GENETIC APPROACHES OF INCREASING NUTRIENT USE EFFICIENCY ESPECIALLY NITROGEN IN CEREAL CROPS – A REVIEW

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Abstract

Nutrient use efficiency determines precisely a certain amount of plant yield in terms of grains or biomass per unit of applied nutrients. Crop plants contain many more elements but for their growth and development basically they require almost sixteen mineral nutrients, among which N, P, K are used in large amount and N covers manifold function for contribution of the yield attributes. In deficiency of any micro- or macro-nutrient and due to few environmental factors plant growth along with its yield are affected adversely. In addition to physiological and breeding strategies genetic progress and implications have characterized this area to some extent. As nutrient efficiency is expressed in several ways, this phenomenon is taken in a wrong sense among the concerned persons and thus, there should be a balance between optimum nutrient use efficiency and optimum crop productivity based on the selected phenotypic characters of crop plants. Nutrient use efficiency is said to be a complex trait. Even its different stages of action are also considered complicated in nature. In such case a single gene can provide huge benefit. This is why modern genetic tools and resources available to the scientists have provided a great possibility for increasing nutrient use efficiency in crop plants. Molecular biology offers possibility for improving the desired characters by introduction of the specific gene(s). Augmentation of nutrient content of crop plants is being caused through genetic engineering. This article makes review and discussion on the genetic approaches in terms of exploited genetic engineering and biotechnological tools for increasing the specific nutrients especially nitrogen in cereal crops.

Key words: Genetic Approach, Nutrient Use, Cereal Crops.

Introduction

Plants nutrients are divided into two groups and that are macronutrient (used in large quantities for plant structure) and micronutrient (used in small quantities for enzymatic process). Mengel and Kirby (1978) classified plant nutrients into four groups on the basis of their biochemical behaviour and physiological function. All these elements are also classified as cations, anions, metals and non-metals based on their specific functions in the plant body. Plants can absorb inorganic nutrients by themselves and organic form of nutrients undergoes mineralization for up-taking by plants. Also transportation of nutrients and their absorption by roots and translocation for assimilation in plant body happens eventually. Nutrient uptake by plant is a catenizing process. In passive process, ions move from higher to lower concentration and in active process, ions move against a concentration gradient.

Nutrient deficiencies vary among soils and areas, and N and P are the most deficient nutrients in temperate as well as tropical soils. Among the essential nutrients, K is absorbed in maximum amount by the modern improved crop cultivars (Fageria et al. 1991), when N is considered as a major limiting factor in plant productivity. Nevertheless, enormous use of N fertilizers in inorganic form for the last few decades showing a detrimental environmental effects. Now it has become essential to reduce N fertilizer pollution, rather than it is strengthening the importance of improving the nitrogen use efficiency of crop plants.

In an edited book by Tandon (1995) it has been said that micronutrients sometimes are of macro importance for producing qualitative high yields. Zinc (Zn) is a good case in point of micronutrient which has greatest importance in plants life. In terms of nutritional constrains Zn's position is next to N and P. Zinc deficiency is

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actually a great problem to crop plant as well as animals including human beings of Indian sub-continent. Iron (Fe) and Boron (B) are also next to zinc in its importance to the living word. Hence, it is very important task now for the scientist to ensure that the requirements of plants for micronutrients can be accounted by participation of all these elements in enzymatic reactions and as constituents of growth hormones. Since the functions of all the sixteen essential elements are character specific, they singly or sometimes by interaction among them form part of molecule, and becomes essential in plants life cycle. For example N is essential for protein and Mg is for chlorophyll in addition to their few other functions. Also the element exerts its effect directly on growth or metabolism and not by some indirect effect such as antagonisms of another element present at a toxic level (Fageria et al. 1991).

