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- Review Article

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 genetical 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).

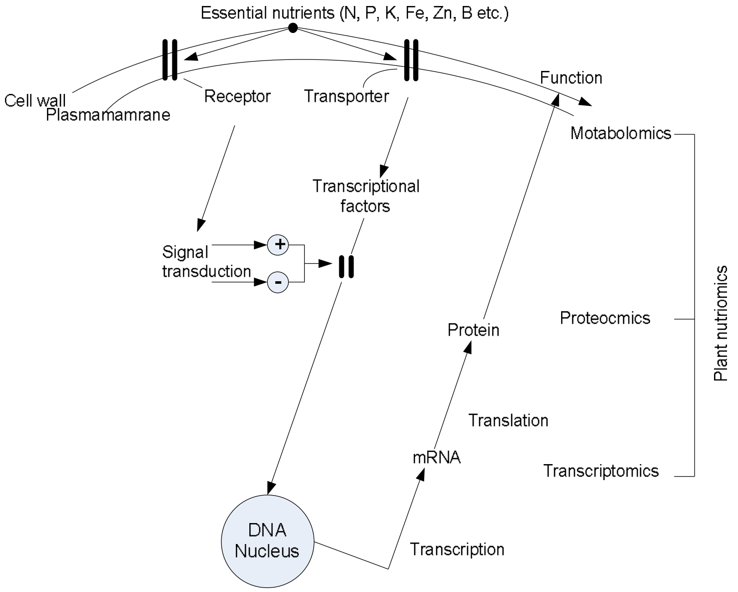

Fig. 1. Line diagram of the basic concepts of plant nutriomics

This line diagram indicates that plant nutriomics is still at a conceptual stage and thus, substantial efforts should be made worldwide for increasing plant nutrition efficiency through genetic and molecular approaches (Yan *et al.* 2006).

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 asses 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 NRTI 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 nitrite 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.

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

Source: Pathak *et al.*, 2011

Remobilization of N is considered as one of the important step in improving NUE in plants (Mascluaux-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 remobilizatin 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 C_4 rice or C_4 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 (Gallais and Hirel 2004). Brugiere *et al.* (2000) suggested that cytosolic GS (GS1) reassimilates amonium 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 transgeneic 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 retrotransposon 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 filing in rice, and GS1; 2 and GS1;3 are not able to compensate the function of GS1;1 (Tabuchi *et al.* 2005, 2007).

Microarry 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 microarry 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 Phy*A gene. To ensure targeting to the cell wall, Holm *et al.* (2002) introduced a signal sequence from barley *a*-amylase upstream of the *Phy*A 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 upto 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 upto 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 Della 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).

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.

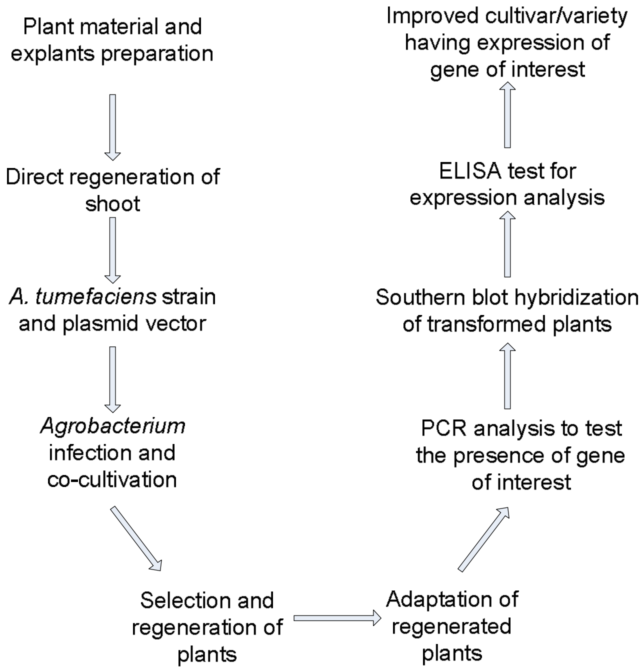

Fig. 2. *Agrobacterium* mediated transformation of cereal crops for a specific gene.

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 from 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 can not 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 underirable traits, which are often associated with the essential aminoacids, 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 riece 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 lysime) in rice. As it has been shown by pyrimiding transgenes (Xa21, Bt, PR-protein genes) functioning in a homozygous single elite cltivar (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 nutritions 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.

