



## Review Article

## Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches

Sushil Kumar<sup>a,\*</sup>, Adinath Palve<sup>a</sup>, Chitra Joshi<sup>a</sup>, Rakesh K. Srivastava<sup>b</sup>, Rukhsar<sup>a</sup><sup>a</sup> Centre of Excellence in Agricultural Biotechnology, Anand Agricultural University, Anand, India<sup>b</sup> International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India

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## ABSTRACT

Micronutrient malnutrition is an important issue in the developing countries especially in Asia and Africa where millions of school-going children and pregnant women are affected. Poor people are more exposed to risks of malnutrition and hidden hunger due to intake of carbohydrate rich but micronutrient deficient plant based food. The expansion of high yielding but micronutrient poor cultivars further intensified the malnutrition. The existing approaches viz., supplementation and food fortification of staple food with minerals and vitamins can address the issue of adequate nutrition security. But supplementation and fortification is neither feasible for each nutrient specially iron nor viable due to recurrent cost. Recently, genetic bio-fortification of crops is emerged as self-targeted and non-recurrent approach to address the micronutrient malnutrition. Most of the traditional breeding approaches were limited due to non-availability of enough genetic variation in the crossable gene pools. Additionally, it also lacks the modulation of target gene expression underlying the micronutrient accumulation. At this juncture, genetic engineering based food biofortification is promising way to address the hidden hunger especially, where breeding is not rewarding due to lack of genetic variability. Genetic modification through gene technology is swift and accurate method to develop nutrient denser crops without any recurrent investment as compared to different strategies.

## 1. Introduction

The spread of micronutrient malnutrition will be more pronounce in the growing population and presently widespread and known to affect almost three billion people worldwide [1, 2]. Malnutrition deficiency, which is also called as “hidden hunger”, increases the general risk of infectious illness and of dying from diarrhoea, measles, malaria and pneumonia in the people living in low income countries [3]. The consequences of malnutrition are severe and long lasting; sometimes moving from generation to generation [4]. Malnutrition during pregnancy increases the risk of mortality, as well as affecting fetal growth, resulting in low birth weight (LBW), risking the survival of the child. The prevalence of malnutrition remains a major public health problem in developing and underdeveloped countries [5, 6].

Presently, people consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially iron (Fe), Zinc (Zn), iodine (I), selenium (Se), and vitamin A [7, 8]. Among trace elements, Fe and Zn are essential for a variety of metabolic processes [9, 10]. Zn and Fe deficiencies ranked fifth and sixth respectively, among the top

ten risk factors contributing to disease burden globally (Kumar, 2011). Micronutrient deficiencies are common in children and even more common in women because of blood losses occurred during menstruation and child birth [11]. Further, lack of awareness and affordability to diverse and balanced foods, dietary practices, and high incidence of infectious diseases are exaggerating micronutrient deficiency in the developing countries [12, 13, 14, 15]. To combat nutrient deficiency especially minerals, various interventions such as food diversification, pharmaceutical supplementation, and fortification has been emphasized.

## 2. Main text

## 2.1. Current strategies to combat malnutrition

The best way of preventing and even eliminating micronutrient malnutrition is to ensure consumption of a micronutrient rich balanced diet. The common yet effective approaches to addressing malnutrition and micronutrient deficiencies are through supplementation, fortification of foods, homestead food production, and biofortification of crops.

\* Corresponding author.

E-mail address: [sushil254386@yahoo.com](mailto:sushil254386@yahoo.com) (S. Kumar).<https://doi.org/10.1016/j.heliyon.2019.e01914>

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Micro-nutrient fortification of food is the most practical way of preventing nutrient deficiency in the people. Food fortification- a strategy to fill nutrients gap- has the dual advantage of being able to deliver nutrients to large segments of the population without requiring radical changes in food consumption patterns.

