhizobium</i> sp. RC3	<i>C. arietinum</i>	Significant increase in yield and grain protein content by 36% and 16%, respectively, whereas nitrogen uptake in root and shoots by 46% and 40%, respectively	Wani et al. (2008)
7.	<i>A. amazonense</i>	<i>O. sativa</i>	Increases the grain dry matter by 7–11.6%, number of panicles from 3 to 18.6 and nitrogen content up to 3.5–18.5% in grain	Rodrigues et al. (2008)
8.	<i>A. xylooxidans Ax10</i>	<i>B. juncea</i>	Significant increase in plant growth and Cu uptake in plants	Ma et al. (2009)
9.	<i>R. phaseoli</i>	<i>V. radiata</i>	Significant increase in biomass, plant height, grain yield, and nitrogen uptake in grain	Zahir et al. (2010)
10.	<i>Pseudomonas</i> sp.	<i>G. max</i> , <i>T. aestivum</i>	Significant increase in soil enzyme activity, grain productivity, and nutrient bioaccumulation	Sharma et al. (2011)
11.	<i>Psychrobacter</i> sp. SRS8	<i>R. communis</i> , <i>H. annuus</i>	Stimulate growth and Ni bioaccumulation, with significant increase in protein and chlorophyll content, and biomass	Ma et al. (2009)
12.	<i>P. putida</i> , <i>P. fluorescens</i> , <i>B. subtilis</i> , <i>B. amyloliquefaciens</i>	<i>L. esculentum</i>	Stimulated overall plant growth, fruits per plants and increased essential mineral uptake under biotic stress condition	Omar et al. (2013)
13.	<i>P. alcaligenes</i> , <i>B. polymyxa</i> , and <i>M. phlei</i>	<i>Z. mays</i>	Enhances the uptake of N, P and K in plant	Singh (2013)
14.	<i>Bacillus M-3</i>	<i>T. aestivum</i>	Increases uptake of P, K, Mn, Fe, and Zn contents in the leaf, grain and straw of plant	Sahin and Turan (2014)
15.	<i>Bacillus cereus</i>	<i>O. sativa</i>	Enhanced grain Zn content by 18–49% in two rice varieties	Shakeel et al. (2015)
16.	<i>S. liquefaciens</i> FA-2, <i>B. thuringiensis</i> FA-3, <i>S. marcescens</i> FA-4	<i>T. aestivum</i>	Observed significant increase in grain yield and zinc content	Abaid-Ullah et al. (2015)
17.	<i>Streptomyces</i> sp.	<i>C. arietinum</i>	Promote overall plant growth, enhanced up to 24–28% of Fe, 25–28% of Zn, 12–14% of Ca, 28–35% of Cu, 160–167% of Mn and 34–37% of Mg content in plant	Sathya et al. (2016)
18.	<i>Bacillus</i> sp.	<i>Z. mays</i>	I Increase the total N, P, and K content in shoot and root	Vejan et al. (2016)
19.	<i>P. syringae</i> -S5, <i>P. fluorescens</i> -S20	<i>Z. mays</i>	Positive effect on seed germination to grain development, and significant increase in P and Zn content in plant on maturity	Hussain et al. (2017)
20.	<i>E. aurantiacum</i> MS-ZT10	<i>T. aestivum</i>	Enhances the N, P, and K concentration up to 2.66%, 1.03% and 0.37%, respectively	Shaikh, and Saraf. (2017)

Fig. 1 Systematic scheme showing the holistic interaction between the rhizosphere and its components for targeting rhizo-engineering of specific crop

genotypes mainly depends upon their genetic potential for NUEs, yet it varies from one agroecosystem to another agroecosystem (Ramzani et al. 2016; Liu et al. 2017).

Despite various agricultural limitations, breeding has played significant role in improvement of cultivars with desired traits. But traditional breeding approaches for developing genotypes are time consuming as well as expensive (Peleg et al. 2008; Shivay and Prasad 2012). So, in this context, it is of immense importance to maximize the efficiency of classical breeding. Several high-throughput technologies such as, transgenics, allele mining (Association analysis and TILLING/EcoTILLING), gene or genome editing, etc. are available to improve the effectiveness of classical breeding; however, the molecular marker-assisted breeding has been considered most successful and acceptable in the cultivar developments and, therefore, it needs elaboration (Tariq et al. 2014). The wide variation for micronutrient levels in crop species including wild relatives could be utilized for biofortification. In wheat, using the transcription factor NAM-B1 identified in wild emmer (*Triticum turgidum* sp. *dicoccoides*) resulted in increased Fe and Zn content by 18 and 12%, respectively (Uauy et al. 2006). This gene has been widely used in breeding programs across the globe. Molecular breeding approaches have been used in several crop improvement program for multiple traits viz., drought, salinity, and cold tolerance in wheat and barley (Kosova et al. 2014) and barley (Kaur et al. 2017), submergence tolerance in rice (Alpuerto et al. 2016), salinity tolerance in soybean (Patil et al. 2016), barley (Zhang et al. 2017), rice (Omisan et al. 2018), improving NUEs in maize (Zhu et al. 2016) and barley (Rajala et al. 2017), protein quality improvement in maize (Babu et al. 2005; Huang et al. 2006), Iron and zinc content in rice, wheat, lentil, and millets (Velu et al. 2017; Khazaei et al. 2017; Anuradha et al. 2017) and likely for agronomic traits in rice (Chang et al. 2016) and