Tansgenic 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.

References

Altpeter F, Baisakh N, Beachy R, Bock R, Capell R, Christou P. 2005. Particle bombardment and the genetic enhancement of crops: myths and realities. *Mol Breed* 15, 305-327. http://dx.doi.org/10.1007/s11032-004-8001-y

Andrews M, Raven JA, Lea PJ, Sprent JI. 2004. A role of shoot protein in shoot-root dry matter allocation in higher plants. *Ann Appl Biol* 145, 25-40. http://dx.doi.org/10.1111/j.1744-7348.2004.tb00356.x

Baudo MM, Lyons R, Powers S, Pastori GM, Edwards KJ, Holdsworth MJ, Shewry PR. 2006. Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. *Plant Biotechnol J* 4, 369-380. http://dx.doi.org/10.1111/j.1467-7652.2006.00193.x, PMid:17177803

Beatty PH, Shrawat AK, Carroll RT, Jhu T, Good AG. 2009. Transcriptome analysis of nitrogen-efficient rice over-expressing alanine amino-transferase. *Plant Biotechnol J* 7(6), 562-576. http://dx.doi.org/10.1111/j.1467-7652.2009.00424.x, PMid:19508275

Bouis H. 1996. Enrichment of food staples through plant breeding: A new strategy for fighting micronutrient malnutrition. *Nutr Rev* 54, 131-137. http://dx.doi.org/10.1111/j.1753-4887.1996.tb03915.x, PMid:8783879

Brinch-Pedersen H, Olesen A, Rasmusen SK, Holm PB. 2000. Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus phytase. Mol. Breed*. 6, 195-206. http://dx.doi.org/10.1023/A:1009690730620

Brugiere N, Dubois F, Masclaux C. 2000. Immunolocalization of glutamine synthetase in senescing tobacco (*Nicotiana tabacum* L.) leaves suggest that ammonia assimilation is progressively shifted to the mesophyll cytosol. *Planta*. 211, 519-527. http://dx.doi.org/10.1007/s004250000309, PMid:11030551

Cakmak I, Ekiz H, Yilmaz A, Torun B, Koleli N, Gultekin I, Alkan A, Eker S. 1997. Differential response of rye, triticale, bread and durum wheat to zinc deficiency in calcareous soils, *Plant Soil*. 188, 1-10. http://dx.doi.org/10.1023/A:1004247911381

Cakmak I, Torun B, Erenogiu B, Ozttirk K, Marschner H, Kalayci M, Ekiz H, Yihmaz A. 1998. Morphological and physiological differences in cereals in response to zinc deficiency. *Euphytica* 100, 349-357. http://dx.doi.org/10.1023/A:1018318005103

Capell T, Christou P. 2004. Progress in plant metabolic engineering. *Curr. Opin*. *Biotechnol*. 15, 148-154. http://dx.doi.org/10.1016/j.copbio.2004.01.009, PMid:15081054

Chardon F, Barthelemy J, Daniel-Vedele F, Masclaux-Daubresse C. 2010. Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. *Journal of Experimental Botany*. 61, 2293-2302. http://dx.doi.org/10.1093/jxb/erq059, PMid:20237091

Datta K, Baisakh N, Oliva N, Torrizo L, Abrigo E, Tan J, Rai M, Rehana S, Al-Babili S, Beyer P, Potrykus I, Datta SK. 2003. Bioengineered 'golden' indica rice cultivars with β-carotene metabolism in the endosperm with hygromycin and mannose selection systems. *Plant Biotechnol. J.* 1, 81-90. http://dx.doi.org/10.1046/j.1467-7652.2003.00015.x, PMid:17147745

Datta K, Baisakh N, Thet KM, Tu J, Datta SK. 2002. Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. *Theoretical and Applied Genetics*. 106, 1-8.

Datta SK. 2004. Rice biotechnology: A need for developing countries. *Ag. Bio. Forum* 7(1&2), 31-35.

Drakakaki G, Christou P, Stoger E. 2000. Constitutive expression of soybean ferritin cDNA in transgenic wheat and rice results in increased iron levels in vegetative tissues but not in seed. *Transg. Res*. 9, 445-452. http://dx.doi.org/10.1023/A:1026534009483

Fageria NK, Baligar VC, Jones CA. 1991. Growth and Mineral Nutrition of Field Crops. Marcel Dekker, Inc. New York. Basel. Hong-Kong. p. 476.

