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Biofortified staple food crops as a need of new era

Shakra Jamil[1*], Ayesha Naseem[2], Shamsa Kanwal[1], Sajid Ur Rahman[1], Rahil Shahzad[1], Muhammad Zaffar Iqbal[1]

[1] *Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan*

[2] *University of Agriculture, Faisalabad, Pakistan*

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Abstract

More than one billion people of the world have no direct access to the nutrient enriched food. So, biofortification is an upcoming feasible, cost effective and sustainable way of providing the essential nutrients to the poor people of the developing world by fortifying the staple food crops. In this review, different approaches of biofortification are discussed just to improve the already growing crops with the essential nutrients like iron, zinc, and vitamin A. These improved crops help to combat the micronutrient deficiencies that cause severe health issues like cognitive impairment, growth failure, and weak eye sight, weakened immune system and reduced productivity in humans. Biofortification approaches like transgenic, agronomic and breeding are being used to improve the nutrients in staple crops like Vitamin A in rice, Zinc in wheat, Iron in wheat and maize. Now more than 20 billion people of the developing world are using biofortified crops and improved their health status. Success stories of biofortification include the biofortification of wheat with lysine using transgenic approach, provitamin improved rice and tryptophan enriched maize. Emphasis is being lead on transgenic approach and its acceptability and adoptability among the farmers for biofortification of food crops. Besides all the challenges, biofortification still has bright future and the potential to feed the world with essential micronutrient enriched food.

* **Corresponding Author:** Dr Shamsa Kanwal ✉ Kanwalshamsa32@yahoo.com

Introduction

Access to the healthy food is the right of every human being. But the micronutrient deficiencies affect the health status of the population, especially in the developing countries (Stein, 2010). Micronutrient deficiencies are very common worldwide and almost 1 Billion people are undernourished due to the scarcity of food in the developing countries of the world (Approximately above 50% of the human population worldwide has no access to a healthy diet(Zhu *et al.*, 2007). One of the reasons of nutrient deficiencies is the soil fertility which produces poor quality of food that in return causes malnutrition in the human population (Nube *et al.*, 2011; Kumssa *et al.*, 2015). Such foods which are poor in nutrients are widespread all over the world (FAO *et al*, 2015; WHO, 2016). This major deficiency of the nutrients leads to many hidden diseases or health issues particularly in children and women (Black *et al.*, 2013). Over two billion people suffer from the deficiency of Zn, Fe and other required nutrients (Black, 2003). The situation is more severe in countries where people live with low income and the risk of nutrient deficiencies is very high like Zn (40%), and Fe (5%) (Muthayya *et al.*, 2013; Joy *et al.*, 2014). Severe human health problems, generally related to sub-optimal metabolic functioning slow down the defense mechanism leading to increased susceptibility to the infections, growth failure, cognitive impairment and, finally reduced productivity in humans (Welch and Graham, 1999; Cakmak *et al.*, 2008). Malnutrition related health issues now become the major hurdle in getting the Millennum Development Goals (MDG) like reduction of poverty, improvement of maternal health and reduced child mortality rate (White *et al*, 2005; White *et al.*, 2011; Wessells *et al.*, 2012).

