Bio fortification: A sustainable approach to mitigate the problem of malnutrition

[1]Abbadi Aruna Prabha, [2]Harshal Avinashe and [3]Nidhi Dubey

[1]M.Sc. Student, Department of Genetics and Plant Breeding, Lovely Professional University, Phagwara (Punjab), India 144411

[2&3]Assistant Professor, Department of Genetics and Plant Breeding, Lovely Professional University, Phagwara (Punjab), India 144411

Abstract

Recent time plant breeding has been oriented towards achieving high agronomic yieldsrather than nutritional quality. So, efforts like fortification and pharmaceutical supplementation are being made to improve the concentration of essential elements in edible portions of crops by various agronomic methods or by genetic selection to reduce the problems of malnutrition. Due to poor economic conditions, all people can't follow a variety of nutrient rich diet. For this purpose, this biofortification of staple crops like rice and wheat had much scope in solving the problems of malnutrition.Here I'm providing a brief study on methods of biofortification which include conventional breeding, agronomy and genetic modification. Biofortification is a cost effective and sustainable process of improving the concentration of essential elements in crop plants.Work of biofortification on staple crops is being done under the Consultative Group on International Agricultural Research and many International & National initiatives (Harvestplus). In this essay,biofortification methods, their approaches in rice, wheat shall be pictured. The problems involved and coming prospects of biofortification will also be discussed.

Keywords: Biofortification, malnutrition, rice, iron, zinc, wheat, challenges, future prospects

Introduction

"Biofortification" or "biological fortification" is a method for enhancing food crops nutritionally there by availing them to human needs by producing them using improved biotechnological methods, conventional approaches of breeding and agronomic practices. As per the records of the United Nations Food and Agriculture Organization about 792.5 m are malnourished throughout the world (1).Concentrating more in providing enough amount of nutritious food than on higher yields would support in fighting "hidden hunger" or "micronutrient malnutrition" countries that are poor, whose nutrient sources are staple crops rice and wheat (2). There will be threat for food security with changing climatic conditions and rapid growth in population rate in developing countries (3, 4).About 40 nutrients are needed for healthy human (Table 1). Essential nutrients which are Na, K, Ca, Mg, P, Cl & Sare needed for humans in little quantities.

Necessity for biofortification research:

Table 1: Micro and macro nutrients which are essential for human health

Micronutrients		Macronutrients		
Micro-minerals	**Vitamins**	**Amino acids (essential)**	**Fatty acids (essential)**	**Macro-minerals**
Fe	A (Retinol)	Histidine	Linoleic acid	K
Zn	D (Calciferol)	Isoleucine	Linolenic acid	Ca
Cu	E (α-Tocopherol)	Leucine		Mg
Mn	K (Phylloquinone)	Lysine		S
I	C (Ascorbic acid)	Methionine		P
Se	B_1 (Thiamin)	Phenylalanine		Na
Mo	B_2 (Riboflavin)	Threonine		Cl
Co	B_3 (Niacin)	Tryptophan		
Ni	B_5 (Pantothenic acid)	Valine		
	B_6 (Pyridoxine)			
	B_7 (Biotin)			
	B_9 (Folic acid, folacin)			
	B_2 (Cobalamin)			

Approachesof biofortification:

Crop plants are biofortified by three main methods which are genetic modification, traditional breeding and agronomic in addition to usage of biotechnology, breeding and fertilization. Transgenic, conventional breeding and agronomical biofortified approaches are done in staple crops like paddy, wheat, corn, jowar, lupine, common bean, potato and tomato (Figure 1). In banana, cauliflower and tapioca biofortification is by both genetic modification and by breeding approaches while in hordeum, soyabean, and rape seed it is transgenic and agronomic approach. Biofortification is aimed at producing healthy nutrient rich foods in adequate amounts sustainably.