barley (Nice et al. 2017). similarly, Hirel et al. (2001) and Masclaux et al. (2001) has studied independently and analyzed for recombinants in breeding lines in maize for various agro-morpho-physiological traits such as NR, GS activities, nitrate content and yield, and found a positive correlation between these traits. Molecular marker technology also offers some opportunities in cultivar development by tracking introgression of associated QTLs/genes underplaying the traits. With the advancement of molecular markers, it can also facilitate the identification or evaluation of potential gene/quantitative trait loci (QTLs) (Table 2). To this, QTLs for nutrient use efficiency (NUE) and nitrogen use efficiency (NiUE) has been identified and reviewed in several crops such as in Arabidopsis, rice, maize, and barley (Hirel et al. 2007). Likely in rice and wheat, QTLs associated with GS1 activity indicates the higher nitrogen content and grain yield (Yamaya 2002). Quantitative studies of NUEs, NiUEs, other nutrient use efficiency and genetic variability in other traits using marker technology will be increasingly useful for future to identify new candidate genes or loci markedly involved in the regulation or homeostasis of nutrient assimilation pathways. This strategy could be used as candidate approach for breeding or designing “super cultivars” responsive for better productivity under nutrient limiting condition.

Nutrigenomic and genetic engineering in crop biofortification

A large section of world’s populations suffered from various nutrition-related disorders, are linked with differential interaction of the nutrients (Stover 2006). In the post-genomic era, nutritional biology research revealed that some extrinsic factors like human diet can influence morpho-physiological modulation of genes. Capitalizing on this behavioral genetics, new scientific area of research ‘nutrigenomics’ come

Table 2 Development of biofortified crop through genomic assisted breeding approach using different omics platform in various crops

S. No.	Plants	Target traits	Omics platform	Major finding	References
1.	Maize	Both lysine and total protein	Transgenics	Lysine-rich protein gene sb401 significantly increases both lysine and total protein content in maize seeds	Yu et al. (2005)
2.	Rice	Tryptophan content	Transgenics and metabolite profile	High-level tryptophan accumulation in seeds of transgenic rice	Wakasa et al. (2006)
3.	Maize	Lysine and tryptophan	Transgenics	High lysine and tryptophan maize resulting from the reduction of both 19- and 22-kD alpha-zeins	Huang et al. (2006)
4.	Maize and soybean	Low phytic acid	Gene silencing	Reduces phytic acid content of maize and soybean seeds to Improved grain Fe content	Shi and Whetstone (2007)
5.	Foxtail millet	Starch content	NGS sequencing and SNP discovery	Allelic variation in <i>Wx</i> gene encodes a key enzyme, granule-bound starch synthase 1 (GBSS1) catalyzes the formation of amylose	Van et al. (2008)
6.	Maize	Provitamin A	Transgenics	Transgenic maize with enhanced provitamin A content	Aluru et al. (2008)
7.	Foxtail millet	Starch content	Metabolite analysis	Observed starch content ranged from 67.5–68.7% in four Foxtail millet genotypes with 3.3–11.4% amylose content and 69.6 °C gelatinization temperature	Kim et al. (2009)
8.	Wheat	Amylose content	Gene silencing	Increasing grain amylose content through silencing of the <i>SBEIIa</i> genes	Sestili et al. (2010)
9.	Maize	Low phytic acid	Transgenics	Low phytic acid 1-1 maize to enhance iron content and bioavailability	Aluru et al. (2011)
10.	Soybean	β -carotene	Transgenics	Enhance the β -carotene content through seed-specific expression	Kim et al. (2012)
11.	Wheat	Grain iron content	Transgenics	Improved grain Fe content of wheat by soybean ferritin gene overexpression	Xiaoyan et al. (2012)
12.	Rice	Grain iron content	Transgenics	Observed significant increase in grain Fe content in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene	Masuda et al. (2013)
13.	Finger millet	Grain protein and calcium content	Marker-assisted selection using EST-SSRs	Established significant negative correlation between calcium and protein content across finger millet genotypes	Nirgude et al. (2014)

Table 2 (continued)

S. No.	Plants	Target traits	Omics platform	Major finding	References
14.	Finger millet	Calcium content	Transcriptome profiling	The study reveals 17 ORFs encoding for CDPKs, 21 for CIPKs, 2 for CaMKs, 1 for CCaMKs, 2 for PEPRKs, 4 for CRKs, 9 for CBLs and 24 for CaM and CaML proteins in GP-1 transcriptome. Similarly, 16 ORFs encoding for CDPKs, 22 for CIPKs, 2 for CaMK, 1 for CCaMK, 2 for PEPRKs, 4 for CRKs, 8 for CBLs and 23 for CaM and CaML proteins were identified in GP-45 transcriptome	Singh et al. (2014)
15.	Finger millet	Calcium content	Transcriptome profiling	Expression analysis reveals CAX1, TPC1, ATPase, CaM, CaMK1, CaMK2 and 14-3-3 genes involved in calcium sequestrations in root, stem and leaf at different reproductive stages of two finger millet genotypes viz. GP 1 and GP45	Mirza et al. (2014)
16.	Barley	PUFA content	Transgenics	Transgenic barley producing essential polyunsaturated fatty acids	Mihálik et al. (2014)
17.	Finger millet	Tryptophan content	Comparative genomic and association mapping	EST-SSRs significantly associated with QTLs viz., OM5 and FM8 for Tryptophan content	Babu et al. (2014)
18.	Maize	Carotenoids	Transgenic, Proteome, and metabolite analysis	Maize seeds engineered for enhanced carotenoid biosynthesis	Decourcelle et al. (2015)
19.	Foxtail millet	Protein content	Genome-wide investigation and comparative genomic	Identified <i>Setarin</i> gene encoding 16 prolamins which is crucial for quality protein accumulation in Foxtail millet seed	Muthamilarasan and Prasad. (2015)
20.	Rice	Folic acid (vitamin B9)	Metabolic engineering	Obtained folate concentrations up to 150 fold higher than those of wild-type rice	Blancquaert et al. (2015)
21.	Finger millet	Calcium content	Transcriptome profiling	Genes encoding Calcium transporters (CDPKs, CIPKs, CaXs and Ca ²⁺ ATPase) were identified	Kumar et al. (2015)
22.	Finger millet	Seed storage proteins	Transcriptome profiling	Genes encoding seed storage proteins in high (prolamin, globulin and glutenin) and high and low (gliadin, kafirin, legumin and albumin) grain calcium genotype respectively, were identified	Kumar et al. (2015)