However, it is estimated that over 60% and 30% people of the world are iron and zinc deficient respectively. Micronutrient deficiencies or “hidden hunger” affect about 38% of pregnant women and 43% of preschool children worldwide and this mostly happens in the developing countries. More than 30% population of world is anemic. The studies estimate that approximately half of this is due to iron-deficiency anemia (IDA). Iron deficient anemia causes serious health issues, including cognitive impairment in children, weak immune system and increased death rate. The major cause of stunted growth among children is zinc deficiency. Twenty two mineral elements which are required by the human body for the proper functioning (Takahashi *et al.*, 2001) can be provided through healthy food. Micronutrient malnutrition is a very serious and ongoing problem in the developing countries so to overcome this problem different intervention programs are introduced including food fortification, supplementation and dietary diversification (Stein, 2010). These programs are inexpensive and are enough to fulfill the nutrition requirements of the population of the developing countries. Now the mostly used strategy to deplete the problem of malnutrition is biofortification (Stein, 2010). The scientists come with the solution to ensure food security by improving the quality status of the crops through a process called biofortification (Nestel *et al.*, 2006). Biofortification is the process of improving the nutritional quality of the staple food crops through conventional plant breeding, agronomic practices and modern techniques by using biotechnology. Biofortification of the staple food crops for the delivery of micronutrients has been proposed to tackle the existing efforts for the alleviation of micronutrient deficiencies. The affect of biofortification on the nutritional status has rarely been tested but the application of mineral fertilizers like iron and zinc fertilizer has very significant effect on the people with malnutrition (Jiang *et al.*, 1997; Cakmak *et a.l*, 2004; Raymanetal., 2008). Two types of approaches mainly used in biofortification one is agronomic approach and the second one is transgenic approach. Agronomic approach helps in the optimization of the application of fertilizers and helps in the movement of minerals/nutrients in the soil. The second approach used is transgenic which aims at the development of crop having the ability to store minerals in their edible tissues of the plant. In transgenic approach, the concentration of different promoters such as β-carotene, ascorbate etc which enhance the uptake and absorption of different nutrients by gut. These promoters help in reduction of nutrients which are not helpful or anti-nutrients like phytates, oxalates etc that cause hurdle in the absorption of nutrients (Philip *et al.*, 2008). Biofortification through transgenic approach relies on improving the phyto-availability of essential micronutrients of the soil, uptake from the rhizosphere, their translocation to the plant parts like shoots and finally their accumulation in the edible portion of the plants (White *et al.*, 2008; Davies, 2007; Puig *et al.*, 2007; Zhu *et al.*, 2007). Biofortification differs from conventional fortification in that it aims to increase nutrient levels in crops during plant growth rather than through manual means (Nestel *et al.*, 2006). Biofortification may therefore present a way to reach populations where supplementation and conventional fortification activities may be difficult to implement. Biofortification emerges with great importance in different aspects of life like this strategy that directly targets the low income people by providing a good quality food in low price and improve the health status. After the one time investment, upcoming cost on its production is very low and germplasm can be shared internationally. Nutritionally improved varieties will continue to be grown and consumed year after year, even if government attention and international funding for micronutrient issues fade. Biofortification mainly provides the direct and easy access of better quality food to the unnourished people of remote areas who have no access to the markets or commercial areas (Nestel *et al.*, 2006).

Biofortified crop development

In plant breeding, the crop development process involve germplasm collection, screening of germplasm, Prebreeding of germplasm, testing and evaluation of that germplasm for particular nutrients for which it would be used in future without compromising the traits and yield. Markers which are cost effective and economic are developed to identify the traits. These markers are tested in different locations to test the Genotype × Environment interaction to check the effect of environment on the nutrients that are introduced in that crop(Bouis and Saltzman, 2017). In the very beginning of the concept of biofortification, the biotechnologist determined the losses of nutrients associated with the biofortification. The main target of biofortification is to sum up all the existing nutrients and the added nutrients in that crop and maintain the nutrient level in that crop (Bouisn and Saltzman, 2017) Table 1.

Table 1. Biofortification targets achieved through breeding (Parts per million).

Micronutrients	Nutritional Status	Crops		
		Beans	Pearl Millet	
Iron	Baseline nutrients	50	47	
	Added Nutrients	44	30	
	Target Achieved	94	77	
		Rice	Wheat	
Zinc	Baseline nutrients	16	25	
	Added Nutrients	12	12	
	Target Achieved	28	37	
		Sweet Potato	Maize	Cassava
Pro-vitamin A	Baseline nutrients	2	0	0
	Added Nutrients	30	15	15
	Target Achieved	32	15	15

Feasibility of biofortification through breeding

Conventional breeding has the potential to increase the micronutrients which are essential for human body by proper utilization of genetic variation in iron, zinc, carotenoids. Both high micronutrient density traits and yield can also be brought together through breeding. Biofortification through breeding is very easy and genetically controlled. So, through this it is possible to improve the lacking nutrients in the staple foods and improve the nutritional balance (Nestel *et al.*, 2006).

Biofortification through breeding

Conventional breeding the most accepted method of biofortification because of cost effectiveness and sustainability. Conventional breeding is effective because it uses the variation present in the parental genotypes and improves the specific target (Garg *et al*., 2018). Rice is of great importance and breeding is done to improve the rice to diminish the malnutrition from the world. In rice breeding, the rice varieties with high zinc and iron are selected and screened. Later on these genotypes are combined with the mineral traits (Garg *et al*., 2018).