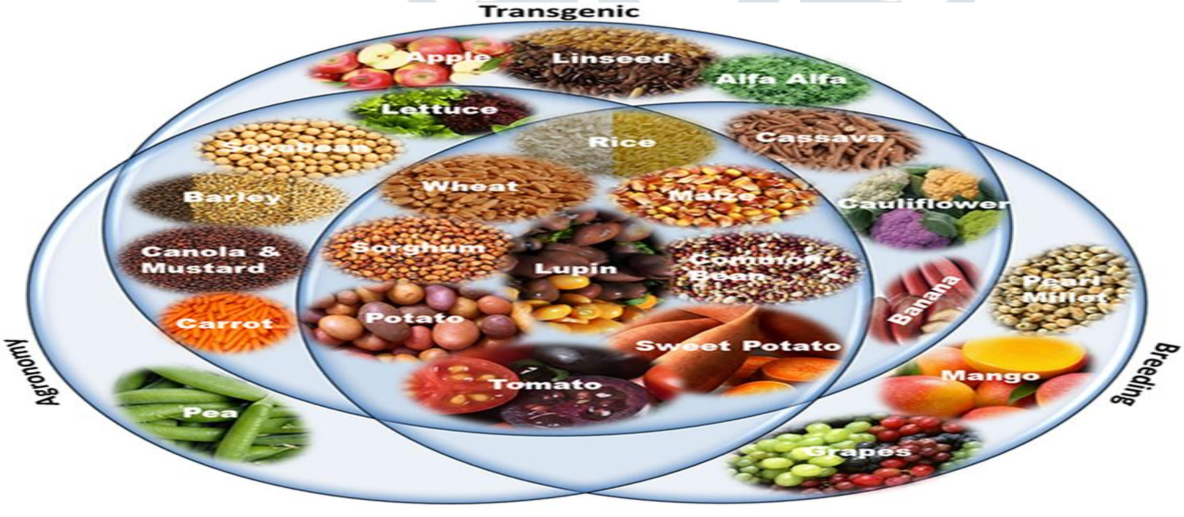


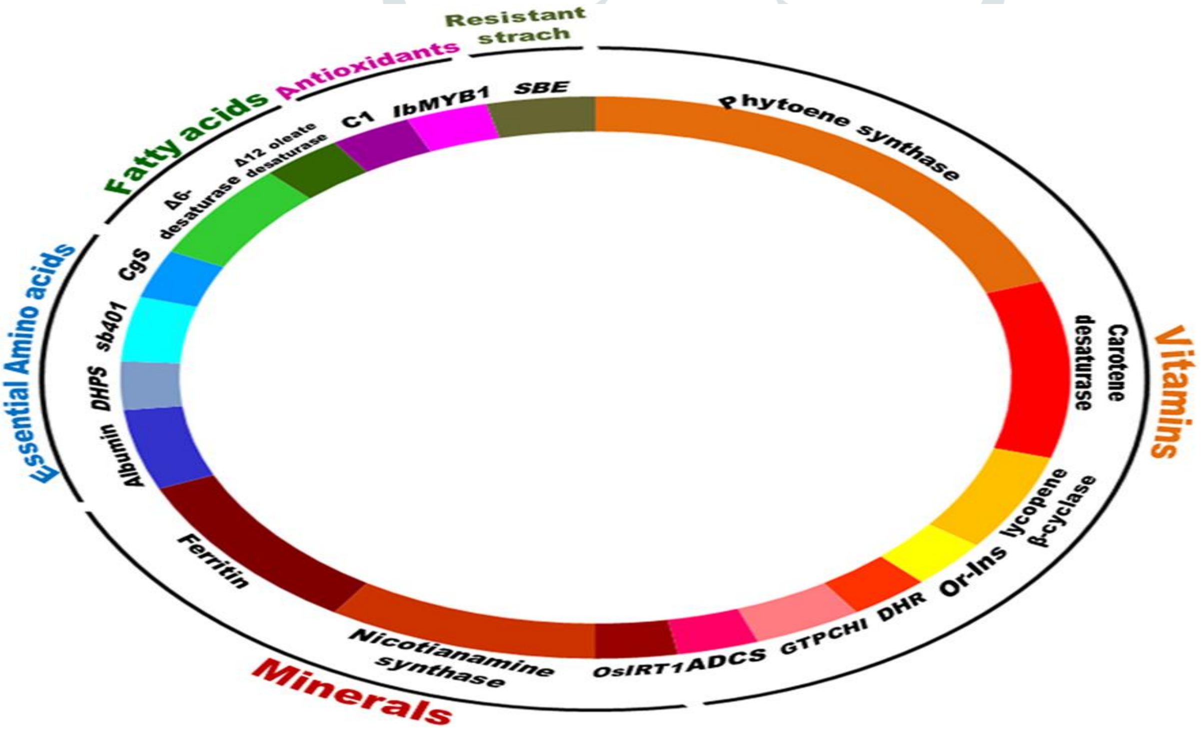
Figure (1): Biofortification methods and crops developed by agronomic, genetic modification and crop improvement approaches

All three methods of biofortification have been used in vegetables, beans, and fruits.