Fixen PE. 2006. Turning challenges into opportunities. In: Proceedings of the Fluid Forum. Fluids: Balancing Fertility and Economics. Fluid Fertilizer Foundation, February 12-14, 2006. Scottsdale, Arizona.

Gallais A, Hirel B. 2004. An approach to the genetics of nitrogen use efficiency in maize. *J. Exp. Bot*. 55, 295-306. http://dx.doi.org/10.1093/jxb/erh006, PMid:14739258

Good AG, Johnson SR, De Paw M. 2007. Engineering nitrogen use efficiency with alanine amino-transferase. *Canadian Journal of Botany*, 85, 252-262. http://dx.doi.org/10.1139/B07-019

Good AG, Shrawat AK, Muench D. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science*. 9, 597-605. http://dx.doi.org/10.1016/j.tplants.2004.10.008, PMid:15564127

Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F. 1999. Iron fortification of rice seed by the soybean ferritin gene. *Nat. Biotechnol*. 17, 282-286. http://dx.doi.org/10.1038/7029, PMid:10096297

Graham RD, Rengel Z. 1993. Genotypic variation in zinc uptake and utilization by plants, In: Robson, A.D. (ed.), Zinc in Soils and Plants, Kluwer Academic Publishers, Dordrecht. pp. 107-118. http://dx.doi.org/10.1007/978-94-011-0878-2_8

Graham RD, Welch RM. 1996. Breeding for staple-food crops with high micronutrient density. Agricultural Strategies for Micronutrients. Working Paper No. 3. International Food Policy Research Institute, Washington, DC.

Graham RD, Ascher JS, Hynes SC. 1992. Selecting zinc efficient cereal genotypes for soils of low zinc status. *Plant Soil*. 146, 241-250. http://dx.doi.org/10.1007/BF00012018

Grusak MA, DellaPenne D. 1999. Improving the nutrient composition of plants to enhance human nutrition and health. *Annu. Rev. Plant Physiol*. 50, 133-161. http://dx.doi.org/10.1146/annurev.arplant.50.1.133, PMid:15012206

Grusak MA, Pearson JN, Marentes E. 1999. The physiology of micronutrient homeostasis in field crops. *Field Crops Res*. 60, 41-56. http://dx.doi.org/10.1016/S0378-4290(98)00132-4

Hibberd JM, Sheehy JE, Langdale JA. 2008. Using C4 photosynthesis to increase the yield of rice-rationale and feasibility. *Current Opinion in Plant Biology*. 11, 228-231. http://dx.doi.org/10.1016/j.pbi.2007.11.002,PMid:18203653

Hirel B, LeGuis J, Ney B, Gallais A. 2007. The challenge of improving nitrogen use efficiency in crop plants. Towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot*. 58, 2396-2387.http://dx.doi.org/10.1093/jxb/erm097,PMid:17556767

Hirose N, Makita N, Kojima M, Kamada-Nobusada T, Sakakibara H. 2007. Overexpression of a Type-A response regulator alters rice morphology and cytokinin metabolism. *Plant Cell Physiol*. 48, 523-539. http://dx.doi.org/10.1093/pcp/pcm022, PMid:17293362

Holm PB, Kristiansen KN, Pedersen HB. 2002. Transgenic Approaches in Commonly Consumed Cereals to Improve Iron and Zinc Content and Bioavailability. *J. Nutr*. 132, 514-516.

Hoshida H, Tanaka Y, Hibino T. 2000. Enhanced tolerance to salt stress in transgenic rice that over-expresses chloroplast glutamine synthetase. *Plant Molecular Biology*. 43, 103-111. http://dx.doi.org/10.1023/A:1006408712416. PMid:10949377

Khoshgoftarmanesh AH, Rainer S, Rufus LC, Bahareh D, Majid A. 2010. Micronutrient efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. *Agron. Sustain. Dev*. 30, 83-107. http://dx.doi.org/10.1051/agro/2009017

Layrisse M, Martinez Torres C, Leets I, Taylor P, Ramirez J. 1984. Effect of histidine, cysteine, glutathione or beef on iron absorption in humans, *J. Nutr.* 114, 217-223.

Li Q. 1985. Red soils in China. Beijing. China Science Press.