Table 2 (continued)

S. No.	Plants	Target traits	Omics platform	Major finding	References
23.	Barnyard millet, little millet and kodo millet	Grain iron and zinc content	Transcriptome, expression profiling and association analysis	Expression of three genes (YSL 2, NAS 1, NAS 3) was found to be correlated to high grain Fe contents, one gene (NAAT) to high grain Zn contents whereas ten genes (IRT 1, NAS 2, ZIP 7, ZIP 5, ZIP 1, FER 1, FER 2, NAC 5, NRAMP 5, NRAMP 7) was found to be correlated to both high Fe and Zn contents	Patel et al. (2015)
24.	Finger millet	Calcium content	MAS, candidate gene-based AM	Nine SSR markers (M2, M6, M11, M16, M26, M27, M36, M45 and M65) were found to be significantly associated with the calcium content trait	Kumar et al. (2015)
25.	Lentil	Iron and zinc content	Marker-trait association analysis using SNP markers	MTA analysis detected 2 SNP tightly linked to grain Fe and 1 SNP linked to Zn concentration	Khazaei et al. (2017)
26.	Pearl millet	Iron and zinc content	Marker-trait associations (MTAs)	SSR markers viz., <i>Xpsmp</i> 2261, <i>Xipes</i> 0180 and <i>Xipes</i> 0096 were associated with grain iron and zinc content	Anuradha et al. (2017)
27.	Wheat	Zinc content	Marker-assisted selection using DArT markers	Dissected two major QTLs on chromosome 1B and 6B across RILs and observed grain Zn efficiency up to 31–90%	Velu et al. (2017)
28.	Barnyard Millet	Iron content	Genome-wide and comparative genomic analysis	<i>In silico</i> analysis of EfrT2 gene showed similarity with other IRT2 proteins of Poaceae family and could be a potential candidate gene for iron biofortification	Manape et al. (2017)

into existence that can explore the intrinsic association of nutritional molecules with genomics. Basically, Nutrigenomics reveals the action of single nutrient on different molecular targets which result in alteration of nutrient-gene expression within a biological system. It uses quantitative techniques for genome-wide identification of genes, having crucial role in alteration of nutrient homeostatic pathways (Kumar et al. 2016). Other technologies such as functional genomics along with bioinformatics can help in exploration of genetic architecture of genes and their effect on human health in nutritional environment (Mutch et al. 2005). Also, through complete profiling of food-responsive gene and by understanding individual preferences, dietary lifestyle, etc., nutrigenomics can help scientists to understand how the individualistic responses varied with genic variations co-ordinately influenced by diet (Kumar et al. 2016). Thus, based on this individualistic health response to a particular nutrient, scientists could be able to design targeted biofortification program to enrich cereal-based diet with nutrient of interest.

In the era of growing nutritional crisis, with the elucidation of available biological information, the crop improvement has realized the importance of genome or genetic engineering. Recently, these technologies revolutionized the biological research and their potential, especially in the field of basic biology, medicine and health care, agricultural research, nutritional biology, and many more (Voytas and Gao 2014; Rajendran et al. 2015). Basically, genetic engineering or transgenic technology facilitates us an opportunity to maneuver gene or genome within a living systems. Genetic engineering performed by precise manipulation of plant, animal and human genomes, whereas genome editing is performed by triggering DNA repair mechanisms within the cell (Rajendran et al. 2015). Since 2015, these high-throughput technologies have made significant impact on nutritional biology research. Transgenics has been extensively used for improving various nutritionally important traits such as starch content in sorghum (McIntyre et al. 2008), maize and millets (Van et al. 2008; Kim et al. 2009) and wheat (Sestili et al. 2010); quality proteins in maize (Yu et al. 2005; Gibbon and Larkins 2005); mineral contents in Carrot (Morris et al. 2008), Bottle gourd (Han et al. 2009), Tomato (Chung et al. 2010), wheat (Xiaoyan et al. 2012; Borg et al. 2012), rice (Paul et al. 2014; Masuda et al. 2013; Trijatmiko et al. 2016) and in Finger millet (Mirza et al. 2014; Vinoth and Ravindhran, 2017). Recently, several research shows that transporters/gene family such as *FER-ITIN*, *PHYTASE*, *IRO*, *IRT*, *NRAMP*, *FRO*, *NAC*, *IDS*, *NAS*, *NAAT* and *YSL* were successfully employed for developing transgenics for Fe and Zn biofortification in rice (Masuda et al. 2013; Paul et al. 2014; Trijatmiko et al. 2016). Similarly, Calcium sensor genes such as *CAX* were targeted in rice, tobacco, tomato, potato, carrot, bottle gourd and finger

millet, whereas *TPC*, *CaMK*, *EcCIPK* gene family were targeted in finger millet for Ca biofortification (Morris et al. 2008; Han et al. 2009; Chung et al. 2010; Mirza et al. 2014; Vinoth and Ravindhran 2017). Likewise, several successful efforts have been made in recent past, which are summarized in Table 2. Thus, based on these efforts, it is now revealed that genetic engineering or transgenics can be used as efficient tool for designing future biofortified crops.