Biofortification through transgenic means:

If a target gene was found it can be used in multiple crops. Transferase enzyme Phytoene synthase family (PSY), carotene desaturase, nicotinamidemono nucleotide synthase, and ferritin (iron storage protein) are some genes that have been utilized in many crops. Conventional breeding works only if there is some genetic variation in the nutrient content among gene pool instead transgenic approach is valid(6-9).It depends upon the availability of the unlimited genetic pool for the integrating desirable geneof a plant into another.Any micro nutrient if is absent in crops then it can only be fortified into those crops by genetic modification of that crop (10).

If micronutrients are available within the plant but are not directed towards the economic product then also transgenic approaches can be used by incorporating genes for increasing the amount of essential micro nutrients and by reducing the anti nutrients (phytic acid)that reduce the bioavailability of plant nutrients. Also, genetic modifications spreads micronutrients among body tissues;increasing the efficiency of metabolic reactions in edible tissues (11-13). PSY, carotene desaturase, and lycopene β-cyclase increases vitamins, iron storing protein (ferritin) and nicotinamine mono nucleotide synthase for minerals, essential amino acids by albumin, and delta sixdesaturase for essential fatty acids have been identified as marked sites for biofortification. Crops fortified by transgenic method are lysine rich maize, unsaturated fatty acid soybean, provitamin A and iron rich cassava, and provitamin Arich Golden rice.

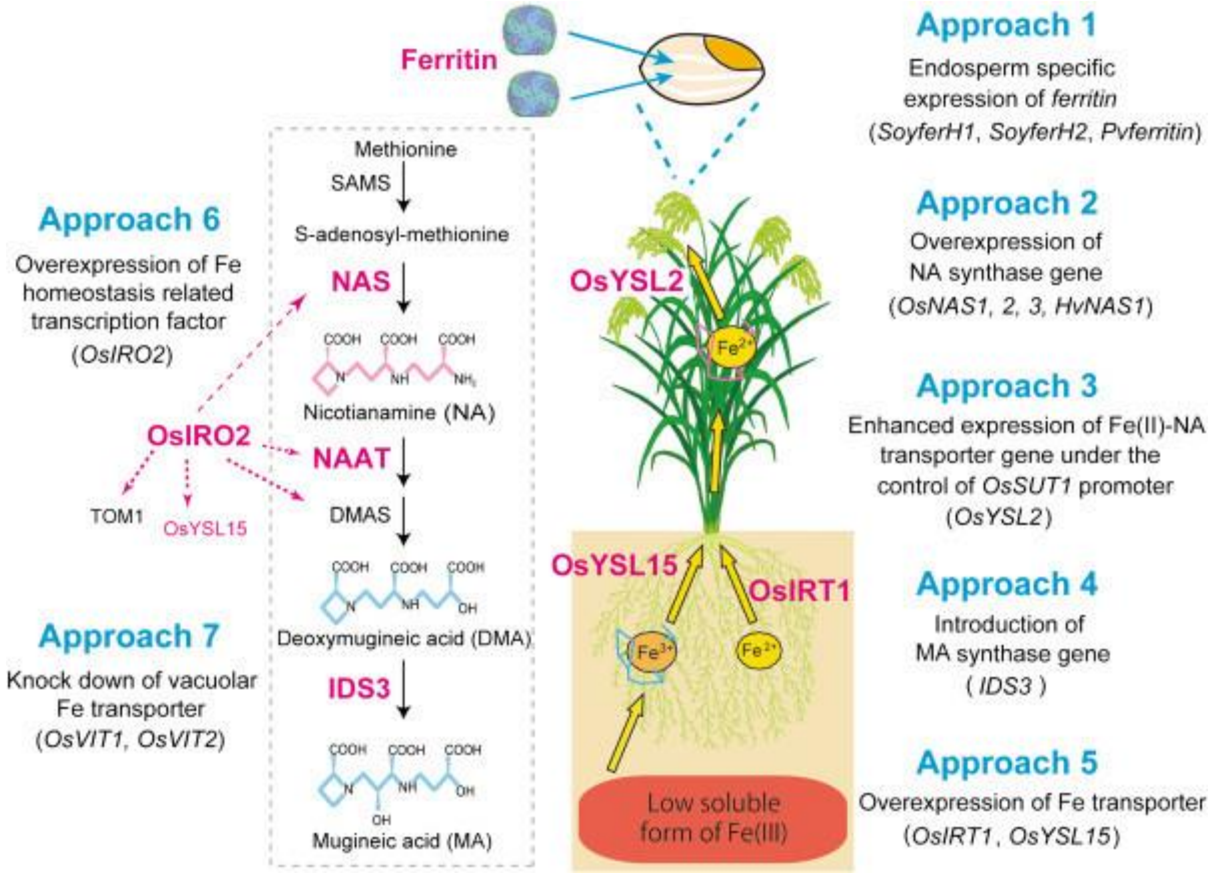


Figure(2): crop biofortification is done using many genes under transgenic approach and if once a useful gene got discovered can be used in fortifying multiple crops.