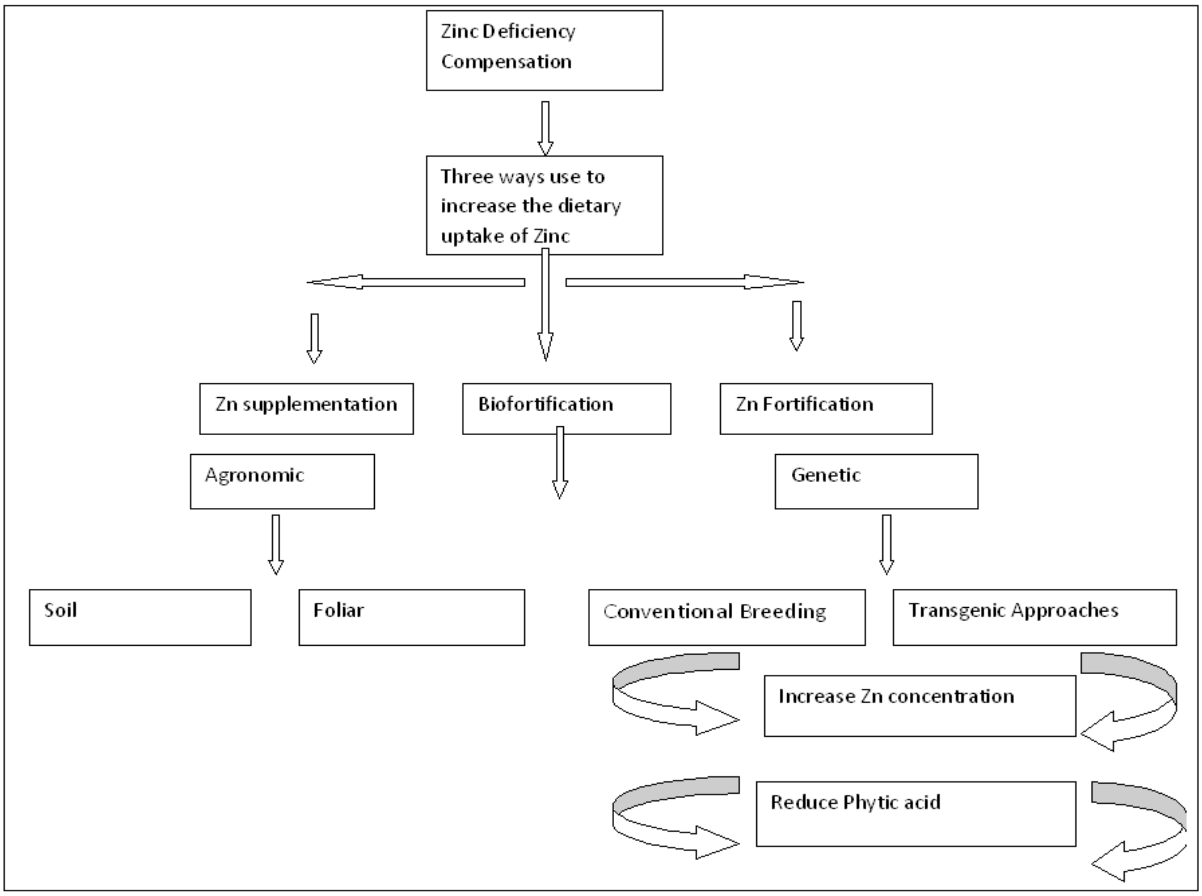

Fig. 1. Different approaches to overcome dietary Zn deficiency (Nakandalage *et al*., 2016).

Wheat as a staple crop is also very important cereal crop that is bred for iron and zinc. Wild species of wheat are identified for high zinc and iron and are used to improve the current varieties (Monasterio and Graham, 2000; Welch and Graham, 1999).

Different varieties have been developed across the world which are rich in zinc and are high yielding. For example six varieties with high zinc like (BHU 1, BHU 3, BHU 5, BHU 6, BHU 7, and BHU 18) released in India in 2014 and four varieties (NR 419, 42, 421, and Zincol) in Pakistan in 2015 (Garg *et al*., 2018). Another purpose of wheat breeding is the increase of provitamin A in the wheat. Wheat is improved for beta carotenes because yellow pigment contents are antioxidant and a good quality trait (Garg *et al*., 2018).