With a long history, fortification of butter, margarine and sugar with vitamin A, salt fortification with iodine, vitamin fortified milk, vitamin B fortified cereals is already being implemented in some countries. Mandatory food fortification compared to voluntary fortification is more effective and has been successfully practiced for decades across the globe. Milk and oil fortification is mandate in 14 and 27 countries, respectively. Similarly, salt fortification with iodine and Fluoride is mandatory in 134 countries. Currently, around 83 countries are exploiting fortification legislations for micronutrient enrichment of the staple food. But still there are drawbacks in current food fortification practices. In most instances, food fortification is only feasible in countries that possess well-developed, efficiently monitored and properly regulated pharmaceutical and food processing sectors. Another important drawback to supplementation and fortification approaches, however, is that they incur the same recurrent costs year after year and its success depends on funding. This approach is especially effective in countries where the poorest people mostly purchase small amounts of processed foods to supplement their diets. Unfortunately, though, in the poorest developing countries, where the majority of the poor especially farming community are dependent on their own produce for food, consumption of industrially processed foods is minimal. Therefore, the reach of these food fortification interventions can be quite limited, especially in rural areas of developing countries where most of the poor live. The mandatory fortification in India inaugurated in 1953 through fortification of hydrogenated vegetable oil with vitamin A and D was mandated [16]. Later on, in 1998, salt fortification through iodization came in existence to control Goiter. In 2000, wheat flour fortification started in West Bengal followed by Andaman and Nicobar Islands.

In industrialized societies, micronutrient deficiencies are addressed through supplementation and fortification [17]. However, in developing countries widespread implementation of dietary diversification and food fortification/supplementation programs are constrained by low income coupled with the poor access of the target population to market [18, 19]. Unfortunately, none of these socioeconomic development derived strategies is an effective remedy against micronutrient malnutrition. Moreover, success of above strategies also involves behavioural changes of societies which depend on literacy, communication, social-marketing, and repeated investments [15].

The global experience of several investigators has verified that instead of single costlier approach a combination of several cost effective approaches is requisite to cap the nutrient deficiency [19]. Therefore, to address the occurrence of micronutrient deficiencies in human populations, plant scientists are concentrating to formulate methods to apply fertilizers and/or use plant breeding strategies to increase the concentrations and/or bioavailability of mineral elements in edible portion of crop plants [13, 20, 21, 22, 23, 24]. These approaches are termed 'agronomic' (fertilizer based) and 'genetic' (breeding based) biofortification, respectively.

Biofortification, an approach to enrich micronutrient content of agriculture produce, involves strategies which spin around targeting and modulation of movement pathways (root uptake, transport, remobilization, storage and enhanced bioavailability) of mineral nutrients, 'pulling' nutrients from soil and 'pushing' them to economic parts of plants in their bioavailable forms. The three major strategies followed till date for biofortifying food crops include: agronomic biofortification, conventional plant breeding and genetic engineering. The key focus of agronomical method is on optimization of the application of mineral fertilizers and/or the improvement of the solubilisation and mobilization of mineral elements in the soil [13]. The latter two approaches target on development and/or improvement of plant cultivars with better micronutrients accumulation capacity and increased levels of their

bioavailability by depressing 'anti-nutrients' concentrations.

To combat the drawbacks of supplementation/fortification, "Biofortification" (breeding for increased mineral and vitamin content) of staple food is a promising, viable and effective route for delivering nutrient rich food to compete hidden hunger particularly in rural population. This strategy can complement the other approaches through providing a sustainable and cheaper means of reducing under-nourished mass which depends on supplementation and commercial fortification for nutrition. The biofortification approach involves fixed one-time costs in developing breeding methodologies, breeding nutritional quality traits into current crop varieties, and adapting these varieties to diverse environments. This approach will require minimum recurrent investments after nutritious varieties have been initially disseminated. Moreover, the costs do not increase with the number of people, and the benefits can be made available globally especially to all developing countries. Finally, breeding for higher trace mineral density in the consumed plant parts will not incur a yield penalty [12, 15].