Transgenic Cereals:

Rice (*Oryza sativa*)

Inappropriate amount of vitamin intake through diet is a major problem and rice has been taken to improve its nutrient status and as a staple food crop it can reduce the problem of under nutrition.



Figure(3): Transgenic approach in Biofortification of rice

With an abundant source of provitamin A (beta-carotene) Golden Rice was a great achievement with marked potential to provide disease fighting ability by expressing genes encoding phosphatase synthetase family and desaturases of carotene (14-18). Rice being changed its genetic makeup increased the concentration upto 150-folds by over expressing genes expressing Arabidopsis GTP-cyclohydrolaseI (GTPCHI) and amino deoxychorismate synthase [ADCS(19,20)]. For an adult in a day 100g of fortified rice with folate is found to be enough. Genes modified for increasing iron content in rice are nicotianamine amino transferase (21), iron transporter OsIRT1 (22). Multiple genes involvement can also biofortify rice with increased iron content(23-25). Zinc in rice is increased by over expressing OsIRT1 (26) and genes which result in synthesis of mugineic acid in barley [HvNAS1, HvNAAT-A, HvNAAT-B (27)]. To reduce bad cholesterol in the body the quality of oil in grains of rice can be improved by increase in polyunsaturated fatty acids (28).

Transgenic Wheat:

Most popular staple food crop grown in our nation is Wheat (*Triticum aestivum*L.). To meet the challenges of malnutrition due to poor vitA, ferrous as well as proteins in wheat research have been increased by expressing genes from bacterial genome PSY, carotene desaturase (CrtI) (29,30). For fortifying wheat with iron expression of iron storage gene of soybean i.e ferritin (31)and in wheat[TaFer1-A (32)]. Contentof protein by increasing amino acids that are essential namely Met, tyr, lys and cys. Using amaranthus albumin gene i.eama1, concentration of wheat grains were enhanced (33). Gene silencing served the purpose of masking the overnutrition and obesity by improving the quality of slow digesting amylase starch by expressing SBE [SBEIIa (34)].

Biofortification through Agronomic Approach:

In order to enhance the human health, crops nutrient status should be improved. This can be achieved temporarily by providing nutrients externally by various agronomic methods (35). Increase in the solubilization and application of fertilizers rich in minerals mobilization of nutrients from soil to plant parts. N, P, K supplements led to Green Revolution and saved livesfrom death of hunger in 1960s. Micro nutrients Zn, Fe, Mn, Se, Co, Mo and Ni are available in different amountsin plants which will be taken from the soil when soil micronutrient status is improved which would help meet sufficient nutrition to human diet (36).Apart from micronutrients, plant growth promoting micro organisms which are Rhizobium, Azotobacter, Bacillus, Pseudomonas in soil would enhance nutrient transport to economic part of plant.In nitrogen deficient soils application of N_2 would enhance the productivity (37). Mycorrhizal fungi releases organic acids, enzymes, side rophores which are able to degrade organic matter and build the mineral nutrients quantities in crop produce (38-41).

Cereals:

In order to avoid iron and zinc deficiency, biofortification of rice plants is done by spraying of iron which helps to increase the amount of iron in rice grains. Similarly, treating germinating seedlings with ferrous sulfate helps improve iron in unpolished rice up to 15.6 times to that of control (42). Zinc provision by spraying as well as in fertilizer form improves zinc amount in rice grain.

Wheat

Foliar urea fertilizers improve inclusion of iron resulted inmore iron accumulation (43). Foliar spray of zinc also minimize the antinutritional elements like phytic acid (44).