In developing countries micronutrient deficiencies result many types of health irregularities. A significant number of peoples take three cereals (wheat, rice and maize) as major source of dietary energy, but they are said to be very much poor for Fe nd Zn and even for vitamin A. So it has become purely an important task now to incorporate extra nutrients in food, as a matter of public health policy. Scientist always has targeted higher crop yields but nutrient contents on the contrary have never been considered as prime goal. Practically increase in crop yield as a result of chemical fertilizers (like N, P, K) application has been ultimately provide lower state of micronutrients. Now-a-days micronutrient deficiency is considered as limiting factor for crop yield and thus, to find out the yield potential crop, genetical correcting measure is very much necessary.

Fixen (2006) stated that the value of improving nutrient use efficiency is dependent on the effectiveness in meeting the objectives of nutrient use. Here, objectives are economical optimum nourishment to the crop, minimizing nutrient losses from the field, sustainability of soil quality components etc. However, in order to identify and understand the regulation of genes involved in enhancing nitrogen use efficiency (NUE) proper evaluation of combined genetic and transgenic approaches to improving NUE should be required as component of any crop improvement programme. The benefits of growing NUE efficient crops will not be realized until breeders evaluate N metabolism and nitrogen use efficiency in economically important crop plants (Shrawat and Good 2008).

Scientists have made remarkable improvement to unfold many plant genotypes with increased amount and uses of micronutrients in staple food crops. These are being exploited now-a-days for increasing micronutrient levels and also for eliminating antinutrient substances (phytic acids, tanins etc.) by breeding as well as genetic engineering techniques. Currently many transgenic plants have been obtained with increased amount of Fe, Zn, vitamin A and S-containing amino acids.

Transgenic strategies for nutritional enhancement may offer rapid and more selective way to introduce the desirable traits in crop plants. Molecular biologists are trying to provide tools for completely molecular and physiological analysis of transformed plants, and some of them are being used to increase nutrient content of crop plants, obviously for better quality food. This article makes review and discussion on the genetic and biotechnological tools, exploitable to increase the content of specific nutrients especially nitrogen in specific cereal crops.

**Genetic specificity and mineral nutrients**

Efficiency of using certain mineral elements for biomass production is considered as one of the important factors and it is highly related to genetic specificity of plants. Due to advancement of different experimental approaches, experiments on varietal specificity of mineral nutrition has been conducted in certain plant species, such as wheat, maize, sugar beet etc. from various methodological aspects. It has now become evident that individual plant species show specific requirements for certain mineral nutrients.

Micronutrient efficiency is genetically controlled, and the physiological and molecular mechanisms of micronutrient efficiency of plants are just beginning to be understand (Khoshgoftarmanesh et al. 2010). Large
ranges of genotypic variation in response to micronutrients deficiency stress have been reported in different plant species, particularly in cereals (Graham et al. 1992, Cakmak et al. 1997, 1998). Such large variation is said to be promising for developing the plant genotypes. Graham and Rengal (1993), Bouis (1996) and Graham and Welch (1996) stated that micronutrient efficient genotypes may provide a number of other benefits, such as reduction in the use of fertilizers, improvement in seedling vigour, resistance to pathogens, and enhancement of grain nutritional quality.

**Plant nutriomics**

Li (1985) stated that a second green revolution is required that does not rely on intensive fertilization; rather it would aim at improving crop yields in soil with reduced fertilizer application. This would be possible if new crop varieties are developed with enhanced adaptation to low fertility soils. This approach may be applicable for both soils with over fertilization in high input areas, and soils of low fertility in low input areas that are deficient in a number of major nutrients such as N, P, K and other essential elements. However, it would be preferable to identify and select specific traits that are directly related to a specific nutrient efficiency (Yan et al. 2006). Once clearly identified, these traits could be used for more efficient screening in controlled environments, or tagged with molecular markers and then improved through marker assisted selection or gene transformation. The author also suggested that useful traits of nutrient efficiency may be associated with altered physiological and biochemical pathways in adaptation to nutrient stress. To achieve it, a systemic study is needed to understand genomic, transcriptomic, proteomic and metabolic aspects of nutrient efficiency, which is called as a whole *plant nutriomics*.