Genetics to genomics: approaches to develop nutritional superiority in cereals

Genomics is one of the important areas of genetical science and assume to be more effective and feasible approach in revamping classical plant breeding to achieve the target of reducing health concerns under nutritional insecure environment (Perez-de-Castro et al. 2012). Recently, several advanced genomic technologies have emerged that can make nutrient biofortification programs rather more smart. These include SNPs array, next-generation sequencing, transcriptome profiling, oligo-directed mutagenesis, gene-silencing (RNA interference), genome-editing (CRISPR-Cas9 system, MegaN, ZFNs and TALENs) technologies, etc. (Abdallah et al. 2015; Schaart et al. 2016; Peng et al. 2016) (Fig. 2). In current practice, these technologies in combination with next-generation sequencing methods facilitate specific and non-specific gene and allele mining (Carvalho and Vasconcelos 2013). In addition, it is of immense importance to integrate different molecular breeding approaches with genomic tools for trait-targeted crop biofortification. The combined breeding programs mainly aim to multiply micronutrient concentration without affecting other essential nutritional components or agronomic per se performance.

For example, using combined genomic resources with bioinformatic strategies may facilitate increased bioavailability of both vitamin C and iron content in tomato (Ruggieri et al. 2016). These combined strategies can also be employed for biofortification of different crops for other target traits. Similarly, genome-wide association studies (GWAS) have revealed tightly linked QTLs in rice for iron and polyphenols contents in sorghum (Rhodes et al. 2014). It is well known that polyphenols are crucial anti-nutritional factor which determine the status of iron bioavailability in different plant parts. Thus, by screening QTLs using GWAS, it may be used as novel candidate genes for improving iron bioavailability in the grain by decreasing polyphenols concentrations (Fig. 2). In the recent aspect, genetically modified (GM) crops are facing different biosafety issues and a lack of consumer preference, especially in south-east Asia. Newly emerged high-throughput molecular platforms such as gene- or genome-editing methods (CRISPR-Cas9 system, MegaN, ZFNs and TALENs) could be efficient tool to design future biofortification programs, because nutrient enrichment and tracking through

Fig. 2 Systematic scheme of genomic-assisted breeding approaches to develop nutritional superiority in cereals

these tools can be obtained much faster and more efficiently without any harmful effects (Wang et al. 2014). The major advantage of such improved cultivars is its modified nature which does not require regulatory frameworks as in the case of genetically modified crops, because the improved gene-edited traits' performance is similar to traits improved from conventional breeding approaches and it does not impose any harmful threats (Huang et al. 2006). Although most of genome engineering tools used to date for crop improvement is in nascent stage, some crop have been developed using these technologies. Gene knockout using diverse site-specific nucleases (SSNs) system has been employed to elucidate important genes that could be used for crop improvement in different plant species, including important crops, such as rice, wheat, maize, barley, sorghum and soybean (Voytas and Gao, 2014; Abdallah et al. 2015). Previously, gene- or genome-editing methods especially CRISPR/Cas9 technologies were confined to some model crops, but now, CRISPR/Cas9-mediated genome editing is adopted significantly for many other crop improvement programs such as for extending the shelf life of vegetables and fruits, improving yield potential, enhancing biotic and abiotic stress tolerance, increasing NUEs, improved nutritional values, decreasing anti-nutritional factors and for other important agronomic traits (Shan et al. 2013; Abdallah et al. 2015). Likewise, these genome editing tools were tested in various crops and realized to be as potential tool to improve nutritional values by reducing antinutritional factors in crops

(Shi and Whetstone 2007; Aluru et al. 2011). Accumulations of unwanted anti-nutritional factors in food crops have negative impact in nutritional quality and food processing (Abdallah et al. 2015; Vinoth and Ravindhran, 2017). Some plants such as mustard, cabbage and cassava produce antinutrients such as glucosinolates, phytate and cyanide, which can cause acute intoxication, ataxia and even partial paralysis in high doses (Hannoufa et al. 2014; Abdallah et al. 2015). Similarly, through pathway engineering, site-directed mutagenesis, exome sequencing and transcriptome profiling of essential nutrient homeostasis-related genes in concern plant species would provide all possible information about best allelic variants of target genes (Fig. 2), which can be used in crop biofortification programs (Abdallah et al. 2015). Thus, based on these studies, it is now revealed that genome engineering technologies along with other genomic tool and techniques can be used as efficient tool for designing future biofortified crops. However, these techniques are still emerging and needs further scientific efforts to realize and to accomplish the full potential of the nutritional biology research.

Conclusion and future prospects

Since realization of green revolution, we are witnessing a qualitative shift in crop research with heavy emphasis on getting higher yield with dynamic adjustment in agronomic

traits. Using the yield efficient cultivars, breeding efforts could only be able to solve food security concerns. But due to recent agronomic and environmental concerns, providing sufficient amount of nutrient-enriched food for an ever-growing population is very difficult. To feed these growing mouths, conventional breeding needs paradigm shift in their breeding strategies, so that it could be able to address nutritional insecurity concern. For this, breeders have to acquire recent breeding strategies so that it can able to develop nutrient responsive cultivars. We know that biofortification through improved nutrient efficient cultivar is the only possible and effective way to fight this concern. Thus, in this review, we have discussed the most recent and promising aspects of biofortification that have contributed significantly or has vast potential in nutrient enrichment in grains of different crops. Here, we have also discussed the recently emerging strategies that we believe could be the most promising aspects for future biofortification program. Thus, with these and other strategies in place, for future perspective, the existing knowledge about the strategies and homeostasis pathway-related hurdles needs to be resolved. Apart from recent developments in translational crop research, the new biofortification strategies need to be developed to tackle the future nutrient insecurity in growing agricultural and environmental constraints.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. All authors read and approved the manuscript.