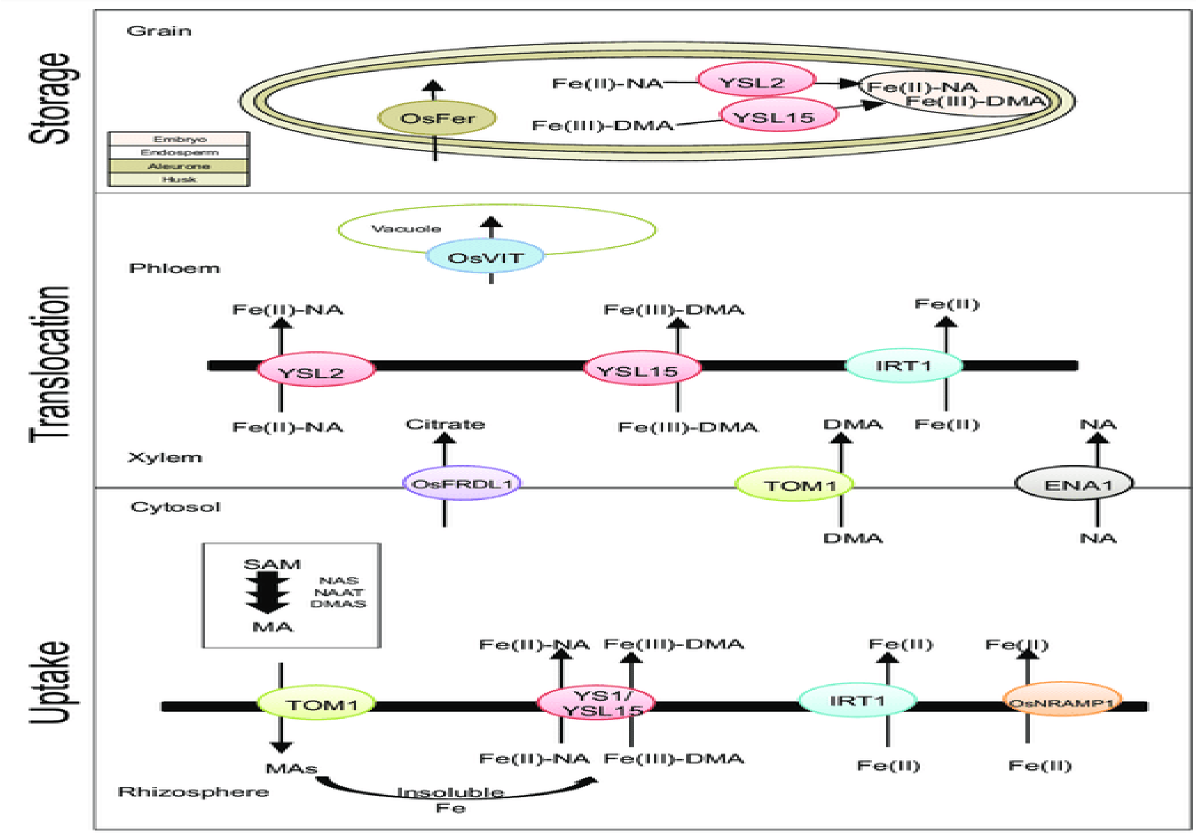


Figure (4): Basic scheme of iron uptake, translocation and storage in rice

Biofertilizers helps in enhancing the yield of grains and fertilizers containing mycorrhizal fungi are extensively being used for biofortification. Iron biofortification in wheat is done by using organic as well as chemical fertilizers and through usage of bacterium Bacillus aryabhattai (45,46).

Biofortification through Conventional Breeding

Conventional breeding method of biofortification is mostly accepted method as it a sustainable, less costly and is used in place of transgenic- and agronomic-based approach. Traditionalplant breeding involves combining parent lines with high nutrient lines and the line that receives having required agronomic traits with good heritability till plants with desired nutrient status and agronomic characters aredeveloped. There should be sufficient genotypic diversity in the character that is being selected, this helps increase vitamin and minerals among crops. If there is limited genetic variation within the genetic material then by crossing with distant relatives of variety can mobilize the trait into the present growing cultivar, also by mutagenesis new traits can be introduced into them. A project named Health gain(2005–2010) in 15 countries with 44 partners has been donein the European Union to spread health promoting and safe cereal foods of high eating quality. The Harvest plus program launched by (CGIAR), the international tropical agriculture center and CIAT, the institute of food policyto produce biofortified staple food crops through breeding approach boosting major essential nutrients –vitamin A, Iron, Zinc incommon food crops in Asia and Africa(47).

Cereals:

Rice

Being most consumed common food crop, its biofortification shall meet the challenge of malnutrition. screening of various traditional rice varieties rich in ferrous and Zn content along with good mineral base are added with better agronomic traits using breeding methods, the harvest plus in 2013 developed world's first zinc rich rice varieties by the Bangladesh Rice Research Institute (BRRIdhan 62, BRRIdhan 64, and BRRIdhan 72), which contains 20–22 ppm zinc in brown rice. A traditional variety Jalmagna showing twice the concentration of iron than in original rice variety and 40% more Zn concentration has been identified and used for rice improvement with increasediron and zinc concentration through various crop improvement approaches(48).