Conventional and molecular breeding; and genetic engineering techniques are the two approaches to biofortify the crops with minerals such as iron and zinc [24, 25, 26, 27, 28]. Since the uptake and accumulation of micronutrients in edible parts of crops are controlled by polygenes having minor effects, the conventional breeding based biofortification approaches have met with only marginal success [17]. Moreover, the success achieved by using this approach depends chiefly on natural variation that exists in the gene pool. In the absence of adequate genetic variability and fixable major gene effects, genetic engineering will be a viable alternative for enhancement of micronutrients at desired levels [29, 30].

Despite the huge efforts made through conventional plant breeding programmes, exploiting the field of genetics which includes quantitative genetics, heterosis, transgressive segregants, mutational breeding, marker assisted breeding, QTL mapping etc. to entrap the natural genetic variations for micronutrients and vitamins accumulation, there still remains a long way to top: dream of nourishing the future. This is mainly because of several weakness and threats to conventional breeding which are: requirement of sufficient genetic variations for a trait in the species which may not be available for many economically important crops, need of genes targeting the trait in sexually compatible plants, long time period required for breeding to introduce a single as well as multiple traits (pyramiding traits) into locally adapted elite varieties without the risk of linkage drag, lack of appropriate knowledge of QTL  $\times$  environmental interactions and effects of epigenetic and genetic background, inability to target nutritional traits to specific organs (e.g. cereal seeds, tubers etc.) and the dependence on the phytoavailability of the mineral nutrients in the soil. Moreover, the presence of inverse relationship between grain yield and grain mineral concentration has posed a major challenge for conventional breeding methods to break such trade-offs [31, 32, 33, 34].

As a modern weapon to fight against mineral deficiency, genetic engineering to generate transgenics has also been deployed to transfer genes directly into elite genotypes. Transgenic technologies are the tools to improve the genotypes by making changes in focussed metabolic pathways. These technologies pave the way to modify proteins [35], vitamins, carbohydrate, fats, minerals, and other metabolites and will be considered in coming sections. However, to develop transgenics for nutrient biofortification, two criteria should be ideally considered: (1) selection of widely adapted genotype of economically important crop; (2) accumulation of nutrient in the edible portion of the crop plant without adverse effect on plant physiology or development and economic yield [36].

Conventional breeding based biofortification is well accepted method to improve the micronutrients in crops. A good number of crops have been targeted for fortification using conventional breeding [37, 38]. The genetic engineering techniques, offers a valid alternative to conventional breeding approaches when there is absence of sufficient genotypic variation for the desired trait within the species (e.g. provitamin A in rice), or

when the crop itself is not amenable to conventional breeding (due to a lack of sexuality; e.g. banana). With the advent of powerful 'omics' technologies, new gene editing tools like transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 and increased availability of fully sequenced genomes in staple crops have created new rooms for this biofortification strategy [39]. Transgenes can be specifically targeted for redistribution of micronutrients between tissues, increasing the efficiency of biochemical pathways in edible tissues, reconstruction of selected pathways (e.g. field of system biology), increasing bioavailability of micronutrients by removal of antinutrients and multigene transfer (e.g. 'multivitamin corn' having high levels of  $\beta$ -carotene, ascorbate (vitamin C) and folate (vitamin B9) in a single plant [1, 17]. Therefore, bridging the gap between plant breeders and molecular biologists in order to harness the power of genetic modification for biofortification of crop plants proves pivotal.

## 2.2. Transgenic approaches for increasing to Fe, Zn and vitamin-A content

Plants acquire Fe and Zn from surrounding rhizosphere and immediate environment as minerals are not synthesized in the plant [40]. Genetic engineering has been deployed in various crops to increase mineral content especially Fe and Zn. The transgenic strategies to increase the Fe and Zn content in the crops have been mainly focused on to increase the uptake and utilization efficiency of plants through modulation of transporters expression [41], and reducing the anti-nutritional factors concentration like phytic acid. Unlike Fe and Zn, transgenic approaches are quite simple for vitamins. The introduction of limiting step in the metabolic pathway of seed to facilitate the production of precursor of vitamin-A i.e.  $\beta$ -carotene or modifying the pathway for its increased production are the most adopted transgenic approaches. In the following sections, we briefly reviewed various genes and transgenic approaches employed for Fe, Zn and vitamin-A in major cereals (Table 1).

