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Transgenics for Nutritional Quality

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Abstract:

Malnutrition is a universal issue holding back development with unacceptable human consequences. Over 2 billion people worldwide suffer from a chronic deficiency of micronutrients, a condition known as hidden hunger. Unfortunately, major food crops are poor sources of micronutrients required for normal human growth. Dietary diversity, fortification and bio fortification supplementation with vitamins and minerals might be the best ways to tackle malnutrition. Bio fortification include agronomic, conventional transgenic approaches. Many transgenic strategies are also available to enhance the nutritional value of crops. Transgenic strategies differ from other approaches in that novel genetic information is introduced directly into the plant's genome.

Transgenic technology is used to describe the process by which the genetic makeup of an organism can be altered using "recombinant DNA technology". This involves the use of laboratory tools and specific enzymes to cut out, insert, and alter pieces of DNA that contain one or more genes of interest. Numerous crops have been genetically modified to enhance their micronutrient contents. Among micronutrients, vitamins, minerals, essential amino acids, and essential fatty acids have been targeted using various genes from different sources to enhance the food crop nutritional level. It has been found that *PSY*, carotene desaturase, and lycopene β-cyclase for vitamins, ferritin and nicotinamine synthase for minerals, albumin for essential amino acids, and Δ6 desaturase for essential fatty acids have been widely reported as targets for biofortification (Fig. 4). Successful examples of transgenic method are high lysine maize, high unsaturated fatty acid soybean, high provitamin A, and high provitamin A Golden rice.Transgenic technology is one of the powerful weapons for fighting

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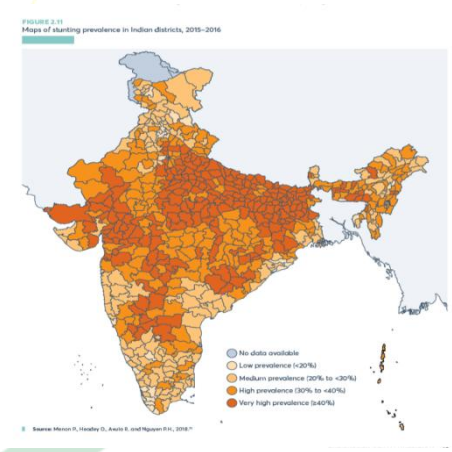
against for food, nutritional and health security. Due to several social, ethical, and technological reasons, the discussion is going on around the world whether transgenics are boon or bane but further advancement on technological front, there are ample scope and opportunities for public acceptance for the transgenics.

Introduction:

Malnutrition is a universal issue holding back development with unacceptable human consequences. The burden of malnutrition across the world remains unacceptably high, and progress unacceptably slow. Over 2 billion people worldwide suffer from a chronic deficiency of micronutrients, a condition known as hidden hunger.Malnutrition is responsible for more ill health than any other cause.17.3% of the population is at risk for zinc deficiency and 1.8 billion people have insufficient iodine intakeVitamin A deficiency affects an estimated 190 million preschool-age children Children under five years of age face multiple burdens: 150.8 million are stunted, 50.5 million are wasted and 38.3 million are overweight.



India holds almost a third (31%) of the world's burden for stunting, and because India is so diverse from state to state, it is important to understand how and why stunting prevalence differs. Researchers used mapping and descriptive analyses to understand spatial differences in distribution of stunting. The mapping showed that stunting varies greatly from district to district having stunting levels above 40%

So far, our agricultural system has not been designed to promote human health, instead it only focuses on increasing grain yield and crop productivity. This approach has resulted in a rapid rise in micronutrient deficiency in food grains, thereby increasing micronutrient

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malnutrition among consumers. Now agriculture is undergoing a shift from producing more quantity of food crops to producing nutrient-rich food crops in sufficient quantities. This will help in fighting "hidden hunger" or "micronutrient malnutrition" especially in poor and developing countries, where diets are dominated by micronutrient-poor staple food crops

What is Nutritional Quality: Quality refers to the suitability or fitness of an economicplant product in relation to its end use. Definition of quality varies according to our needs from the viewpoint of seeds, crop growth, crop product, post-harvest technology, consumer preferences, cooking quality, keeping quality, transportability etc. (Gupta *et al.*,2001). The nutritional qualityrefers tothe suitabilityor fitness of a Plant product for human and animalconsumption.