Plant nutrition studies look at nutrient efficiency mainly at the whole plant level, although useful studies with whole plant can not provide sufficient insight into the genetic nature and its specific modification of the nutritional processes (Yan et al. 2006).

Recent studies in molecular biology have provided possible means to tackle the complex plant nutritional problems through genetic approaches, which together with phenotypic analysis can elucidate the functions and interactions of plant nutrients at the molecular, cellular, organ and whole plant levels. This concept of plant nutriomics was presented schematically by Yan et al. (2006). However, this concept based on essential mineral nutrients is redrawn as follows (Fig. 1).
Nitrogen use efficiency (NUE)

In case of nitrogen use efficiency (NUE) transgenic overexpression of primary N assimilatory genes in model plants did not yield any significant improvement (Lightfoot 2009, Pathak et al. 2008), although physiological experiments on wheat indicated that the expression level of primary N assimilatory enzymes might matter for NUE in some cases (Pathak et al. 2011). They stated further, marker studies indicated that genes of secondary N metabolism could be more critical for NUE than the genes of primary N assimilation, especially in cereals which mobilized internal N pools from senescing leaves during grain filling. Apart from genes belonging to the N assimilatory pathway, various other candidates are being identified through screening of different varieties for their NUE under limiting conditions (Chardon et al. 2010).

Biotechnological interventions to improve nitrogen use efficiency (NUE) have largely revolved around manipulation and overexpression of many crucial candidate genes apart from using knockout mutations to assess its effect on biomass and plant N status and overall yield (Good et al. 2004; Masclaux-Daubresse et al. 2010, Pathak et al. 2008). In case of cereals, the total grain biomass or grain N content would be most suitable indicators of NUE, rather than any other organ (Pathak et al. 2011). The same authors suggested that areas where NUE can be targeted is to improve the distribution of N between leaves and stem and roots, better photo-synthetic rate/unit leaf N, reduced leaf senescence, transgenic developing C_4 options for rice and wheat.

Pathak et al. (2011) stated that there are two types of nitrate uptake system and these are (i) low affinity transport system encoded by NRT1 gene family and (ii) high affinity transport system encoded by NRT2 gene family. In addition, a number of ammonium transporters and putative amino acid transporters have also been
identified in Arabidopsis. Though the various nitrate and ammonium transports in plants are very well characterized and their differential regulation mechanisms are well known, overexpression studies involving their genes have not been conclusive. Pathak et al. (2011) have presented several attempts at transgenic manipulation of the enzymes of primary and secondary assimilation, from there the plant transgenics and the observed phenotypes of cereal crops only are mentioned below (Table 1).

**Table 1.** Plant transgenics and observed phenotypes in cereal crops.

<table>
<thead>
<tr>
<th>Gene product</th>
<th>Gene source</th>
<th>Promoter</th>
<th>Target plant</th>
<th>Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS2-Chloroplastic glutamine synthetase</td>
<td>O. Sativa</td>
<td>CaMV35S</td>
<td>O. Sativa</td>
<td>Enhanced photorespiration, salt tolerance</td>
</tr>
<tr>
<td>GS1-Cystolic glutamin synthetase</td>
<td>P. vulgaris</td>
<td>Rubisco</td>
<td>T. aestivum</td>
<td>Entrance capacity to accumulate nitrogen</td>
</tr>
<tr>
<td>NADH-GOGAT-NADH dependent glutamate synthetase</td>
<td>O. sativa</td>
<td>O. sativa</td>
<td>O. sativa</td>
<td>Enhanced grain filling, increased grain weight</td>
</tr>
<tr>
<td>GDH-glutamate dehydrogenase</td>
<td>E. coli</td>
<td>OsUBI</td>
<td>Z. mays</td>
<td>Increased N assimilation, herbicide tolerance, biomass, grain aa content</td>
</tr>
<tr>
<td>GDH-glutamate dehydrogenase</td>
<td>C. sorokiana</td>
<td>CaMV35S</td>
<td>T. aestivum</td>
<td>Schmidt and Miller, 2009 (patent No. 627, 886)</td>
</tr>
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</tr>
<tr>
<td>OsENOD93-1</td>
<td>nodulin gene</td>
<td>35sC4PDK</td>
<td>O. sativa</td>
<td>Increased shoot mass and seed yield.</td>
</tr>
</tbody>
</table>