### 2.2.1. Iron (Fe)

Globally, rice, wheat and maize provide more than 50% of caloric requirement and are targeted in major biofortification programmes to alleviate micronutrient deficiencies [59]. Studies were carried out to increase the Fe content of the endosperm by expressing *lactoferrin* (Fe-chelating glycoprotein) and *ferritin* [60]. Human milk is a rich source (1–2 g/l) of lactoferrin (LF). For future application in infant formula, Nandi et al [61] developed transgenic rice grains with human LF gene under the control of the rice glutelin-1 promoter to increase the Fe content. The expression of heterologous protein was at significantly higher levels than control, reaching 0.5% of the grain weight and the

bioavailability was confirmed through human Caco-2 bioassay. Lee et al [62] also confirmed the expression of the hLF gene in transgenic *japonica* rice and accounted for approximately 1.5% of total soluble protein.

Ferritin, a localized protein in plant plastid, is a major non-toxic storage form for Fe which can releases Fe for metabolic functions as and when needed. Being ubiquitous protein, ferritin stores about 4,500 Fe atoms in bioavailable form [63]. Therefore, enhancement of Fe accumulation in by *ferritin* gene expression under the control of endosperm-specific promoters is an important strategy to Fe biofortification. Studies found that overexpression of ferritin in several crops increased Fe content as well as bioavailability [45, 46, 54, 55, 64, 65, 66, 67, 68]. To increase the Fe accumulation in endosperm of brown rice seeds, Goto et al. [45] generated rice transformants of *SoyferH1*, under endosperm-specific GluB1 rice promoter and reported a threefold increase in grain Fe content as compared to non-transformed lines. Similarly, soybean ferritin cDNA was also transferred in wheat and rice under the control of maize ubiquitin promoter [54]. However, resulted transformants showed high Fe content in leaves than seeds owing to strong role of leaves as sink. Which also hints that excess ferritin sequesters the Fe in the leaves reducing the Fe mobilisation to the seeds. Qu et al. [67] introduced soybean ferritin into rice with strong endosperm specific globulin promoter, and this led to an increase of up to 13 times in ferritin protein expression than in Goto et al. [45]. However, there was only a moderate enhancement (30%) in Fe content. These results indicated need to improve Fe in seeds, in addition to increased Fe storage, it is necessary to increase the Fe transport from the soil and enhanced translocation within the plant system [47].

Above experiments in rice provided genetic engineering as a viable option for Fe biofortification in cereals. However, as compare to rice, a very little work has been carried out to improve the Fe content of wheat and maize grains especially in endosperm. Borg et al [55] cloned and analysed wheat ferritin genes (*TaFer1-A*) and showed the capability of ferritin overexpression in the wheat endosperm to increase the Fe content. Endosperm targeted overexpression of the *TaFer1-A* gene under the control of HMW glutenin 1DX5 promoter resulted in 50–85% higher content in wheat grain.

### 2.2.2. Zinc (Zn)

Zn is an essential element; and is also a co-factor for ~300 enzymes and more than 1000 transcription factors [69]. There is limited natural diversity in grain Zn content of cereals [70]. Thus, increasing the Zn content of cereal grains will be important for improving human nutrition and metabolism. However, manipulation of the Zn content of the cereal grain may be less straightforward than Fe [59]. Interestingly, study of

**Table 1**

A list of transgenic crops developed for iron, zinc and vitamin A and targeted genes.