References

- Abaid-Ullah M, Hassan MN, Jamil M, Brader G, Shah MKN, Sessitsch A, Hafeez FY (2015) Plant growth promoting rhizobacteria: an alternate way to improve yield and quality of wheat (*Triticum aestivum*). *Int J Agric Biol* 17:51–60
- Abdallah N, Prakash C, McHughen A (2015) Genome editing for crop improvement: challenges and opportunities. *Statew Agric Land Use Baseline* 1:183–205
- Ahkami AH, White RA, Handakumbura PP, Jansson C (2017) Rhizosphere engineering: enhancing sustainable plant ecosystem productivity. *Rhizosphere* 3:233–243
- Alpuerto JB, Hussain RMF, Fukao T (2016) The key regulator of submergence tolerance, SUB1A, promotes photosynthetic and metabolic recovery from submergence damage in rice leaves. *Plant Cell Environ* 39(3):672–684
- Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Rodermel S (2008) Generation of transgenic maize with enhanced provitamin A content. *J Exp Bot* 59(13):3551–3562
- Aluru MR, Rodermel SR, Reddy MB (2011) Genetic modification of low phytic acid 1-1 maize to enhance iron content and bio-availability. *J Agric Food Chem* 59(24):12954–12962
- Anuradha N, Satyavathi CT, Bharadwaj C, Nepolean T, Sankar SM, Singh SP, Meena MC, Singhal T, Srivastava RK (2017) Deciphering genomic regions for high grain iron and zinc content using association mapping in pearl millet. *Front Plant Sci* 8(412):1–17
- Babu SS, Specht ED, David SA, Karapetrova E, Zschack P, Peet M, Bhadesia HKDH (2005) In-situ observations of lattice parameter fluctuations in austenite and transformation to bainite. *Metall Mater Trans A* 36(12):3281–3289
- Babu BK, Agrawal PK, Pandey D, Kumar A (2014) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. *Mol Breed* 34:1261–1279
- Babu AG, Shea PJ, Sudhakar D, Jung IB, Oh BT (2015) Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil. *J Environ Manag* 151:160–166
- Bauer P, Hell R (2006) Translocation of iron in plant tissues. In: Abadia J (ed) *Iron nutrition in plants and rhizospheric micro-organisms*. Springer, Berlin, pp 279–288
- Bavandpour F, Zou Y, He Y, Saeed T, Sun Y, Sun G (2018) Removal of dissolved metals in wetland columns filled with shell grits and plant biomass. *Chem Eng J* 331:234–241
- Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol* 28(4):1327–1350
- Blancquaert D, Van Daele J, Strobbe S, Kiekens F, Storozhenko S, De Steur H, Van Der Straeten D (2015) Improving folate (vitamin B 9) stability in biofortified rice through metabolic engineering. *Nat Biotechnol* 33(10):1076
- Borg S, Brinch-Pedersen H, Tauris B, Madsen LH, Darbani B, Noeparvar S, Holm PB (2012) Wheat ferritins: improving the iron content of the wheat grain. *J Cereal Sci* 56(2):204–213
- Bouis HE, Welch RM (2010) Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci* 50:20–32
- Carvalho SMP, Vasconcelos MW (2013) Producing more with less: strategies and novel technologies for plant-based food biofortification. *Int J Food Res* 54:961–971
- Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, Luo Y (2016) Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* 22(1):78
- Chung MY, Vrebalov J, Alba R, Lee J, McQuinn R, Chung JD, Giovannoni J (2010) A tomato (*Solanum lycopersicum*) APETALA2/ERF gene, SIAP2a, is a negative regulator of fruit ripening. *Plant J* 64(6):936–947
- DalCorso G, Fasani E, Manara A, Visioli G, Furini A (2019) Heavy metal pollutions: state of the art and innovation in phytoremediation. *Int J Mol Sci* 20:3412
- Decourcelle M, Perez-Fons L, Baulande S, Steiger S, Couvelard L, Hem S, Sandmann G (2015) Combined transcript, proteome, and metabolite analysis of transgenic maize seeds engineered for enhanced carotenoid synthesis reveals pleiotropic effects in core metabolism. *J Exp Bot* 66(11):3141–3150
- Dessaux Y, Grandclément C, Faure D (2016) Engineering the rhizosphere. *Trends Plant Sci* 21(3):266–278