Maize along with the cash crop is also used as a multipurpose crop as for human consumption, animal feed and for industrial use. There is a vast genetic diversity present in maize and scientists discovered varieties which are enriched with provitamin A naturally (Garg *et al*., 2018). Biofortification of maize with high provitamin A is the big achievement in the field of breeding. The purpose of sorghum breeding is reported for rich micronutrients and high beta carotenes by Reddy *et al*., 2005.

Breeding for biofortified material

The very first step for the breeding of the biofortified material is the exploration of the existing material like already present varieties to find out the genetic diversity for the required nutrients like zinc, iron and pro-vitamin A. Another objective of breeding the biofortified germplasm is to test the existing varieties in that pipeline for fast-tracking (the method of commercializing the obtained genotypes having the targeted nutrients with the required practices and the end products without any delay (Bouis, H. E. and A. Saltzman, 2017).Pre breeding is required if the variation is present in the unadapted material.

The next step in the breeding for biofortified crop is to test the micronutrient improved germplasm, develop molecular markers and conduct genetic studies to facilitate breeding and determines the Genotype × Environment interaction. The last step includes the selection of the most promising varieties and testing them at different national research institutes to check their agronomic performance (Bouis, H. E. and A. Saltzman, 2017).

Transgenic approaches for crop improvement

Transgenic plant breeding approaches are very promising for the development of the biofortified crops enriched with required micronutrients like transgenic iron and zinc rice with the desired agronomic traits (Trijatmiko *et al.*, 2016). There are some issues associated with the release of biofortified crops like Golden rice which is the good source of Vitamin A and carotenoids but has not been introduced in the large part of the world due to the risky regulatory processes (Wesseler, J. and D. Zilberman, 2014). Although transgenic breeding approaches have high nutritional status but conventional breeding is mostly used because it does not face the same regulatory issues for its release (Bouis, H. E. and A. Saltzman, 2017).

In biofortified rice, two genes are introduced that encode two enzymes phytoene synthase (PSY) and phytoene desaturase (CRTI). Two types of biofortified rice are produced Golden Rice 1 and Golden Rice 2. Golden rice 1 contains PSY gene from daffodil and CTRI gene from the Erwinia Uredovora bacterium (Ye *et al*, 2000). In Golden Rice 2, the PSY gene is replaced with CTRI genes with 23 times more beta carotenes in the rice (Paine *et al.*, 2005). Folic acid which is the precursor of beta carotenes is important for anemic patients and for the normal pregnancy (Bibbins-Domingo *et al.*, 2017). Genes encoding GTP-cyclohydrolaseI (GTPCHI) and aminodeoxychorismate synthase are over expressed to increase the foliate contents in rice (Storozhenko*et al.*, 2007; Blancquaert *et al.*, 2015).Rice is also targeted to reduce the rate of anemia in the poor population. Different genes that encodenicotianamine aminotransferase (Takahashi *et al.*, 2001).

Iron transporter OsIRT1 (Lee, S. and G. An, 2009), nicotinamine synthase 1(OsNAS1) and OsNAS 2(Lee *et al.*, 2009; Zheng *et al.*, 2010), soybean feratin (Goto *et al.*, 1999) have been introduced to increase the iron contents in rice.

Zinc biofortification is carried out using two strategies1: Agronomic biofortification and 2: Genetic biofortification. In genetic biofortrification, the cultivars are developed with the ability to store the essential nutrients like Zn in the grains while agronomic biofortification is done through the soil application of zinc at the very high rate, time period and rate of Zn application is maintained (Phattarakul *et al.*, 2009). Agronomic biofortification is actually the process of enriching the grains with the Zn by the application of Zn fertilizer during growth stages of crop and genetic biofortification is the breeding of the crop through conventional or the modern biotechnological approaches (Cakmak, 2008).

Different genes like OsIRTI and mugineic acid synthesis gene from barleyHvNAS1, HvNAS1, HvNAAT-A,HvNAAT-B, IDS (Masuda *et al.*, 2008). Different approaches have been used to overcome the dietary zinc deficiencies.Figure1 (Nakandalage*et al.*, 2016).To fortify the rice with lysine needs three main approaches like 1) Accumulate lysine, 2) Manipulate the seed storage proteins and 3) Overexpress lysine in the seeds. This happens by using two key enzymes that are used in lysine biosynthesis like Asparate Kinase and dihydrodipicolinate synthase (DHPS)

(Galili, 2002).