**Source:** Pathak et al., 2011

Remobilization of N is considered as one of the important step in improving NUE in plants (Masclaux-Daubreasse et al. 2010). In case of cereals, grains main source of N is found to be N remobilized from the vegetative parts. It accounts for 60-92% of the N accumulated in grain and the remobilization rate depends upon availability of N and remobilization efficiency. However, it is know that environmental factors along with genotype affect the phenomenon and eventually genes take part for translocation and remobilization attractive targets for improving nitrogen use efficiency.

There are several reports of transgenic overexpression of AS genes that are actively involved in remobilization and translocation of amino acids resulting in enhanced seed protein content and total protein content (Pathak et al. 2011). Molecular manipulation of asparagine synthetase (encoded by Aln 1 gene) has been attempted recently along the reports of genetically engineered plants overexpressing alanine amino transferase (Good et al. 2007). As translocation and remobilization of N discussed by Pathak et al. (2011), genetically engineered rice (Oryza sativa) was developed by introducing barley alanine aminotransferase complementary DNA (cDNA) driven by tissue specific OsAnt1 promoter (Shrawat et al. 2008). These plants showed improved biomass and grain yield along with significant change in key metabolites and nitrate content confirming increased NUE. However, Beatty et al. (2009) reported the involvement of candidate gene through root and shoot transcriptome analysis in engineered rice over expressing alanine amino transferase (AlaAT) under the control of tissue specific promoter showing a strong NUE phenotype. The role of GDH in N remobilization is said to be controversial, but transgenic plants over expressing gdhA gene were shown to have improved amino acid content and thereby higher yields in maize and wheat (Lightfoot 2009).

The genes act for N metabolism and nitrate signaling are found to be tightly regulated by sugar signaling mechanism. Pathak et al. (2011) said that a coordination between N and C metabolism is required at the amino acid synthesis level due to requirement of carbon skeletons for their synthesis, SnRK1, a principal
regulator in carbon signaling, is know to be linked to N and amino acid metabolism. Nitrate is a potential signal that affects N and C metabolism as well as organ growth and development in plants.

However, overexpression of cystolic glutamine synthetase GS2 genes was performed in Oryza sativa using CaMV35S promoter (Hoshida et al. 2000). More recently, overexpressing a GS1 isoenzyme of maize in maize under the control of the CsVMV promoter increased kernel number and kernel size, thus increasing yield by 30% (Masclaux Daubresse et al. 2010). Although overexpression of GOGAT genes has been rare, Yamaya et al. (2002) reported a spectacular effect of the overexpression of NADH-GOGAT under the control of its own promoter in rice. Transgenic plants showed an increase in grain weight. They concluded the studies saying that overexpression of GS or GOGAT genes can improve biomass and grain yields depending on which gene allele and which promoter are used.

For nitrogen storage under anaerobic stress alanine performs the function as a major amino acid. Overexpression of barley alanine aminotransferase under the control of root promoters in canola and rice had interesting effects, considerably increasing plant biomass, seed yield, NUE and shoot nitrogen concentration when plants were grown at low nitrate supply (Good et al. 2007; Shrawat et al. 2008). Hibbered et al. (2008) suggested that engineering C4 rice or C4 wheat would be a good way to improve NUE.