Strategies to Tackle Malnutrition

1. **To increase diversity of food intake:** Dietary diversity and supplementation with vitamins and minerals might be the best ways to tackle malnutrition but these are impractical in developing countries, where poverty is widespread.
2. **Fortification**: In this nutrient are added artificially to the food. For example, Salt iodization was introduced in the early 1920s in both Switzerland and the United States of America and has since expanded progressively all over the world to the extent that iodized salt is now used in most countries, but it requires government co-ordination and distribution.
3. **Bio fortification**: An alternative approach is to tackle the problem of nutritionally poor staple crops at its source by increasing their nutritional qualities through a strategy known as 'bio fortification', which should translate into improved diets.
    a. **Agronomic Augmentation:** The simplest of these tactics relies on an increase of the mineral content of plants through the addition of the appropriate mineral as an inorganic compound to the fertilizer. This method has been successful in some instances but depends on the crop species and cultivar, the mineral itself and the quality and properties of the soil, making the strategy difficult to apply generally.
    b. **Conventional breeding**: To improve the nutrient content of plants by conventional breeding, sometimes in combination with mutagenesis. The identification of mineral-dense cereal varieties and the use of markerassisted selection to such traits into widely cultivated, adapted germplasm have recently been reviewed Mutagenesis

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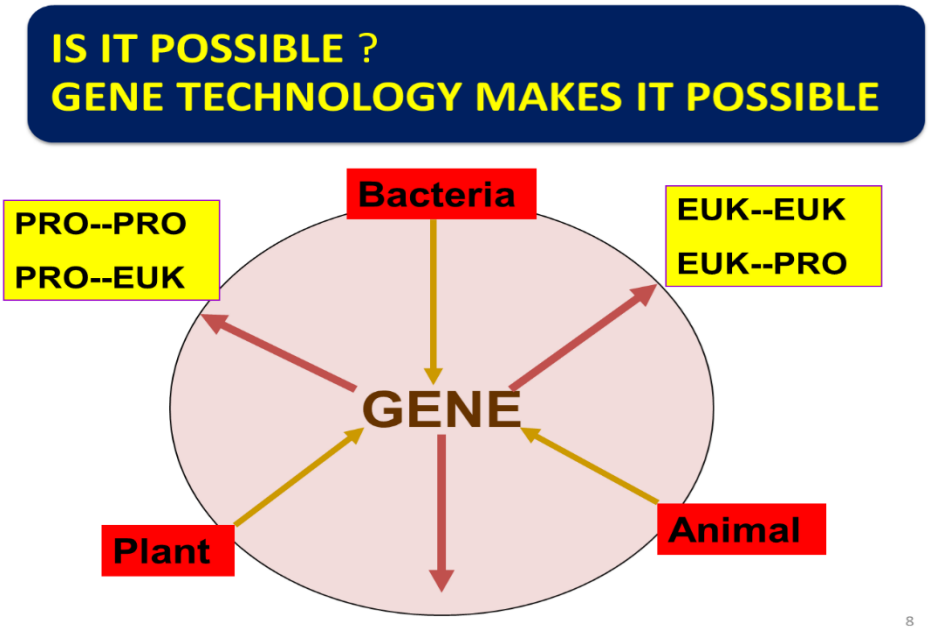
has also been used to produce crops with higher nutrient levels, including the lysine-enriched maize opaque-2 mutant. One problem with these conventional breeding approaches is the time taken to identify useful traits and breed them into elite cultivars.

c. **Genetic engineering**: A rapid way to introduce desirable traits into elite varieties. Transgenic strategies differ from other approaches in that novel genetic information is introduced directly into the plant's genome.