Wheat is one of the most important cereals used as a staple food in the world and biofortified wheat is a rich source of readily available nutrients for the consumer Galili, G. and R. Amir, 2013).Fortification with pro Vitamin A by using the bacterial PSY and carotene desaturase genes (Cong *et al.*, 2009; Wang *et al.*, 2014). Maize is improved for carotenoids to upgrade the level of Vitamin A in the micronutrient deficient population by performing Genome-Wide Association of the seeds for carotenoids from a wide range of colour of the grains of the inbred lines of the maize (Owens *et al.*, 2014).

The grains of maize has significant variations for the carotenoids (Harjes *et al.*, 2008; Berardo *et al.*, 2009; Burt *et al.*, 2011). Pro vitamin A biofortification efforts for carotenoids in maize provide various loci that can be used further in Marker Assisted Selection and Genome Sequencing programs (Owens *et al.*, 2014).Maize is fortified with lysine because lysine is not in sufficient amount in the cereal crops (Joint, F. and W. H. Organization, 2007).Opaque 2 gene is used to increase the lysine contents in the maize for the development of Quality Protein Maize (QPM) (Glover, 1992).

This still not accepted as a good approach for lysine improvement in QPM and attempts are made for better approaches (Antisense dsRNA targeting alpha zeins are used to increase the lysine and tryptophan contents in maize (Haung *et al.*, 2006).

Barley is fortified with zinc by using Zn transporters to compensate the Zn deficiencies (Ramesh *et al.*, 2004). Phytase gene is also over expressed in barley to increase the iron and Zn contents (Holme *et al.*, 2010).

Sorgham is one of the most important staple foods of millions of people of the world because it has the ability to grow in harsh conditions. Sorgham is fortified for Vitamin A by overexpressing Homo188 A to fulfill the vitamin A requirements of the people (Garg *et al.*, 2018). Sorgham is also improved for lysine contents by introducing high lysine protein (HT12).

Agronomic approaches for biofortification

Agronomic approaches require the physical application of the nutrients to fulfill the nutrition requirements temporarily (Cakmak I. and Kutman UB, 2017). In this, the nutrients are easily available and improve the health status of the humans.

The plants take nutrients from the soil so the nutrients are added to the soil so that the plants take the nutrients and help to achieve the nutritional status. Different crops are improved agronomically for different nutrients like wheat, sorgham, barley, rice etc. Rice biofortification by agronomic practices is another way to improve the rice for zinc and Vitamin A. Two main approaches are used to improve the vitamin A and zinc in rice is through foliar application and the application of zinc to the soil (Garg *et al.*, 2018).

Wheat is also biofortified through agronomic approach like inclusion of iron in urea fertilizer that has positive correlation with the iron in the grains and improves the grain quality of wheat (Aciksoz *et al.*, 2011). Foliar application of zinc to the wheat helps to cope with the nutrients deficiency and also helps to reduce the phytic acid that is not good for the humans (Yang *et al.*, 2011).

Zinc is the most important micronutrient which is required for the good grain quality and helps to improve the yield of the crops. So sorgham is also improved for the nutrients both by organic and inorganic application of the fertilizer. Plant growth-promoting bacteria and arbuscular mycorrhizal fungi are used to improve the metabolic status of sorgham.

Maize which is a very important and widely used staple food crop of the people is also improved by foliar application of zinc (Dhawi *et al.*, 2015). Plant growth promoting rhizobacteria is also used to improve the nutritional status of the plants (Garg *et *al.*, 2018). Barley is also improved agronomically by the application of organic and inorganic fertilizers. By the application of biofertlizer along with inorganic fertilizer increase the zinc and iron concentration in the plants (Garg *et al.*, 2018).

Nutrient availability of biofortified crops

Nutrients which are essential for human body transfer through proper channel like soil to crop, crop to food and then at last from food to human (Valenca *et al*, 2017). Soil to crop:

The availability of nutrients from soil to the crop depends upon different factors like soil fertility, quantity of dry matter in the soil, soil aeration and also on the variety of the crop (Alloway *et al.*, 2009). Plants do some modifications in their structure to increase the availability of nutrients from soil to the crop (Zhang *et al.*, 2010). The interaction of the micronutrients with each other also affects the availability of nutrients to the food. For example the soil phosphorous enhances the Zn uptake while at the same time the application of the phosphorous fertilizer initiates the Zn deficiency in the plants (Zingore *et al.*, 2008).