**Manipulation of nitrogen remobilization**

Nitrogen remobilization is a complex metabolic process, but it is important for plant productivity because through this mechanism organic nitrogen is recycled to developing leaves and storage organs (Masclaux et al. 2001). Therefore, grain yield of cereals does not depend only on uptake of nitrate before flowering, depends also on the remobilization of leaf N during the maturation of seed. About 80% of the total nitrogen in panicle of rice arises due to remobilization through phloem from senescing organs (Tabuchi et al. 2007). Consequently, efforts have been made for identifying genes encoding proteins, which are activated specifically during remobilization of nitrogen, carbon and minerals at the time of leaf senescence (Gaills and Hirel 2004). Brugiere et al. (2000) suggested that cytosolic GS (GS1) reassimilates ammonium released from protein hydrolysis. GS1 facilitates synthesis of Gln, which is the major form of reduced N in phloem sap, and NADH-GOGAT1 is important for developing sink organs for Gln remobilization in rice (Andrews et al. 2004). This is why, in case of senescing organs of plants synthesis of Gln is said to be an important step for recycling of nitrogen.

Martin et al. (2006) investigated the roles of two genes encoding cytosolic maize GS1 (gln-3 and gln-4) by examining the impact of knockout mutations on kernel yield and by overexpressing gln1-3 in maize. They found gln1-4 and gln1-3 double mutants to exhibit reduced kernel size and kernel number, without reduction in shoot biomass production at maturity. Hirel et al. (2007) reported that transgenic maize plants overexpressing gln1-3 gene produced greater kernel numbers under both high and low nitrogen conditions compared to that of wild type. These findings in maize suggest that GS1 plays important role for kernel yield under both low and high nitrogen fertilization.

In case of rice GS1 knockout mutants made by inserting retrotransposin Tso17 into exon-8 or exon-10 of Osgs 1;1 exhibit a remarkable reduction both in growth and grain filling, grown under normal N fertilization. But reintroduction of Osgs 1;1 cDNA under the control of its own promoter into the mutants complement successfully the slow growth of the plants. This study indicates that GS1;1 is important for normal growth and grain filling in rice, and GS1; 2 and GS1;3 are not able to compensate the function of GS1;1 (Tabuchi et al. 2005, 2007).

**Microarray analysis**

It is a technology for measuring transcriptome expression analysis and it has been applied to some important cereal crops to examine the genetic response. Lian et al. (2006) measured the effect of low nitrogen stress
on rice by reducing the applied ammonium nitrate for 2 hours. They found over 400 overexpressed sequence tags (ESTs) to be regulated differentially in rice roots, and nitrogen uptake and assimilation transcript were found to be unaffected. Transcriptome changes between nitrate-fed ammonium-fed rice were detected by Zhu et al. (2006). They found 198 differentially expressed genes (DEGs) involved in signal transduction, stress response, transcriptional regulation and metabolism. Lu et al. (2005) made an analysis on the gene expression changes in wheat, applying both organic and inorganic nitrogen separately. They found organic nitrogen treatment to show the largest number of up-regulated nitrogen uptake and assimilation genes, including AlaAT. On the contrary, they found low expression of nitrogen metabolism genes in case of low inorganic nitrogen treatments.

Beatty et al. (2009) stated that there have been relatively few microarray studies measuring the effect of a transgene on the plant transcriptome and most of the reports have analysed overexpressed regulatory proteins. Effect of the overexpression of the Zea mays transcriptional factor DREB2A on the Zea mays transcriptome was measured by Maruyama et al. (2004). They reported 51 genes to be expressed differentially in transgenic relative to wild type plants under non-stressed conditions.

Hirose et al. (2007) overexpressed the response regulator OsRR6 (negatively regulates cytokinin signaling) in rice and found 667 and 641 transcripts up-regulated in roots and shoots, respectively. They also found 591 and 962 transcripts down regulated in roots and shoots, respectively. In both the cases they used a two-fold cut-off. Baudo et al. (2006) overexpressed the storage protein glutenin in wheat using a 1.5 fold expression difference cut-off. They found five differentially expressed transcripts from a 9426 EST cDNA wheat microarray. Their findings indicate very little effect of the overexpressed storage protein gene on the wheat transcriptome.