Transgenic Technology: Art and science of creating designer crops.

- **TRANSGENESIS:** The process of introducing an exogenous gene called transgene into living organism so that the organism exhibits a new property and transmit the property to its offspring.
- **TRANSGENIC PLANTS:** Genetically modified plants in which foreign gene have been introduced into the targeted plants.
- The production of transgenic plants is achieved by recombinant technology that enables the transfer of transgenes from any biological system into the plant cells and integrate them in to the plant genome. It even removes kingdom barriers to transfer a gene from one organism to another.



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STATUS OF GM CROPS IN WORLD: Commercial cultivation of transgenic crops started in the early 1990s. GM crops are now commercially planted on about 100 million hectares in some 22 developed and developing countries. To date, nearly 525 different transgenic events in 32 crops have been approved for cultivation in different parts of the world. In the last 22 years, the global area of transgenic crops has increased significantly from 1.7 million hectare in 1996 to 191.7 million hectares in 2018, i.e. around 113-fold increases (ISAAA 2018). There were 95.9 million hectares (50%) of transgenic soybean, 58.9 million hectares (31%) of transgenic maize, 24.9 million hectares (13%) of transgenic cotton, 10.1 million hectares (5.3%) of transgenic canola and 1.9 million hectares other crops. Thus, transgenic technology is regarded as the fastest crop technology to be adopted in modern agriculture.

STATUS OF GM CROPS IN INDIA: Bt cotton is the only genetically modified (GM) crop that has been approved for commercial cultivation in 2002 by the Government of India. Network Project on Functional Genomics and Genetic Modification in Crops was launched by ICAR in 2005 for development of GM crops. Bt Brinjal resistant to brinjal shoot fly was approved by GEAC in 2009, no further action on commercialization has been taken.The Parliamentary Standing Committee on Science and Technology, Environment and Forests, recommended GM crops should be introduced only after criticalscientific evaluation (August 25, 2017). Two new transgenic varieties of indigenously developed Bt Brinjal namely Janak and BSS-793, containing Bt Cry1Fa1 gene have been developed by the NIPB New Delhi, released commercially only after taking no-objection certificate (NOC) from concerned states.The indigenous transgenic varieties of mustard Dhara Mustard Hybrid 11 (DMH 11) developed by Delhi University is pending for commercial release.

DEVELOPMENT OF TRANSGENIC CROPS

1. Nucleic acid extraction

Nucleic acid extraction, either DNA or ribonucleic acid (RNA) is the first step in the genetic engineering process. It is therefore important that reliable methods are available for isolating these components from the cell. In any isolation procedure, the initial step is the disruption of the cell of the desired organism, which may be viral, bacterial or plant cells, in

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order to extract the nucleic acid. After a series of chemical and biochemical steps, the extracted nucleic acid canbe precipitated to form thread-like pellets ofDNA/RNA.

2. Gene cloning

➢ **PRODUCTION OF DNA FRAGMENTS**

- cDNA library

Complementary DNA is a copy of messenger RNA may be produced with the help of reverse transcriptase. The cDNA may be cloned in *E.coli* to create cDNA library. It is essential to use cDNA copy of the gene when the expression of a eukaryotic gene is required in a bacterium.

- Oligo nucleotide synthesizers / gene machine

The base sequence of a gene can be deducted from amino acid sequences of protein, or the base sequence of the m-RNA produced by it a DNA molecule having base sequence can be chemically synthesized by oligo nucleotide synthesizers/ gene machine.

- Genomic library

Genomic library of an organism is collection of plasmidclones phage lysates containing recombinant DNA molecules so that the sum total of such DNA molecules in the collection represents the entire genome of the organism in question.

➢ **JOINING TO A VECTOR**

This genomic DNA is treated with specific enzymes called restriction enzymes cutting it into smaller fragments with defined ends to allow it to be cloned into bacterial vectors.