- Dimpka CO, Bindran PS (2016) Fortification of micronutrients for efficient agronomic production: a review. *Agron Sustain Dev* 36(7):1–26
- Egamberdieva D (2008) Plant growth promoting properties of *Rhizobacteria* isolated from wheat and pea grown in loamy sand soil. *Turk J Biol* 32:9–15
- Ehsanullah AT, Randhawa MA, Anjum SA, Nadeem M, Naeem M (2015) Exploring the role of zinc in maize (*Zea Mays* L.) through soil and foliar application. *Univ J Agric Res* 3(3):69–75
- FAO (2017) The state of food insecurity in the World—Meeting the 2016 International Hunger Targets: taking stock of uneven progress. Food and Agriculture Organization of the United Nations, Rome
- Gibbon BC, Larkins BA (2005) Molecular genetic approaches to developing quality protein maize. *Trends Genet* 21(4):227–233
- Global Hunger Index (2018) <http://www.ifpri.org/publication/2018-global-hunger-index-inequalities-hunger>. Accessed 9 Apr 2019
- Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biol Biochem* 39(8):1968–1977
- Han JS, Park S, Shigaki T, Hirschi KD, Kim CK (2009) Improved watermelon quality using bottle gourd rootstock expressing a $\text{Ca}^{2+}/\text{H}^{+}$ antiporter. *Mol Breed* 24(3):201–211
- Hannoufa A, Pillai BV, Chellamma S (2014) Genetic enhancement of *Brassica napus* seed quality. *Transgenic Res* 23:39–52
- Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gallais A (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol* 125(3):1258–1270
- Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J Exp Bot* 58(9):2369–2387
- Huang S, Frizzi A, Florida CA, Kruger DE, Luethy MH (2006) High lysine and high tryptophan transgenic maize resulting from the reduction of both 19- and 22-kD α -zeins. *Plant Mol Biol* 61:525–535
- Hussain S, Hussain MB, Gulzar A, Zafar-ul-Hye M, Aon M, Qaswar M, Yaseen R (2017) Right time of phosphorus and zinc application to maize depends on nutrient-nutrient and nutrient-inoculum interactions. *Soil Sci Plant Nutr* 63(4):351–356
- Kaur Y, de Souza RJ, Gibson WT, Meyre D (2017) A systematic review of genetic syndromes with obesity. *Obes Rev* 18(6):603–634
- Khazaei H, Podder R, Caron CT, Kundu SS, Diapari M, Vandenberg A, Bett KE (2017) Marker-trait association analysis of iron and zinc concentration in lentil (*Lens culinaris* Medik.) Seeds. *Plant Genome* 10(2):1–8
- Kim S, Sohn E, Lee I (2009) Starch properties of native foxtail millet, *Setaria italica* Beauv. *J Crop Sci Biotechnol* 12(1):59–62
- Kim MJ, Kim JK, Kim HJ, Pak JH, Lee JH, Kim DH, Ha SH (2012) Genetic modification of the soybean to enhance the β -carotene content through seed-specific expression. *PLoS One* 7(10):e48287
- Kong Z, Glick BR (2017) The role of plant growth-promoting bacteria in metal phytoremediation. *Adv Microb Physiol* 71:97–132
- Kong ZY, Mohamad OA, Deng ZS, Liu XD, Glick BR, Wei GH (2015) Rhizobial symbiosis effect on the growth, metal uptake, and antioxidant responses of PGPB in Metal Phytoremediation *Medicago lupulina* under copper stress. *Environ Sci Pollut Res* 22:12479–12489
- Kong ZY, Deng ZS, Glick BR, Wei GH, Chou MX (2017) A nodule endophytic plant growth-promoting *Pseudomonas* and its effects on growth, nodulation and metal uptake in *Medicago lupulina* under copper stress. *Ann Microbiol* 67(1):49–58
- Kosova K, Vitamvas P, Prasil IT (2014) Wheat and barley dehydrins under cold, drought, and salinity - what can LEA-II proteins tell us about plant stress response? *Front Plant Sci* 5:343
- Kumar A, Gaur VS, Goel A, Gupta AK (2015) De novo assembly and characterization of developing spikes transcriptome of finger millet (*Eleusine coracana*): a minor crop having nutraceutical properties. *Plant Mol Biol Rep* 33:905–922
- Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S, Singh M, Gupta S, Babu BK, Sood S, Yadav R (2016) Nutraceutical value of finger millet [*Eleusine coracana* (L.) Gaertn], and their improvement using omics approaches. *Front Plant Sci* 7:934
- Kumssa DB, Joy EJM, Ander EL, Watts MJ, Young SD, Walker S, Broadley MR (2015) Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. *Int J Sci Rep* 5:10974
- Liu W, Wang Q, Wang B, Hou J, Luo Y, Tang C et al (2015) Plant growth promoting rhizobacteria enhance the growth and Cd uptake of *Sedum plumbizincicola* in a Cd-contaminated soil. *J Soils Sediment* 15(5):1191–1199
- Liu D, Zhang W, Pang L, Zhang Y, Wang X, Liu Y, Chen X, Zhang F, Zou C (2017) Effects of zinc application rate and zinc distribution relative to root distribution on grain yield and grain Zn concentration in wheat. *Plant Soil* 411:167–178
- Ma Y, Rajkumar M, Freitas H (2009) Isolation and characterization of Ni mobilizing PGPB from serpentine soils and their potential in promoting plant growth and Ni accumulation by *Brassica* spp. *Chemosphere* 75(6):719–725
- Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. *J Environ Manage* 174:14–25
- Malagoli M, Schiavon M, Acqua S, Pilon-Smits EH (2015) Effects of selenium biofortification on crop nutritional quality. *Front Plant Sci* 6:1–5
- Manape TK, Varanavasiappan S, Raveendran M, Robin S, Rajeswari S, Sudhakar D (2017) Isolation and *In Silico* Analysis of Iron Regulated Transporter 2 Gene from Indian Barnyard Millet (*Echinochloa frumentacea* L.). *Int J Curr Microbiol Appl Sci* 6(4):1576–1587
- Masclaux C, Quilleré I, Gallais A, Hirel B (2001) The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Ann Appl Biol* 138(1):69–81
- Masuda H, Kobayashi T, Ishimaru Y, Takahashi M, Aung MS, Nakaniishi H, Mori S, Nishizawa NK (2013) Iron-biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. *Front Plant Sci* 4:132
- Mattarozzi M, Manfredi M, Montanini B, Gosetti F, Sanangelantoni AM, Marengo E, Careri M, Visioli GA (2017) Metaproteomic approach dissecting major bacterial functions in the rhizosphere of plants living in serpentine soil. *Anal Bioanal Chem* 409:2327–2339
- McIntyre CL, Drenth J, Gonzalez N, Henzell RG, Jordan DR (2008) Molecular characterization of the waxy locus in sorghum. *Genome* 51(7):524–533
- Mihálik D, Gubišová M, Klempová T, Čertík M, Ondreičková K, Hudcovicová M, Kraic J (2014) Transgenic barley producing essential polyunsaturated fatty acids. *Biol Plant* 58(2):348–354
- Mirza N, Taj G, Arora S, Kumar A (2014) Transcriptional expression analysis of genes involved in regulation of calcium translocation and storage in finger millet (*Eleusine coracana* L. Gaertn). *Gene* 550:171–179
- Morris J, Hawthorne KM, Hotze T, Abrams SA, Hirschi KD (2008) Nutritional impact of elevated calcium transport activity in carrots. *PNAS* 105(5):1431–1435