**Transgenic efforts to nutritional enhancement**

Transgenic efforts may enhance the nutritional value of crops. This approach can make the way very much rapid to introduce desirable traits into elite varieties. Zhu et al. (2007) stated that transgenic approaches differ from other approaches in that novel genetic information is introduced directly into the plants genome. The chosen approach depends predominantly on whether the nutritional compound is synthesized de novo by the plant or obtained from the environment. They further stated that organic molecules, such as amino acids, fatty acids and vitamins are synthesized by plant. Capell and Christou (2004) said that increasing the nutritional value requires some form of metaboling engineering with the aim of increasing the amount of these desirable compounds, decreasing the amount of a competitive compound or even extending the existing metabolic pathway to generate a novel product.

Modern genetic and molecular technologies provide a number of tools that can be utilized for the development of staple foods with a higher iron and zinc content and improved bioavailability of these minerals (Holm et al. 2002). Their research summarizes current strategies aimed at increasing the iron-sequestering capacity of the endosperm and improving mineral bioavailability via in planta synthesis of microbial phytases. They have presented a case study of wheat and have discussed the future strategies addressing the importance of phytase thermostability. Their work was to engineering wheat for constitutive and endosperm-specific expression of an A. niger phytase (Brinch-Pedersen et al., 2000). Their design was comprised of the strong constitutive promoter from the maize ubiquitin 1 gene and the A. niger PhyA gene. To ensure targeting to the cell wall, Holm et al. (2002) introduced a signal sequence from barley a-amylase upstream of the PhyA gene (Ubi-Sp-phyA). The constructs were then introduced into wheat mature embryos by particle bombardment and transgenic regenerable cell lines selected using the bar-Bialaphos selection technique. In their study, western immunoblotting with polyclonal antibodies raised against the Aspergillus phytase indicated that a phytase of the expected molecular weight had been synthesized and further that the protein as expected was glycosylated. The A. niger phytase contains 10 Asn-linked consensus sites (Asn-
Xaa-Ser/Thr) and the mature fungal enzyme is known to be secreted, glycosylated protein (Van Hartingveldt et al., 1993). However, Holm et al. (2002) stated in their case study that at the early and mid stages of grain filling, the heterologous phytase was primarily synthesized in one or more tissues of the pericarp, seed coat and aleurone, whereas the endosperm was the primary site for phytase synthesis toward the end of grain filling. Their study on progeny analyses revealed that the transgenic trait was transferred to the next generation and that there was an up to fourfold phytase activity than measured in wild type seeds.

However, in recent transformation studies, endosperm specific expression of soybean (Goto et al. 1999) or Phaseolus vulgaris (Lucca et al. 2001b) ferritin gene in rice resulted in an up to threefold increase or doubling, respectively, of the iron content of the seed. This implies that the low iron concentration in the seed may not result from low iron availability for transport, but rather from a lack of sequestering capacity in the seed (Holm et al. 2002). On the other hand, Drakakaki et al. (2000) stated that when the soybean ferritin gene expression was driven by a so-called constructive promoter to increase ferritin synthesis throughout the plant, there was only an increase in the iron content of the vegetative parts but not in the seed.

In developing countries, the most widely used cereal crops are wheat and rice. In these crops, very small fraction (Wheat 20% and Rice 5%) of iron (Grusak and Dela Penne 1999, Grusak et al. 1999, Miller et al. 1993) is transported from the senescing leaves to the grain. In contrast >70% of zinc is mobilized (Grusak et al. 1999). In cereals the two minerals are almost exclusively stored in the husk, the aleurone and the embryo and therefore, large proportions of minerals are lost during milling and polishing (Welsh and Graham 1999). This implies that the full potential of the genotype determined increments in iron and zinc content is not related for improving human nutrition and in this context, the mechanisms underlying iron and zinc transport and deposition in different tissues of grain are of particular importance (Holm et al. 2002), from transgenic point of view.

However, in genetic studies many sorts of drawbacks are being alleviated by using molecular markers associated with the traits of interest. Molecular marker genotype can eliminate the undesirable trait and important for selection of complex traits where multiple genes are involved. Marker assisted selection for complex trait like yield with essential nutrients may be an essential tool in producing new varieties.