Lian X, Wang S, Zhang J, Feng Q, Zhang L, Fan D, Li X, Yuan D, Han B, Zhang Q. 2006. Expression profiles of 10422 genes at early stage of low nitrogen stress in rice assayed using a cDNA microarray. *Plant Mol. Biol*. 60, 617-631. http://dx.doi.org/10.1007/s11103-005-5441-7, PMid:16649102

Lightfoot DA. 2009. Genes for improving nitrate use efficiency in crops. In: Jenks, M.A, Woods, A.J. (eds). Genes for plant abiotic stress 167-184. Ames. IA: Wiley Blackwell. http://dx.doi.org/10.1002/9780813809380.ch7

Lonnerdal B. 2000. Dietary factors influencing zinc absorption. *J. Nutr.* 130, 13785-13835.

Lonnerdal B. 2002. Phytic acid-trace element (Zn, Cu, Mn) interactions. *Int. J. Food. Sci. Tech*. 37, 749-758. http://dx.doi.org/10.1046/j.1365-2621.2002.00640.x

Lonnerdal B. 2003. Genetically modified plants for improved trace element nutrition. *J. Nutr*. 133, 1490-1493.

Lu C, Malcolm J, Hawkesford MJ, Peter B, Barraclough PB, Paul R, Poulton PR, Ian D, Wilson ID, Gary L, Barker GL, Keith J, Edwards KJ. 2005. Markedly different gene expression in wheat grown with organic or inorganic fertilizer. *Proc. R. Soc. B.* 272, 1901-1908. http://dx.doi.org/10.1098/rspb.2005.3161, PMid:16191595 PMCid:PMC1559878

Lucca P, Hurrell R, Potrykus I. 2001a. Approaches to improving the bioavailability and level of iron in rice seeds. *J. Sci. Food Agric*. 81, 828-834. http://dx.doi.org/10.1002/jsfa.886

Lucca P, Hurrell R, Potrykus I. 2001b. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *TAG*. 102, 392-397. http://dx.doi.org/10.1007/s001220051659

Martin A, Lee T, Kichey T. 2006. Two cytosolic glutamine synthetase isoforms of maize are specially involved in the control of grain production. *Plant Cell.* 18, 3252-3274. http://dx.doi.org/10.1105/tpc.106.042689, PMid:17138698 PMCid:PMC1693956

Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K. 2004. Identification of cold-inducible downstream genes of the Arabidopsis DREB1A/CBF3 transcriptional factor using two microarray systems. *Plant J.* 38, 982-993. http://dx.doi.org/10.1111/j.1365-313X.2004.02100.x, PMid:15165189

Masclaus-Daubresse C, Daniel-Vedele F, Decorgnat J. 2010. Nitrogen uptake, assimilation and remobilization in plants. Challenges for sustainable and productive agriculture. *Annals of Botany*. 105, 1141-1157. http://dx.doi.org/10.1093/aob/mcq028, PMid:20299346 PMCid:PMC2887065

Masclaux C, Quillere I, Gallais A, Hirel B. 2001. The challenge of remobilization in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Ann. Appl. Biol*. 138, 69-81. http://dx.doi.org/10.1111/j.1744-7348.2001.tb00086.x

Mengel K, Kirby EA. 1978. Principles of plant nutrition. International Potash Institute, Bern.

Miller RO, Jacobsen JS, Skogly EO. 1993. Aerial accumulation and partitioning of nutrients by hard red spring wheat. *Common Soil. Sci. Plant Ana.* 24, 2389-2407. http://dx.doi.org/10.1080/00103629309368963

Pathak RR, Ahmad A, Lochab S, Raghuram N. 2008. Molecular physiology of plant N-use efficiency and biotechnological options for its enhancement. *Current Science*. 94(11), 1394-1403.

Pathak RR, Lochab S, Raghuram N. 2011. Improving Plant Nitrogen-Use Efficiency in Comprehensive Biotechnology, 2nd Edition Elsevier. *Oxford*. 4, 209-218.

Shrawat AK, Good AG. 2008. Genetic Engineering Approaches to Improving Nitrogen Use Efficiency. *ISB News Report May 2008*.