➢ **PROPAGATION IN A HOST CELL**

Introduction of the recombinant DNA in to a suitable host is called transformation. Almost as a rule all DNA segments are first cloned in a suitable strain of E.coli to generate their multiple copies.

➢ **SELECTION OF TRANSFORMED HOST CELL**

Generally a gene called reporter gene is combined with the gene being transformed with a to facilitate the selection of transformed cells

➢ **EXPRESSION OF THE TRANSFERRED GENE**

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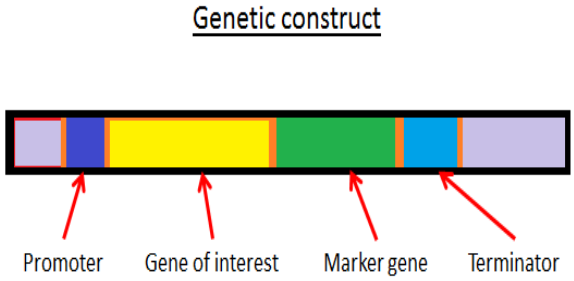
A special class of vectors called expression vectors has to be constructed to permit the expression of the cloned genes in the host cells. Such vector must have suitable regulatory sequences such as promoters, operators and ribosomes binding sites.

3. Designing Genes for Insertion

A typical plant gene consists of promoter,terminating sequences, reporter gene, selectable marker



a. PROMOTER SEQUENCE

It is the ON/OFF switch that controls when & where in the plant the gene will be expressed Promoters allow differential expression of genes. For instance, some promoters cause the inserted genes to be expressed all the time, in all parts of the plant (constitutive) whereas others allow expression only at certain stages of plant growth, in certain plant tissues (Tissue specific), or in response to external environmental signals (Induced promotor) or chemically synthesized. The amount of the gene product to be expressed is also controlled by the promoter.

b. TERMINATING SEQUENCES

The end of the gene coding sequences is specified by chain termination codon TAG (AUG in m-RNA), it is followed by a stretch of nontranslated region and at the end is present the poly adenylation site, which denotes the end of transcription.

a. REPORTER GENE

Reporter genes are cloned into the vector near the gene of interest,tofacilitatetheidentificationoftransformedcellsaswellastodetermine the correct expression of the inserted gene:

i. **SCORABLE REPORTER GENE:** Expression of the genes is detected by highly sensitive enzymatic assays.

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ii. SELECTABLE REPORTER GENE: Expression of genes is detected by resistance to a toxin.

d. SELECTABLE MARKER

Selectable marker genes are usually linked to the gene of interest to facilitate its detection once inside the plant tissues. Genetic engineers used antibiotic resistance and herbicide resistance marker genes to detect cells that contain the inserted gene. Cells that survive the addition of marker agents to the growth medium indicate the presence of the inserted gene.

4. METHODS OF GENE TRANSFER

a. INDIRECT

i. BIOLOGICAL

a. Agrobacterium mediated- *Agrobacterium tumefaciens*, a soil bacterium known as "nature's own genetic engineer".It causes crown gall disease. Plant cells infected with the bacterium will not form galls, but produce cells containing the desired gene which when cultured in a special medium will regenerate into plants and manifest the desired trait.

b. Virus mediated- Through this process, a virus causes harmful transformations of an in vivo cell or cell culture. The term can also be understood as DNA transfection using a viral vector.

b. DIRECT - Vector less & naked DNA

ii. PHYSICAL

- **Gene gun-** A biolistic particle delivery system, originally designed for plant transformation, Device for delivering exogenous DNA (transgenes) directly in to the cells
- **Micro/Macro injection-** Introduction of cloned genes into plant cells by means of very fine needles or glass micropipettes (dia: 0.5-10µm).The microinjection technique is a direct physical approach, and therefore host- range independent, for

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introducing substances under microscopically control into defined cells without damaging them.