Genetic engineering

Substantial genetic variations in different food crops for micronutrient and vitamin A are being exploited now-a-days to increase the micronutrient levels and also for eliminating antinutrient chemicals (phytic acid, tannins) by conventional breeding techniques as well as genetic engineering techniques. So in future priority should be given for developing plant genotypes with enhanced amount of micronutrient through genetic approaches. Genetic engineering techniques can be used to create novel cultivars with desired properties (Lonnerdal 2002, 2003).

Examples of this type of approaches are the insertion of novel genes, enhancement of the expression of genes, present but at low level, and depression of the expression of genes or disruption of the pathways involved in the synthesis of inhibitors of nutrient element absorption. Two novel iron-binding proteins have been incorporated into rice (Lonnerdal 2003). Compared to other cereal crop plants rice proteins have low allergenicity and this crop contains no toxic compound. Insertion of a gene for a protein that can bind more metal ions per molecule is needed to achieve a trace element content that is high enough to meet the iron requirement. Ferritin is that type of protein, whose each molecule can bind as many as 4500 atoms of iron (Theil et al. 1997). The gene for soybean ferritin has been inserted to rice (Goto et al. 1999). These experiments seem to have been done on a smaller scale, but the iron content in a few transformants is 2-3 fold than that of wild type (Lonnerdal 2003). A research group has inserted the ferritin gene from Phaseolus vulgaris into rice (Lucca et al. 2001a).
Increasing nutrient use efficiency in cereal crops

Genetic engineering allows incorporation of gene from unrelated organisms to plants through sophisticated and artificial techniques. It leads to direct access and manipulation of genetic information contained in DNA and also leads to create synthetic genes. This technology reduces time taken to obtain the improved cultivar. A flow chart of Agrobacterium mediated transformation of cereal crops for a specific gene is show below in Fig. 2.

![Flow chart of Agrobacterium mediated transformation of cereal crops for a specific gene.](image)

Genetic engineering techniques are only used when other technique are exhausted and when (i) the trait to be introduced is not present in the crop; (ii) the trait is so difficult, not possible to improve it by conventional breeding methods; and (iii) through conventional breeding method it will take a very long time to introduce the traits.

This technology aims in increasing plant productivity. It has been possible by molecular biologists to identify gene(s) whose products participate in diverse metabolic pathways. It is most applicable to compounds of nutritional importance. It may be called nutritional genomics and the great example of this phenomenon is modification of genes, which resulted synthesis of vitamin E in plants.

**Recombinant DNA technology**

Recombinant DNA technology allows the modification of DNA by the enzyme like endonucleases, lipases and polymerases and these are used by scientists to isolate, characterize and modify the genes, and thereby transferring them into the same or different organism. Hence, the development of transgenic plants involves gene identification and modification of the target gene for expression in crop plants. However, transfer of this modified gene into the plant cells is done by in vitro techniques or natural methods. The in vitro technique
makes the plants containing a high copy number of rearranged or catenated transgenes. This methodology has been found to be simple and efficient in case of monocotyledonous plants, including cereals. For example, recombinant DNA technology was used to improve the provitamin A content of rice grain endosperm in Golden rice (Ye et al. 2000). Goto et al. (1999) transformed rice plants using a phytoferritin (Protein storage form of Fe in plants) gene form soybean and rice endosperm promoter gene to enrich Fe in the rice grain endosperm. Lucca et al. (2001a) also enriched Fe in rice grain endosperm using the phytoferritin gene from pea plants.

Amino acids and transgenes

Most crops are deficient in certain essential amino acids and that cannot be synthesized de novo by humans (Zhu et al. 2007). Cereal grains such as rice and wheat are found to be deficient in lysine and threonine, in this case, a simple approach may be the expression of recombinant storage proteins with desirable amino acid profiles. As an example of this approach include the expression of pea legumin, which has a high lysine content in rice and wheat grains (Singh et al. 1997, Stoger et al. 2001).