Wheat

The first most aim of biofortification is to breed wheat as it the staple food. There is greater variation in ferrous and Zn concentrations of grain in wheat,its near wild relatives.Through this variation HarvestPlusreleased many Zn rich varieties of wheat (BHU 1, BHU 3, BHU 5, BHU 6, BHU 7, and BHU 18) in 2014 in Indiaand in Pakistan in 2015 four varities (NR 419, 42, 421, and Zincol) were developed. Punjab Agricultural University, Indiareleased another variety with high zinc (PBWIZn). High Zn & iron content variety (WB2) has been developed and released by Indian Institute of Wheat and Barley Research, India. Variety (HI 8627)rich in Provit-A has been released by (IARI),India in 2005. Antioxidantproperties increased by anthocyanins has been an important research in wheat. Black colored wheat cultivar took twenty years of trail in breeding and was released in China; it has high protein and selenium content(49). In India purple,white, black, blue lines have been registered in 2017 (50).

Constraints for Biofortification

Constraints in Agronomic Biofortification:

1. Though biofortification through agronomic measures is easy approach but due to varied mechanisms of translocation of minerals, their building up in plant species and various types of soils in different crop regions (51) the results of agronomic biofortification are not constant.
2. As this method does not involve targeting particular plant parts, the accumulation of desired nutrients may occur in the leaves or other least important portions of plants. Hence, the success of thistechnique is limited to some minerals and particular plant species.
3. The threat is that accumulation of fertilizers in soil and water causes harmful effects in environment (52). There is risk of toxicity if these fertilizers are being added continuously. Ferric and ferrous ions get accumulated due to excessive intake of ferrous, this catalyst thereby forms noxious reactive oxygen species (ROS). ROS are strong oxidizing agents that effect DNA, proteins, and lipids in

plants. Therefore, fertilization strategies should be devised and optimized to ensure adequate supply of iron for proper growth of agronomic plants while minimizing accumulation of iron.

Constraints in Conventional Breeding Methods

1. The problem is with the availability of genetic diversity for micro nutrients in the genetic makeup and the time period for developing varieties with required trait(s) which can be overcome by introgressionof traits into present cultivating varitiesbut conventional breeding makes is difficult to incorporate specific trait within time.
2. Though transgenic methods have overcome the limitations of conventional breeding approach yet there is a problem in acceptance of transgenic plants in masses.
3. The current political and economic strategies are not supportive in addition to the time killing regulatory processes which are very expensive, seen in case of genetically modified Brinjal (Bt), 1st developed by Mahyco in Indian. But there was a lot of unacceptance from farmers, few scientists and activists against GMO who didn't accept the release in India until tested thoroughly.
4. Even after 8 years of research on golden rice from the scientists being published details of the Golden rice in Science in 2000, also International Rice Research Institute scientists working on it and still it is not ready for farmers because of lack of permission from government authority.

Challenges and future prospect

To calm down public anxiety and in order to gain acceptance, there is a need to conduct research on consumption of biofortified crops and their effect on human health. Recent technological advances like zinc-finger nucleases, CRISPR-Cas9 and TALENs can be used for genetically manipulating the crops for biofortification without affecting genome of whole plant (53-55). Though reduction in concentration of phytic acid increased iron absorption, alternative strategies have to be found without losing beneficial actions of phytic acids and polyphenols. Ultimately, biofortification would help to improve the efficiency of uptake of nutrients, their translocation and storage in plant parts by increasing the ability of storage parts to gather minerals in a form that does not affect growth and development but are available for humans biologically. Also to minimize the level of antinutritional compounds such as phytic acid, which interferes with the absorption of minerals in the gut.

Conclusion

From the above study, it is revealed that biofortification is a sustainable, comparatively cheaper agricultural strategy which helps in improving the quality of food thereby reducing malnourishment problems in world. Biofortification hold great importance for addressing human malnutrition problems due to lack of required mineral nutrients in food. The nutrients that are lacking in the diets of humans both in poor and rich countries is met by biofortifying the staple crops with ferrous, Zn, Se and Pro vita A contents. National and international programs like Harvest plus contribute to the success of biofortification approach. There is a need for association of crop researchers, nutrition scientists and engineers in genetics and scientists in molecular biology to achieve biofortification. Among different costly and time killing regulatory approval processes for accepting transgenic approaches, breeding methods are found easy and acceptable to improve or producenutritious food. Ultimately, these biofortified crops have been found to have great importance in developing countries where micronutrient malnutrition is quite common and there is need to increase the potentiality of biofortification in future.