- **Electroporation-** Uses electrical pulses (high intensity electric field) to produce transient pores in the plasma membrane (destabilizes the membrane) there by allowing DNA in to the cells. When the electric field is turned off, the pores in the membrane reseal, enclosing the DNA inside.

iii. **CHEMICAL**

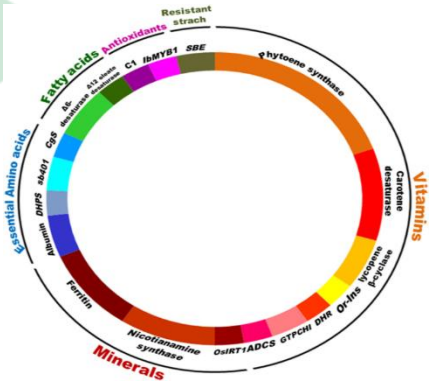
- **PEG-** Usually one cell lacking walls i.e, protoplast are used. Protoplast are incubated with a solution of DNA and PEG (in case of PEG mediated transfer). Catechol was the most potentiential chemical in inducing transformation at concentrations of 1–30µm, followed by hydroquinone (3–30 µm), phenol (10–100 µm) and benzene (only at 100 µm).

5. SELECTION OF SUCCESSFULLY TRANSFORMED TISSUES

Following the gene insertion process, plant tissues are transferred to a selective medium containing an antibiotic or herbicide, depending on which selectable marker was used. Only plants expressing the selectable marker gene will survive, and it is assumed that these plants will also possess the transgene of interest. Thus, subsequent steps in the process will only use these surviving plants.

Nutritional Quality Traits

- **Nutrients (Nutraceuticals)** Macro: Protein, Carbohydrates, Fats, Fiber
- Micro: Vitamins, Minerals, Antioxidants, Isoflavinoids, Phytoestrogens, Condensed tannins
- Anti-nutrients: Phytase, Allergen and Toxin removal



Utilization of different genes for biofortification by transgenic means. Large numbers of genes have been utilized for crop biofortification. Transgenic based approach has

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advantages that a useful gene once discovered, can be utilized for targeting multiple crops. Some important genes like phytoene synthase, carotene desaturase, nicotinamide synthase and ferritin have been utilized in multiple events including multiple crops.

TRANSGENIC CEREALS

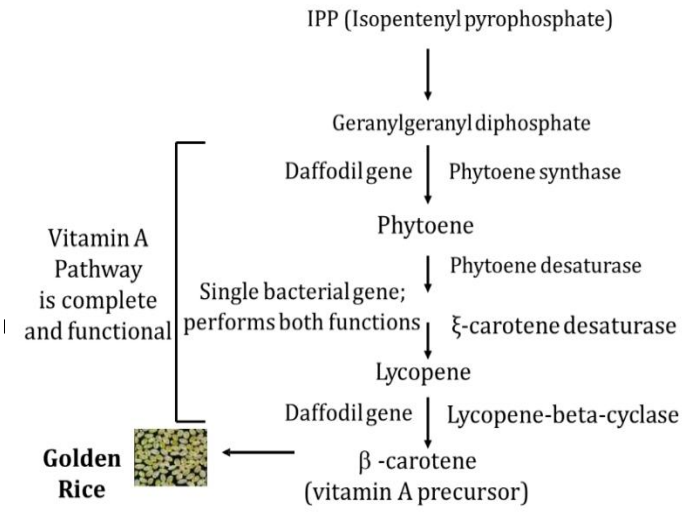
Transgenic Rice (Oryza sativa): Rice has been targeted to address the global challenge of under nutrition.

VITAMIN A

GOLDEN RICE -1

Golden rice was developed in Europe in 2001 by Ingo Potrykus, it is intended to use be used to reduce the vitamin A deficiency. Golden Rice technology is based on the simple principle that rice plants possess the whole machinery to synthesis of β-carotene, and while this machinery is fully active in leaves, parts of it are turned off in the grain endosperm. It is a genetically modified food crop of rice which contains β- carotene synthesis pathway is activated in the rice endosperm that is gives the characteristic golden colour.