Geneticists have been attempting to enhance the lysine content in cereals, but have met with unsatisfactory outcomes. It is because of underrable traits, which are often associated with the essential amino acids, e.g. high lysine content. Zaman and Kabir (2008) made a biochemical analysis of five tip sterile (undesirable trait) varieties of hexaploid wheat. Only four amino acids such as valine, glutamine, glycine and lysine could be identified by them. Proline and aspergine which play important role for fertility of anthers could not be detected in their study. However, high proline content increases in fertile anthers prior to anthesis in contrast to low proline in male sterile anthers. In such case biotechnological progress may allow the use of transgenic approach to increase the content of a specific essential amino acid in a target plant.

Lactoferrin and ferritin are also capable of facilitating uptake of trace element. Various dietary ligands can enhance trace element absorption. One example of this is amino acids, which are largely released from proteins during digestion. Cysteine or cysteine rich peptides are shown to have a positive effect on iron absorption (Layrisse et al. 1984, Tylor et al. 1986) and histidine is shown to facilitate zinc absorption (Lonnerdal 2000). Organic acids such as fumarate, citrate and succinate can also enhance trace element absorption.

Engineered genes are being used by scientists in tropical indica rice using nonantibiotic selectable marker pmi (phosphomenose isomerase) gene (Datta et al. 2003). Moreover, iron has been enhanced in indica rice by introducing ferritin gene driven by the endosperm specific promoter (Vasconcelos et al. 2003). Datta (2004) stated that it is possible to improve the amino acids (such as lysine) in rice. As it has been shown by pyrimiding transgenes (Xa21, Bt, PR-protein genes) functioning in a homozygous single elite cultivar (Datta et al. 2002), it might be possible to incorporate the genes for β-carotene and enhancement of iron and protein in a single rice variety to achieve the dream rice for those who need it most. Datta (2004) stated that nutrition rice with iron and β-carotene in polished seeds has been developed with genetic engineering technology. He further stated that it is now possible to use Bt or Xa21 rice in farmers field with a reduced use of pesticide, and policymakers should look into the potential use of biotechnology, provide access to the intellectual property rights (IPR), and make improved rice seeds available free of IPR for resource-poor farmers.

Transgenic plants are capable of transmitting the incorporated gene to the next generation. Transgenic strategies have been concentrated for increasing the mineral content, particularly iron (Fe) and zinc (Zn) in crop plants. Two distinct approaches are used and these are (i) increasing the efficiency of uptake and transport to harvestable tissues and (ii) increasing the amount of bioavailable mineral accumulating in plant (White and Broadley 2005). Combination of multiple nutritional improvements into cereal crops without
disrupting endogenous metabolic pathways should be achieved in such a way which can ensure transgene and expression stability from generation to generation. This would be achieved through direct integration of multiple genes into a single, permissive transgenic locus (Altpeter et al. 2005).

Transgenic approaches have both advantages and disadvantages. The advantages are (i) rapid progress, (ii) unconstrained by gene pool, (iii) targeted expression in edible organs, and (iv) applicable directly to the elite cultivars. The disadvantages are (i) regulatory landscape, (ii) political and socio-economic issues relevant to transgenic plants, and (iii) possible intellectual property constraints. Compared to that of mineral fertilization and conventional breeding/mutagenesis transgenic strategies could help more to alleviate malnutrition of both crop plants and human beings.

**Conclusion**

Genetically modifying plants for increasing ‘nutrient use’ efficiency is a complementary approach. By increasing the nutritional element of cereal crops and/or enhancing the bioavailability of the essential macro- and microelements in the staple food crops, nutritional necessity in the developing countries may be improved. Genetic modification can be achieved by two different approaches and these are (i) conventional breeding and (ii) genetic engineering. The second one is the most suitable biotechnological tool for increasing the ‘nutrient use’ efficiency. This can be achieved by introducing the genes which code for nutrient element binding proteins, over expression of storage protein and/or increased expression of proteins and responsible for nutrient elements uptake into the plants. Finally it can be said that significant advances will be made in near future in the developing countries.

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