References

1. McGuire S. (2015). FAO, IFAD, and WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. *Rome: FAO. Adv Nutr* 6(5):623–4.
2. Khush GS, Lee S, Cho JI, Jeon JS. (2012). Biofortification of crops for reducing malnutrition. *Plant Biotechnol Rep* 6:195–202.

3. Bazuin S, Azadi H, Witlox F. (2011). Application of GM crops in Sub-Saharan Africa: lessons learned from green revolution. *Biotechnol Adv* 29:908–12.
4. Das JK, Kumar R, Salam RA, Bhutta ZA. (2013). Systematic review of zinc fortification trials. *Ann Nutr Metab* 62(1):44–56.
5. Perez-Massot E, Banakar R, Gomez-Galera S, Zorrilla-Lopez U, Sanahuja G, Arjo G. (2013). The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes Nutr* (2013) 8(1):29–41.
6. Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P. (2007). Transgenic strategies for the nutritional enhancement of plants. *Trends Plant Sci* (2007) 12:548–55.
7. Welch RM, Graham RD. (1999). A new paradigm for world agriculture: meeting human needs-productive, sustainable nutritious. *Field Crops Res* 60:1–10.
8. Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y. (2014). Biofortification: progress toward a more nourishing future. *Glob Food Secur* 2(1):9–17.
9. Brinch-Pedersen H, Borg S, Tauris B, Holm PB. (2007). Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J Cereal Sci* 46:308–26.
10. Perez-Massot E, Banakar R, Gomez-Galera S, Zorrilla-Lopez U, Sanahuja G, Arjo G. (2013). The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes Nutr* 8(1):29–41.
11. Shewmaker CK, Sheehu JA, Daley M, Colburn S, Ke DY. (1999). Seed-specific overexpression of phytoene synthase: increase in carotenoids and metabolic effects. *Plant J* (1999) 20:41–412.
12. Agrawal PK, Kohli A, Twyman RM, Christou P. (2005). Transformation of plants with multiple cassettes generates simple transgene integration patterns and high expression levels. *Mol Breed* (2005) 16:247–60.
13. Yang SH, Moran DL, Jia HW, Bicar EH, Lee M, Scott MP. (2002). Expression of a synthetic porcine alpha-lactalbumin gene in the kernels of transgenic maize. *Transgenic Res* (2002) 11:11–20.
14. Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P. (2000). Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoids-free) rice endosperm. *Science* 287:303–5.
15. Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R. (2002). Golden rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *J Nutr* 132(3):506S–10S.
16. Datta K, Baisakh N, Oliva N, Torrizo L, Abrigo E, Tan J. (2003). Bioengineered ‘golden’ Indica rice cultivars with beta-carotene metabolism in the endosperm with hygromycin and mannose selection systems. *Plant Biotechnol* J 1:81–90.
17. Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G. (2005). Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nat Biotechnol* 23:482–7.
18. Burkhardt PK, Beyer P, Wuenn J, Kloeti A, Armstrong GA, Schledz M. (1997). Transgenic rice (Oryza sativa) endosperm expressing daffodil (Narcissus pseudonarcissus) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *Plant J* 11:1071–8.
19. Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF. (2007). Folate fortification of rice by metabolic engineering. *Nat Biotechnol* 25(11):1277–9.
20. Blancquaert D, Van daele J, Strobbe S, Kiekens F, Storozhenko S, De Steur H. (2015). Improving folate (vitamin B9) stability in biofortified rice through metabolic engineering, *Nat Biotechnol* 33:1076–8.
21. Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S. (2001). Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat Biotechnol* 19:466–9.
22. Lee S, An G. (2009). Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. *Plant Cell Environ* 32:408–16..