Comparision of golden rice and normal rice



Golden rice was developed with introducing three genes encoding the enzymes phytoene synthase, carotene desaturase and lycopene β-cyclase. Phytoene synthase (*psy*) gene derived from daffodils

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(*Narcissus pseudonarcissus). Psy is a* transferase enzyme involved in the biosynthesis of carotenioids. It catalyzes the conversion of Geranylgeranyl diphosphate to phytoene. Carotene desaturase and Lycopene cyclase (*crt1*) gene isolated from soil bacteria *Erwina uredovora,* produce enzymes and catalysts for the synthesis of carotenoids in the endosperm of rice.

β -Carotene Pathway Genes Added

Limitations of golden rice

Original Golden Rice (GR1) does not produce enough ß-carotene (Provitamin A). It produces only 1.6 µg/gm of carotenoids. Unexpected effect seen due to the insertion genes. It was supposed to produce lycopene (as in tomatoes) and so be bright red; instead, it produced ß-carotene due to unexpected metabolic pathway. Health and Environmental risks regarding GM crops. Due to this reason, there is low acceptance in public.

GOLDEN RICE -2

In 2005 Syngenta, produced golden rice -2. Evaluation of phytoene synthase from several plant sources led to the identification of the *psy* gene from maize as the most efficacious source, resulting in the greatest accumulation of total carotenoids and β- carotene. They combined, the phytoene synthetase gene (*psy*) from maize and the carotene desaturase gene (*crtl*) from *Erwinia uredovora were* inserted into rice. Produced up to 23 times more carotenoids than golden rice (up to 37 µg/g), and preferentially accumulates beta- carotene (up to 31 µg/g of the 37 µg/g of carotenoids).

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FOLIC ACID (Vitamin B9)- Rice has been genetically modified to increase folate content (up to 150-fold Up to 150-fold increase by overexpressing genes encoding Arabidopsis GTP-cyclohydrolase I *(GTPCHI*) and aminodeoxychorismate synthase *[ADCS]*.

IRON CONTENT

Rice has also been targeted to address the global challenge of iron deficiency anemia. Multiple reports have indicated an increase in iron content in rice by expressing genes encoding genes - Nicotianamine aminotransferase, iron transporter *OsIRT1*, nicotianamine synthase 1 *(OsNAS1)* and 2 *(OsNAS2)*, soybean ferritin and common bean ferritin. In addition to enhanced iron content, improvement in iron bioavailability was also achieved by reducing antinutrient compounds in rice such as phytic acid.

ZINC CONTENT

Zinc content was also elevated in GM rice by overexpressing OsIRT1 and mugineic acid synthesis genes from barley [HvNAS1, HvNAS1, HvNAAT-A, HvNAAT-B, IDS3].

PROTEIN QUALITY

Improvement in quality protein has been addressed by targeting essential amino acid content in rice by expressing seed-specific genes of bean β-phaseolin, pea legumin etc.

Wheat (*Triticum aestivum*)

Wheat is one of the most widely grown staple food crops in the world. Researchers have tried to address the challenges of most deficient nutrients like vitamin A, iron, and quality proteins through wheat. The **provitamin** A content of wheat has been enhanced by expressing bacterial PSY and carotene desaturase genes [CrtB, CrtI]. The**iron content** in wheat has been enhanced by expression of ferritin gene from soybean and wheat [TaFer1-A]. To increase iron bioavailability phytase activity was increased by the expression of the phytochrome gene [phyA] and phytic acid content has been decreased by silencing of wheat ABCC13 transporter. Protein content, especially essential amino acids lysine, methionine, cysteine, and tyrosine contents of wheat grains were enhanced using Amaranthus albumin gene [ama1].