Shrawat AK, Carroll RT, De Paw M, Taylor GJ, Good AG, 2008. Genetic engineering of improved nitrogen use efficiency in rice by tissue-specific expression of alanine amino-transferase. *Plant Biotechnology Journal*. 6, 722-732. http://dx.doi.org/10.1111/j.1467-7652.2008.00351.x, PMid:18510577

Singh SA, Zheng Z, Murai N. 1997. The pea seed storage protein legumin was synthesized, processed and accumulated stably in transgenic rice endosperm. *Plant Sci*. 130, 189-196. http://dx.doi.org/10.1016/S0168-9452(97)00219-7

Stoger E, Parker M, Christou P, Casey R. 2001. Pea legume overexpressed in wheat endosperm assembles into an ordered para crystalline matrix. *Plant Physiol.* 125, 1732-1742. http://dx.doi.org/10.1104/pp.125.4.1732, PMid:11299354 PMCid:PMC88830

Tabuchi M, Sugiyama K, Ishiyama K, Inoue E, Sato T, Takahashi H, Yamaya T. 2005. Severe reduction in growth rate and grain filling of rice mutants lacking OsGS1; 1, a cytosolic glutamine sysnthetase 1;1. *Plant J.* 42, 641-651. http://dx.doi.org/10.1111/j.1365-313X.2005.02406.x, PMid:15918879

Tabuchi M, Abiko T, Yamaya T. 2007. Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). *J. Exp. Bot.* 58, 2319-2327. http://dx.doi.org/10.1093/jxb/erm016, PMid:17350935

Tandon HLS. (ed.) 1995. Micronutrient Research and Agricultural Production. Fertilizer Development and Consultation Organization. New Delhi, India, p. 164.

Taylor PG, Martinez Torres C, Romano EL, Layrisse M. 1986. The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. *Am. J. Clin. Nutr*. 43, 68-71.

Theil EC, Burton JW, Beard JL. 1997. A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. *Eur. J. Clin. Nutr*. 51, 528-531.

Van Hartingveldt W, Vanzeiji CMJ, Barteveld GM, Gouka RJ, Suykerbuyk MEG, Luitein RGM, VanParidon PA, Selten GCM, Veenstra AE, Gorcom RFM, Vanttondel CAMJJ. 1993. Cloning, characterization and overexpression of the phytase-encoding gene (ohyA) of *Aspergillus niger*. *Gene*. 127, 87-94. http://dx.doi.org/10.1016/0378-1119(93)90620-I

Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, Oliveira M, Goto F, Datta SK. 2003. Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Science*. 164(3), 371-378. http://dx.doi.org/10.1016/S0168-9452(02)00421-1

Welsh, RM, Graham RD. 1999. A new paradigm for world agriculture; meeting human needs; productive, sustainable, nutritions. *Field Crops. Res*. 60, 1-10. http://dx.doi.org/10.1016/S0378-4290(98)00129-4

White PJ, Broadley MR. 2005. Biofortifying crops with essential mineral elements. *Trends Plant Sci*. 10, 586-593. http://dx.doi.org/10.1016/j.tplants.2005.10.001, PMid:16271501

Yamaya T, Obara M, Nakajima H, Saski S, Hayakawa T, Sato T. 2002. Genetic manipulation and quantitative trait loci mapping for nitrogen recycling in rice. *Journal of Experimental Botany*. 53, 917-925. http://dx.doi.org/10.1093/jexbot/53.370.917, PMid:11912234

Yan X, Ping W, Hongqing L, Guohua X, Faugsen X, Qifa Z. 2006. Plant nutriomics in China: An overview. *Annals of Botany*. 98, 473-482. http://dx.doi.org/10.1093/aob/mcl116, PMid:16735410 PMCid:PMC2803557

Ye X, Al-Babili S, Kloti A, Jhang J, Lucca P. 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid free) rice endosperm. *Science*. 287, 303-305. http://dx.doi.org/10.1126/science.287.5451.303 ,PMid:10634784

Zaman S, Kabir G. 2008. Protein profiles and amino acids in seeds of tip sterile wheat (*Triticum aestivum* L.). *Plant Environ. Dev*. 2(2), 119-123.

Zhu C, Shaista N, Sonia GG, Ana MP, Teresa C, Paul C. 2007. Transgenic strategies for the nutritional enhancement of plants. *TRENDS in Plant Science. Elsevier Ltd*. 12, 548-555.

Zhu GH, Zhuang CH, Wang YQ, Jiang LR, Peng XX. 2006. Differential expression of rice genes under different nitrogen forms and their relationship with sulfur metabolism. *J. Integrative Plant Biol.* 48, 1177-1184. http://dx.doi.org/10.1111/j.1744-7909.2006.00332.x