| Crop    | Nutrient  | Gene used                                                                                                          | Total increase in level                            | Transformation method            | Ref                    |
|---------|-----------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------|------------------------|
| Rice    | Vitamin A | <i>Phytoene synthase (PSY)</i> from daffodil, <i>phytoene desaturase (CrtI)</i> gene from <i>Erwinia uredovora</i> | 1.6 $\mu\text{g/g}$ DW                             | <i>Agrobacterium tumefaciens</i> | Ye et al [42]          |
|         |           | <i>Phytoene synthase (PSY)</i> from maize, <i>CrtI</i> from the <i>Erwinia uredovora</i>                           | 37 $\mu\text{g/g}$ DW                              |                                  | Paine et al [43]       |
|         | Fe        | psy and lycopene $\beta$ -cyclase ( $\beta$ -lcy) both from daffodil                                               | 1.6 $\mu\text{g/g}$ DW                             |                                  | Beyer et al [44]       |
|         |           | Over expression of soybean ferritin gene <i>Soyfer H-1</i>                                                         | 38.1 $\mu\text{g Fe/g}$ DW                         |                                  | Goto et al. [45]       |
|         |           | <i>Phaseolus ferritin</i>                                                                                          | 22.07 $\mu\text{g Fe/g}$ DW                        |                                  | Lucca et al. [46]      |
|         |           | <i>Ferritin</i>                                                                                                    | 7 $\mu\text{g/g}$ DW                               |                                  | Masuda et al. [47, 48] |
| Wheat   | Zn        | <i>OsNAS2</i>                                                                                                      | 19 $\mu\text{g/g}$ DW                              | Bombardment method               | Johnson et al. [49]    |
|         |           | HvNAS1 gene from Barley                                                                                            | 35 $\mu\text{g/g}$                                 |                                  | Masuda et al [50]      |
|         | Vitamin A | Soybean <i>ferritin</i> , <i>Aspergillus flavus</i> phytase, <i>OsNAS1</i>                                         | 35 $\text{mg/g}$ DW                                |                                  | Wirth et al. [51]      |
|         |           | Overexpression of <i>OsNAS2</i>                                                                                    | 76 $\mu\text{g/g}$                                 |                                  | Johnson et al [49]     |
|         |           | maize psy1 gene encoding phytoene synthase, bacterial <i>crtI</i>                                                  | 4.96 $\mu\text{g/g}$ DW                            |                                  | Cong et al [52]        |
|         |           | <i>CrtB</i> or <i>CrtI</i>                                                                                         | 3.21 $\mu\text{g/g}$ of seed dry weight            |                                  | Wang et al [53]        |
| Maize   | Fe        | <i>Soybean ferritin</i>                                                                                            | 40 $\mu\text{g/g}$ (in leaves tissues)             | <i>A. tumefaciens</i>            | Drakakaki et al. [54]  |
|         |           | Overexpression of <i>TaFer1-A</i>                                                                                  | 44.5 $\mu\text{g/g}$ (in endosperm)                |                                  | Borg et al [55]        |
|         |           | bacterial <i>crtB</i> and <i>crtI</i>                                                                              | 9.8 $\mu\text{g/g}$ DW                             |                                  | Aluru et al. [56]      |
|         |           | psy1 (maize)                                                                                                       | 59.32 $\mu\text{g/g}$ DW                           |                                  | Naqvi et al. [17]      |
| Cassava | Vitamin A | Bacterial <i>crtB</i>                                                                                              | 6.67 $\mu\text{g/g}$ DW                            | <i>A. tumefaciens</i>            | Welsch et al. [57]     |
| Canola  | Vitamin A | <i>crtB</i> and <i>crtI</i>                                                                                        | 857 $\mu\text{g/g}$ fresh weight $\beta$ -carotene |                                  | Ravanello et al. [58]  |

Ozturk et al. [71] showed strong correlations between protein content, Fe and Zn content. *Gpc-B1* (GRAIN PROTEIN CONTENT B1) is a wheat quantitative trait locus associated with increased grain protein also increased the Zn and Fe content [72]. An increment of 10–34% in concentrations of grain Zn, Fe, Mn and protein was observed in cultivated wheat after introgression of *Gpc-B1* locus from the wild tetraploid wheat *Triticum turgidum* ssp. *dicoccoides* into different recombinant chromosome substitution lines, indicating the role of *Gpc-B1* in remobilization of protein, Zn, Fe and Mn from the leaves to the grains [73].