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Transgenic Maize (*Zea mays*)

Maize is one of the important staple crops in developing countries, and it has been addressed for vitamins, minerals, quality protein, and antinutrient components by means of genetic engineering. Maize endosperm has been enriched with **provitamin A** (carotenoids) by expressing bacterial crtB andmultiple carotenogenic genes by expressing bacterial crtB and multiple carotenogenic genes in Maize endosperm.. Tocotrienol and tocopherol (Vitamin E) content in maize has been increased by overexpression of homogentisic acid geranylgeranyl transferase.Vitamin C level in corn has been enhanced nearly 100-fold times by recycling oxidized ascorbic acid to reduced form by the expression of dehydroascorbate reductase

Transgenic legumes

Transgenic Soybean (*Glycine max*), Soybean is a global source of vegetable oil and high-quality protein. The soybean has been targeted to increase provitamin A (beta-carotene), a monounsaturated ω-9 fatty acid (oleic acid) and seed protein contents by expressing bacterial PSY gene. In a different approach **provitamin A** was enhanced by expressing bacterial PSY [crtB, crtW, bkt1]. Soybean is rich in healthy **oil** and has approximately 20% oil content. But 7–10% of the oil contains unstable fatty acid α-linolenic acids that contribute to reduced soybean seed oil quality. It results in the formation of undesirable trans-fatty acid as a result of hydrogenation. To enhance the agronomic value of soybean seed oil by reducing the levels of α-linolenic acids (18:3), siRNA-mediated gene silencing-based approach has been utilized for silencing of ω-3 FAD3. Soybeans contain approximately 40% protein, but they are deficient in one or more of the essential **amino acids**, especially the sulfur-containing amino acids, cysteine and methionine. The cysteine and methionine content of soybean seeds has been increased through overexpression of, O-acetylserine sulfhydrylase and cystathionine γ-synthase respectively.

Antinutrients, Allergens and toxins

Biotechnology approaches can be employed to down-regulate or even eliminate the genes involved in the metabolic pathways for the production, accumulation, and/or activation of these toxins in plants. For example, the solanine content of potato has already been

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reduced substantially using an **antisense** approach, and efforts are under way to reduce the level of the other major potato **glycoalkaloid, chaconine**. Work has also been done to reduce cyanogenic glycosides in cassava through expression of the cassava enzyme **hydroxynitrile lyase** in roots. When "disarming" plant natural defenses in this way, we need to be cognizant of potentiallyincreased susceptibility to pests and diseases, so the base germplasm should have input traits to counter this.

Genetically Modified Organisms Pitfalls

Low acceptance among masses. Different countries have adopted different regulatory process. The same variety of bt brinjal developed by Indian scientists it released in Bangladesh but it is not released in India. Regulatory processes are very expensive and time consuming.Its success rate in terms of cultivar release is very low.Its dissemination is also being held back due to inability to get approval from Governments.

Problems Associated with production of transgenic plants

Low regeneration frequency associated with albinism and anthocyanin pigmentation and resolved by the use of additives.Low transformation frequency after co-cultivation, varied from 0.0001 to 5% in our experiments due to induction of hypersensitive response by *Agrobacterium*.Large number of escapes when Kanamycin is used as a selectable marker.Segregation problems in subsequent generations if homozygosity is not obtained.Problems of stability in subsequent generations due to gene silencing.

CONCLUSION

There is deficient micronutrient intake in well over half of people globally, notably women and children. Food crops are ideal vehicles for changing the balance of nutrient intake of whole world population. Though we have reached to the highest yield potential but it do not limits the nutritional requirement. Transgenic technology is one of the powerful weapons for fighting against for food, nutritional and health security. Due to several social, ethical and technological reasons, the discussion are going on around the world whether transgenics are boon or bane but further advancement on technological front, there are ample scope and opportunities for public acceptance for the transgenic. Both conventional and molecular

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breeding are effective strategies for potential economic enrichment of nutritional status to overcome malnutrition problem worldwide.

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