Crop to Food: The availability of the nutrients from crop to the food depends upon the variety of the crop whether the crop fixes the nutrients in the edible portion of the food or not.

It also depends upon the crops like in rice nutrients store in the outer layer of the grain and during food processing, it removes. There happens the deficiency of iron and zinc in the crop (Hass *et al.*, 2005). In wheat, the nutrients store in the endosperm of the grain that remains available even after the removal of the seed coat (Ajiboye *et al*, 2015).

Food to human: The availability of the nutrients from food to the human body depends upon the consumption of the food by the human and the interaction between the food components and the micronutrient (SandstroÈm, 2000). There are different factors that are responsible for its availability like age, gender, genotype etc (Gibson, 2007). The availability of the nutrients from soil to crop to food to human also depends upon the agronomic fortification.

Vitamin A availability studies show the proficient conversion of pro Vitamin A to the retinol that is a form of vitamin A used by the human body. The consumption of biofortified Vitamin A crops increases the level of beta carotene in the body (Bouis, H. E. and A. Saltzman, 2017). The example of fortified vitamin A crop is orange sweet potato which is the rich source of Vitamin A (Haskell *et al.*, 2004; Low *et al.*, 2007). Another example of biofortified Vitamin A crop is provitamin a maize. It improves the Vitamin A supplementation in the body and also improves the visual functioning of the children (Gannon *et al.*, 2014; Ruel *et al.*, 2017).The biomarkers studies showed that Zn in the improved wheat is available. Iron fortification in pearl millets and beans improve the health status of the children and available to the targeted population (Hass *et al.*, 2005).

Farmer adoptability

There are different factors which are critical to farmer adoption including trait visibility, colour changes, change in dry matter contents. The main criteria of farmers for adoption are the yield and income associated with that improved variety or crop. Crop and environment specific traits which are related to adoption should be considered in breeding for biofortification. Trait visibility includes colour changes due to vitamin A and dry matter concentration. It requires both the consumer and producer acceptability. In terms of infrastructural changes like information flow and market network, there is need to include the farmer's used breeding methods to identify the biofortified genotypes that are best suited to the local producer and consumer (Nestel *et al.*, 2006).

Impact on nutritional status

The improvement of micronutrients leads to the various health improvements in women and children. For example β-carotene rich sweet potatoes improve vitamin A and iron rice improves the reproductive age of women and eye sight.

Limitation of biofortification

In transgenic point of view although it overcome the genetic variation limits but the main hurdle in this approach is the less acceptance among the people. It is important to adopt this strategy by the farmer community (Al-Babili and Beyer, 2004). In agronomic biofortification, the rate is very high and successfully nutrients are provided to the plants but the main limitation is the variability in the nutrients mobility, accumulation of minerals and the profile of the each crop. Agronomic biofortification is less cost effective but it requires continuous inputs and intensive agronomic approaches.

It is not always results in successful accumulation of minerals in the edible part of the plant (Garg *et al.*, 2018). Another major constrain in agronomic biofortification is that mineral availability is reduced due to the phytic acid (Frossard *et al.*, 2000) and the leaching down of fertilizers in the soil and disturb the environment (Waters and Sankaran, 2011).

Conventional plant breeding programs proved to be the very sustainable and cost effective method of improving the crop but the limitation is the time consumption. Conventional breeding is good for different traits but it's difficult to breed a crop for a specific trait (Lyons *et al.*, 2005; Tulchinsky, 2010; Garg *et al.*, 2018).

Conclusion

It is well stated that biofortification is the most promising and cost effective method of improving the nutrient deficient crops and improve the nutritional status of the malnourished population across the world. Biofortification strategies like agronomic approaches, crop breeding and the transgenic approaches have the potential of improving the crop and addressing the malnutrition of the world. The crops are improved for various nutrients like zinc, provitamin A and others. Still there is a problem with acceptance and adoption of transgenically biofortified crop. Inspite of all these hurdles, biofortified crops have bright future with the improved nutrients and always helpful in improving the health status. It has the potential to tackle malnutrition by providing the sufficient nutrients to the poor people of the world.

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