Over-expression of genes involved in Zn translocation and mobilization with increased bioavailability of Zn without yield penalty in an important way to enhance the grain Zn [14]. Many transporters associated with cations have been identified in rice, but few have been characterized with respect to substrate specificity, expression pattern and cellular localization. Among many identified cation transporter families, members of the ZIP (ZRT, IRT-related protein) and CDF (Cation diffusion facilitator) families are predominant as playing major role in Zn uptake and translocation. IRT1 protein of ZIP family contributes significantly to the uptake of Zn in *A. thaliana* root cells [74]. Over-expression of NA synthase (NAS) by introducing 35S enhancer elements led to 2–3 fold increases in Zn content in paddy [75]. Similarly, transgenic rice expressing barley nicotianamine synthase gene *HvNAS1* under the control of the rice actin1 promoter accumulated 2–3-fold higher Zn in polished rice grains [50]. At IRR1, several thousand transformants of IR64 and IR69428 are produced with soybean or rice ferritin and rice nicotianamine synthase (NAS2) over expression genetic constructs and the content Zn and Fe in those lines have surpassed the target level from the field trials. Thus, overexpression of NAS genes makes nicotianamine an interesting target for Zn biofortification. Moreover, biofortifying cereals with NAS alone or in combination with ferritin have great potential in combating global human mineral deficiency [75, 76].

To understand the pathway of Fe and Zn in grain, sufficient work is reported chiefly in many crop species such as wheat, rice, maize, and barley. Despite many challenges such as the root–shoot barrier and grain filling [69] wheat researchers exploit tools and resources developed in rice to make significant improvements in Zn content in wheat grain to bring improved wheat lines [14].

### 2.2.3. Provitamin-A

There is an absolute requirement of vitamin A in human diet to prevent numerous chronic diseases including cancer. The various plant-derived food accumulate  $\beta$ -carotene, precursor of provitamin-A, in very different. Vitamins content in cereals are low and consequently vitamin deficiency affects up to 50% of the global population [77]. In human, vitamins are the second major group of micronutrients required for the growth and health. Transgenic offers a sustainable approach to intensify the vitamin content of edible part, but thus far it has only been possible to enhance individual vitamins such as vitamin A. Transgenic based biofortification technology can play superior role in cereal crops where metabolic pathways for vitamins are absent, truncated, or inhibited in the endosperm [17]. Biofortification of crops with provitamin A, giving rise to ‘golden’ crops [78]. The generation of “Golden Rice” [79] and sorghum [80] with higher levels of provitamin A, are classic examples of transgenic based biofortification technology.

In plants understanding of carotenoid biosynthesis and metabolism has been dissected and understood at substantial level. Overexpression and upregulation of the bacterial phytoene synthase gene, *CrtB*, is a fruitful strategy to increase high provitamin A content into staple crops [81]. An enhanced accumulation of  $\beta$ -carotene, lutein and violaxanthin was demonstrated through overexpression of *CrtB* in potato [82]. In rice endosperm, overexpression of the daffodil phytoene *PSY* gene coupled with bacterial *crtI* gene and/or the daffodil *LCYB* gene yielded a total carotenoid content of 1.6  $\mu\text{g/g}$  dry weight [42]. Paine et al [43] reported 37  $\mu\text{g/g}$  total carotenoid in Golden rice-2 where daffodil gene was replaced by maize *PSY1*. Similarly, overexpression of the bacterial *crtB* and *crtI* genes under the control of a “super  $\gamma$ -zein promoter” for

endosperm-specific,  $\beta$ -carotene in the maize endosperm increased up to 34-fold [56]. Wheat endosperms transformed with maize *y1* gene encoding phytoene synthase driven by a endosperm-specific 1Dx5 promoter together with the bacterial phytoene desaturase *crtI* gene under the constitutive CaMV 35S promoter control, resulted 10.8-fold increase in the total carotenoids [52]. Further enhancement the provitamin A level in wheat grains, was recorded through co-transformed of the bacterial *CrtB* and *CrtI* genes [53]. In addition to rice, other crops engineered for higher  $\beta$ -carotene content are potato, canola, tomato, carrot, and cauliflower [83]. However, carotenoid get degraded during storage of cereal grains. This degradation is mediated by oxygen and lipoxygenase enzyme [84, 85]. Down regulation of lipoxygenase and/or enhance accumulation of vitamin E through genetic engineering have been revealed to upsurge carotenoid stability during storage [86].