- Mustafa YC, Serdar B, Ramazan Ç, Fikretin Ş, Adil A (2006) Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. *Biol Fertil Soils* 42:350–357
- Mutch DM, Wahli W, Williamson G (2005) Nutrigenomics and nutrigenetics: the emerging faces of nutrition. *FASEB J* 19:1602–1616
- Muthamilarasan M, Prasad M (2015) Advances in *Setaria* genomics for genetic improvement of cereals and bioenergy grasses. *Theor Appl Genet* 128:1–14
- Nice LM, Steffenson BJ, Blake TK, Horsley RD, Smith KP, Muehlbauer GJ (2017) Mapping agronomic traits in a wild barley advanced backcross-nested association mapping population. *Crop Sci* 57:1199–1210
- Nirgude M, Babu BK, Shambhavi Y, Singh UM, Upadhyaya HD, Kumar A (2014) Development and molecular characterization of genic molecular markers for grain protein and calcium content in finger millet (*Eleusine coracana* (L.) Gaertn.). *Mol Biol Rep* 41(3):1189–1200
- Omar AA, Samia IM, Tamer SA (2013) Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi J Biol Sci* 20:57–61
- Omisun T, Sahoo S, Saha B, Panda SK (2018) Relative salinity tolerance of rice cultivars native to North East India: a physiological, biochemical and molecular perspective. *Protoplasma* 255(1):193–202
- Orhan E, Esitken A, Ercisli S, Turan M, Sahin F (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Sci Hortic* 111:38–43
- Patel GS, Dubey M, Chandel G (2015) Characterization of metal homeostasis related rice gene orthologs in nutrient rich minor millets. *Int J Plant Anim Environ Sci* 5(4):14–23
- Patil SS, Mali MG, Tamboli MS, Patil DR, Kulkarni MV, Yoon H, Kale BB (2016) Green approach for hierarchical nanostructured Ag-ZnO and their photocatalytic performance under sunlight. *Catal Today* 260:126–134
- Paul S, Ali N, Datta SK, Datta K (2014) Development of an iron-enriched high yielding *indica* rice cultivar by introgression of a high-iron trait from transgenic iron-biofortified rice. *Plant Foods Hum Nutr* 69:203–208
- Peleg Z, Saranga Y, Yazici A, Fahima T, Ozturk L, Cakmak I (2008) Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. *Plant Soil* 306(1–2):57–67
- Peng Y, Hu Y, Mao B, Xiang H, Shao Y, Pan Y, Zhang G (2016) Genetic analysis for rice grain quality traits in the YVB stable variant line using RAD-seq. *Mol Genet Genom* 291(1):297–307
- Pereira SI, Castro PM (2014) Phosphate-solubilizing rhizobacteria enhance *Zea mays* growth in agricultural P-deficient soils. *Ecol Eng* 73:526–535
- Perez-de-Castro AM, Vilanova S, Cañizares J, Pascual L, Blanca JM, Díez M, Picó B (2012) Application of genomic tools in plant breeding. *Curr Genom* 13(3):179–195
- Rajala A, Peltonen-Sainio P, Jalli M, Jauhiainen L, Hannukkala A, Tenhola-Roininen T, Manninen O (2017) One century of Nordic barley breeding: nitrogen use efficiency, agronomic traits and genetic diversity. *J Agric Sci* 155(4):582–598
- Rajendran SRCK, Yau Y, Pandey D, Kumar A (2015) CRISPR-Cas9 Based Genome Engineering: opportunities in Agri-Food-Nutrition and Healthcare. *OMICS* 19(5):1–16
- Ramzani PMA, Khalid M, Naveed M, Ahmad R, Shahid M (2016) Integrating the organic amendment with iron fertilization for improving productivity and Fe biofortification in rice under acidified calcareous soil. *Pakistan J Agric Sci* 53(2):16
- Rhodes DH, Hoffmann L Jr, Rooney WL, Ramu P, Morris GP, Kresovich S (2014) Genome-wide association study of grain polyphenol concentrations in global sorghum [*Sorghum bicolor* (L.) Moench] germplasm. *J Agric Food Chem* 62:10916–10927
- Rodrigues ML, Nakayasu ES, Oliveira DL, Nimrichter L, Nosanchuk JD, Almeida IC, Casadevall A (2008) Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. *Eukaryot Cell* 7(1):58–67
- Rojas-Tapias DF, Bonilla R, Dussán J (2014) Effect of inoculation and co-inoculation of *Acinetobacter* sp. RG30 and *Pseudomonas putida* GN04 on growth, fitness, and copper accumulation of maize (*Zea mays*). *Water Air Soil Pollut* 225(12): 1–13
- Ruel MT, Alderman H (2013) Maternal and Child Nutrition Study Group. Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition. *lancet* 382(9891):536–551
- Ruggieri M, Polizzi A, Schepis C, Morano M, Strano S, Belfiore G, David E (2016) Cutis tricolor: a literature review and report of five new cases. *Quant Imaging Med Surg* 6(5):525
- Sahin F, Turan M (2014) The effect of Plant Growth Promoting Rhizobacter strain on wheat yield and quality parameters, Paper at: IFOAM Organic World Congress, 2014, Istanbul, Turkey, 13–15 October, 2014.
- Sathya A, Vijayabharathi R, Srinivas V, Gopalakrishnan S (2016) Plant growth-promoting actinobacteria on chickpea seed mineral density: an upcoming complementary tool for sustainable biofortification strategy. *3 Biotech* 6(2):138
- Schaart JG, van de Wiel CCM, Lotz LAP, Smulders MJM (2016) Opportunities for products of new plant breeding techniques. *Trends Plant Sci* 21:438–449
- Sestili F, Janni M, Doherty A, Botticella E, D'Ovidio R, Masci S, Lafiandra D (2010) Increasing the amylose content of durum wheat through silencing of the SBEIIa genes. *BMC Plant Biol* 10(1):144
- Shaikh S, Saraf M (2017) Biofortification of *Triticum aestivum* through the inoculation of zinc solubilizing plant growth promoting rhizobacteria in field experiment. *Biocatal Agric Biotechnol* 9:120–126
- Shakeel M, Rais A, Hassan MN, Hafeez FY (2015) Root associated bacillus sp. improves growth, yield and zinc translocation for basmati rice (*Oryza sativa*) varieties. *Front Microbiol* 6: 1286
- Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL et al (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat Biotechnol* 31:686–688
- Sharma SK, Johri BN, Ramesh A, Joshi OP, Prasad SS (2011) Selection of plant growth-promoting *Pseudomonas* spp. that enhanced productivity of soybean-wheat cropping system in central India. *J Microbiol Biotechnol* 21(11):1127–1142
- Sharma D, Jamra G, Singh UM, Sood S, Kumar A (2017) Calcium biofortification: three pronged molecular approaches for dissecting complex trait of calcium nutrition in finger millet (*Eleusine coracana*) for devising strategies of enrichment of food crops. *Front Plant Sci* 7:2028
- Shi Y, Whetstone JR (2007) Dynamic regulation of histone lysine methylation by demethylases. *Mol Cell* 25(1):1–14
- Shivay YS, Prasad R (2012) Zinc-coated urea improves productivity and quality of basmati rice (*Oryza sativa* L.) under zinc stress condition. *J Plant Nutr* 35(6):928–951
- Singh JS (2013) Plant growth promoting rhizobacteria- potential microbes for sustainable agriculture. *Resonance* 18:275–281
- Singh UM, Chandra M, Shankhdhar SC, Kumar A (2014) Transcriptome Wide Identification and Validation of Calcium Sensor Gene Family in the Developing Spikes of Finger Millet Genotypes for Elucidating Its Role in Grain Calcium Accumulation. *PLoS One* 9(8):e103963