23. Wirth J, Poletti S, Aeschlimann B, Yakandawala N, Drosse B, Osorio S. (2009). Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. Plant Biotechnol J ,7:631–44.
24. Masuda H, Ishimaru Y, Aung MS, Kobayashi T, Kakei Y, Takahashi M. (2012). Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Sci Rep* 2:534.
25. Masuda H, Kobayashi T, Ishimaru Y, Takahashi M, Aung MS, Nakanishi H. (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.
26. Lee S, An G. (2009). Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. *Plant Cell Environ* 32:408–16.
27. Masuda H, Suzuki M, Morikawa KC, Kobayashi T, Nakanishi H, Takahashi M. (2008). Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. *Rice* 1:100–8.
28. Crawford M, Galli C, Visioli F, Renaud S, Simopoulos AP, Spector AA. (2000). Role of plant-derived omega-3 fatty acids in human nutrition. Ann *Nutr Metab* 44:263–5.
29. Wang C, Zeng J, Li Y, Hu W, Chen L, Miao Y. (2014). Enrichment of provitamin A content in wheat (Triticum aestivum L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI. *J Exp Bot* 65(9):2545–56.
30. Cong L, Wang C, Chen L, Liu H, Yang G, He G. (2009). Expression of phytoene synthase1 and carotene desaturase crtI genes result in an increase in the total carotenoids content in transgenic elite wheat (Triticum aestivum L.). J Agric Food Chem 57(18):8652–60.
31. 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* 20:766–73.
32. Borg S, Brinch-Pedersen H, Tauris B, Madsen LH, Darbani B, Noeparvar S. (2012). Wheat ferritins: improving the iron content of the wheat grain. *J Cereal Sci* 56:204–13.
33. Tamas C, Kisgyorgy BN, Rakszegi M, Wilkinson MD, Yang MS, Lang L. (2009). Transgenic approach to improve wheat (Triticum aestivum L.) nutritional quality. *Plant Cell Rep* 28(7):1085–94.
34. Sestili F, Janni M, Doherty A, Botticella E, D'Ovidio R, Masci S. (2010). Increasing the amylose content of durum wheat through silencing of the SBEIIa genes. *BMC Plant Biol* 10:144.
35. Cakmak I, Kutman UB. (2017). Agronomic biofortification of cereals with zinc: a review. *Eur J Soil Sci* 69:172–80.
36. Cakmak I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification. *Plant Soil* 302:1–17.
37. Hardarson G, Broughton WJ. (2004). Maximising the use of biological nitrogen fixation in agriculture. *Ann Bot* (2004) 93(4):477.
38. Rengel Z, Batten GD, Crowley DE. (1999). Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Res* 60:27–40.
39. Hardarson G, Broughton WJ. (2004). Maximising the use of biological nitrogen fixation in agriculture. *Ann Bot* 93(4):477.
40. Cavagnaro TR. (2008). The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. *Plant Soil* 304:315–25.
41. He W, Shohag MJ, Wei Y, Feng Y, Yang X. (2013). Iron concentration, bioavailability, and nutritional quality of polished rice affected by different forms of foliar iron fertilizer. *Food Chem* 141(4):4122–6.
42. Yuan L, Wu L, Yang C, Quin LV. (2013). Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *J Sci Food Agric* 93(2):254–61.
43. Cakmak I. (2010). Biofortification of cereals with zinc and iron through fertilization strategy. In *19th World Congress of Soil Science, Soil Solutions for a Changing World* Vol. 5. p. 1–6.
44. Yang XW, Tian XH, Lu XC, Cao YX, Chen ZH. (2011). Impacts of phosphorus and zinc levels on phosphorus and zinc nutrition and phytic acid concentration in wheat (Triticum aestivum L.),*J Sci Food Agric* 91(13):2322–8.

45. Ramzani PMA, Khalid M, Naveed M, Ahmad R, Shahid M. (2016). Iron biofortification of wheat grains through integrated use of organic and chemical fertilizers in pH affected calcareous soil. *Plant Physiol Biochem* 104:284–93.
46. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. (2014). Inoculation of zinc solubilizing Bacillus aryabhattai strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in vertisols of central India. *Appl Soil Ecol* 73:87–96.
47. Bouis HE, Welch RM. (2010). Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci* 50:S20–32.
48. Gregorio GB, Senadhira D, Htut H, Graham RD. (2000). Breeding for trace mineral density in rice. *Food Nutr Bull* 21:382–6.
49. Li W, Beta T, Sun S, Corke H. (2006). Protein characteristics of Chinese black-grained wheat. *Food Chem* 98:463–72. doi.
50. Garg M, Chawla M, Chunduri V, Kumar R, Sharma S, Sharma NK. (2016). Transfer of grain colors to elite wheat cultivars and their characterization. *J Cereal Sci* 71:138–44.
51. Ismail AM, Heuer S, Thomson MJ, Wissuwa M. (2007). Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Soil* 65:547–70.
52. Waters BM, Sankaran RP. (2011). Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective. *Plant Sci* 180(4):562–74.
53. Abdallah NA, Prakash CS, McHughen AG. (2015). Genome editing for crop improvement: Challenges and opportunities. *GM Crops Food* Oct; 6(4):183-205.
54. Gaj T, Gersbach CA, Barbas CF, III. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology* Jul; 31(7):397-405.
55. Yin H, Kauffman KJ, Anderson DG. (2017). Delivery technologies for genome editing. *Nature Reviews* 16(6):387-399.
56. Khush GS, Lee S, Cho JI, Jeon JS. (2012). Biofortification of crops for reducing malnutrition. *Plant Biotechnol* Rep 6:195–202.