However, genetic engineering to increase the richness of vitamins through single-point interventions generally does not improve the yields of the target vitamin [87]. Therefore, to improve complex traits in crops, multiple transgene expression and combinatorial engineering are the approaches to overcome the challenge where genes encoding pathway enzymes are modified and over expressed [88, 89]. Early examples of multiple transgene through mini-pathway engineering in rice, tomato and potato employed 2–3 transgenes cloned in a single *Agrobacterium* construct. Recently, Naqvi et al [17] created elite inbred transgenic corn plants in which the levels of ascorbate,  $\beta$ -carotene, and folate were increased specifically in the endosperm through the simultaneous modification of three separate metabolic pathways. The transgenic kernels contained 169-fold the normal amount of  $\beta$ -carotene, 6-fold the normal amount of ascorbate, and double the normal amount of folate. More recently, up to seven different bacterial carotenoid genes (from *isopentenyl pyrophosphate isomerase (IPI)* to *CrtW*) have been assembled in a single *Agrobacterium* construct and used to transform canola and *Lilium* [90]. The large accumulation of carotenoid in rice endosperm with combined expression of *ZmPSY1*, *PaCrtI* with *AtDXS* or *AtOR*, hints that the supply of isoprenoid precursors and metabolic sink are imperative rate-limiting steps in carotenoid biosynthesis [91].

An alternative to mini-pathway engineering is combinatorial nuclear transformation which is a novel method for the rapid production of multiplex-transgenic plants, which we have used to dissect and modify a complex metabolic pathway [88] (Zhu et al, 2008). This strategy allows generating a metabolic library for the investigation of metabolite of interest. Using this technology in maize Zhu et al [88] (2008) overexpressed *Zmpsy1* and observed that the total endosperm carotenoid levels were elevated 53-fold, and the expression of 2 sequential enzymes (*ZmPSY1* and *PaCrtI* in Ph-3) resulted in a further tripling of the carotenoid levels.

Provitamin content can also be increased in food crops through redirecting metabolic flux towards carotenoids by genes silencing. The alteration of genome through gene silencing (block strategy) can also be combined with overexpression (push strategy) to address vitamin A deficiency (Zeng et al, 2015). Simultaneous application of push (*CrtB*) and block strategy (carotenoid hydroxylase) to enhance and arrest the metabolic flux, respectively, Zeng et al. (2015b) developed transgenic wheat that accumulated  $\beta$ -carotene up to 5.06  $\mu\text{g/g}$ . Though, the  $\beta$ -carotene was not adequate amount to address vitamin A deficiency.

### 3. Conclusion

Micronutrients are very essential for human nutrition especially for alleviating malnutrition in children and women. Micronutrients such as Fe, Zn and provitamin A are the important component of biofortification program. Transgenic breeding is a strategic tool that can be used to improve the level of these micronutrients by several folds in the staple cereals. Since, genes are available for those target traits it is possible to improve the micronutrients in many staple crops. Proof of concept of improvement of micronutrients through transgenics are available through trait-specific strategies. Opportunity to use combination of genes for



simultaneous enhancement of micronutrients can be possible. Transgenic crops shall widely be cultivated for alleviating the malnutrition after addressing the regulatory issues. Moreover, recently, genome editing tools such as CRISPR-Cas, ZFN, TALEN, etc. to edit plant genes has shown a great potential in crop improvement. The genome editing tools should also be exploited for biofortification of crops. Though, genome editing based biofortification is going on at experimental level but this should be exploited at intensively to swift the biofortification in cereals and horticultural crops especially vegetables. Same time, biofortified crop varieties with enhanced nutritional qualities must be evaluated by clinical trials for bioavailability and its effect on end user's health.

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