- Sobariu DL, Fertu DIT, Diaconu M, Pavel LV, Hlihor RM, Draçgoi EN et al (2016) Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. *New Biotechnol* 39:125–134
- Stover PJ (2006) Influence of human genetic variation on nutritional requirements. *Am J Clin Nutr* 83:436S–442S
- Szymanska S, Płociniczak T, Piotrowska-Seget Z, Złoch M, Ruppel S, Hryniewicz K (2016) Metabolic potential and community structure of endophytic and rhizosphere bacteria associated with the roots of the halophyte *Aster tripolium* L. *Microbiol Res* 182(2):68–79
- Tariq A, Anjum SA, Randhawa MA, Ullah E, Naeem M, Qamar R, Ashraf U, Nadeem M (2014) Influence of zinc nutrition on growth and yield behaviour of maize (*Zea mays* L.) hybrids. *Am J Plant Sci* 5:2646–2654
- Trijatmiko KR, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines FM et al (2016) Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Sci Rep* 6:19792
- Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314(5803):1298–1301
- Van K, Onoda S, Kim MY, Kim KD, Lee SH (2008) Allelic variation of the Waxy gene in foxtail millet [*Setaria italica* (L.) P. Beauv.] by single nucleotide polymorphisms. *Mol Genet Genomics* 279(3):255–266
- Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq Boyce A (2016) Role of plant growth promoting rhizobacteria in agricultural sustainability a review. *Molecules* 21(5):573
- 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 Soil* 411(1–2):81–99
- Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security. *Front Plant Sci* 8:29
- Voytas DF, Gao C (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. *PLoS Biol* 12(6):e1001877
- Wakasa K, Hasegawa H, Nemoto H, Matsuda F, Miyazawa H, Tozawa Y, Miyagawa H (2006) High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. *J Exp Bot* 57(12):3069–3078
- Walker V, Bertrand C, Bellvert F, Moëne-Loccoz Y, Bally R, Comte G (2011) Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytol* 189(2):494–506
- Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat Biotechnol* 32:947–951
- Wani PA, Khan MS, Zaidi A (2007) Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. *J Plant Nutr Soil Sci* 170(2):283–287
- Wani PA, Khan MS, Zaidi A (2008) Chromium-reducing and plant growth-promoting *Mesorhizobium* improves chickpea growth in chromium-amended soil. *Biotech Lett* 30(1):159–163
- Wu SC, Cao ZH, Lib ZG, Cheung KC, Wong MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* 125:155–166
- Xiaoyan S, Yan Z, Shubin W (2012) Improvement Fe content of wheat (*Triticum aestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. *J Agric Biotechnol* 20(7):766–773
- Yamaya T, Obara M, Nakajima H, Sasaki S, Hayakawa T, Sato T (2002) Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. *J Exp Bot* 53(370):917–925
- Yu J, Peng P, Zhang X, Zhao Q, Zhu D, Sun X, Ao G (2005) Seed-specific expression of the lysine-rich protein gene sb401 significantly increases both lysine and total protein content in maize seeds. *Mol Breed* 14:1–7
- Zahir ZA, Shah MK, Naveed M, Akhter MJ (2010) Substrate-dependent auxin production by *Rhizobium phaseoli* improves the growth and yield of *Vigna radiata* L., under salt stress conditions. *J Microbiol Biotechnol* 20(9):1288–1294
- Zhang K, Raboanatahiry N, Zhu B, Li M (2017) Progress in genome editing technology and its application in plants. *Front Plant Sci* 8:177
- Zhu S, Vivanco JM, Manter DK (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. *Appl Soil Ecol* 107:324–333