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Review

Manipulating seed storage proteins for enhanced grain quality in cereals

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The importance of proteins in our diet is well recognized. Cereals, the major group of our food crops have provided the main source of energy and dietary proteins since the period human ancestors resorted to domestication and cultivation of crops. Their proteins have an imbalanced distribution of essential amino acids which is due to the low content of these amino acids in their predominant seed protein fractions. Various strategies using conventional and molecular breeding towards improvement of nutritional value of food crops have been followed by plant scientists from time to time. The enormous information generated through characterization studies of their seed storage proteins and the development of new technologies for genetic engineering and plant transformations have formed the basis of improvement of grain quality in different cereals. Genes for this purpose have been stably integrated and efficiently expressed in transgenic cereals with useful results. Successful approaches towards this end have included manipulation of the expression levels of genes for homologous proteins, use of genes for heterologous proteins, modification of nutritionally inferior polypeptides by inserting codons for essential amino acids in their genes etc. The present review covers information on various achievements by different workers through initial attempts in this direction.

Key words: Seed storage proteins, grain quality, transgenic crops, cereals.

INTRODUCTION

Proteins were the first substance to be recognized as a vital constituent of living cells. Being next to water in terms of their abundance and availability, these are crucial in different biological roles as enzymes and hor-mones and in cell repair, defense mechanisms, transport of many substances, storage and blood clotting etc (Boulter and Derbyshire, 1978). For carrying out these highly diverse functions, proteins occur in various confi-gurations and sizes. Generally, carbohydrates and fats are used as a source of energy but under certain situa-tions like excess dietary proteins or inadequate dietary fats and carbohydrates, proteins may also be utilized to supply energy. An adult human cannot synthesize amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. There-fore, these need to be supplied through foods and have been termed as essential amino acids. Further, the term limiting has been used for a given essential amino acid because its absence or deficiency limits the ability of the body to make proteins despite the presence of all other amino acids. Animal protein sources like egg, milk, poultry, fish and meat are considered balanced in terms of correct ratio of essential amino acids and thus, are nutritionally better sources as compared to plant proteins (WHO/FAO/UNU report, 1985) . As per this WHO techni-cal report, eggs as a protein source have the highest quality rating of 100 as compared to protein rating of 70 for fish, 60 for cow milk, 50 for white rice, 47 for soybean, 44 for whole grain wheat and 34 for potato. In view of their high cost, animal proteins cannot be easily afforded by the people of developing countries and thus, plants provide a cheaper source of dietary proteins for the poor populations. As indicated by different surveys and reports, inadequate intake of nutrients including proteins over a continuously long period may lead to malnutrition among infants, pre-school children, pregnant and lacta-ting women of poor populations.

A simple classification of plant proteins into following four groups is based on the pioneering work of T.B. Osborne (1924), and despite subsequent modifications, this classification has retained its importance over the years:

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1.) Albumins: as proteins soluble in water; the group is mainly represented by enzymes
2.) Globulins: are insoluble in water but soluble in dilute salt solution; these are the major proteins in leguminous seeds.
3.) Prolamins: are soluble in aqueous alcohol and are present as major proteins in maize, wheat and barley.
4.) Glutelins: are insoluble in all the above solvents but soluble in dilute acids or dilute alkalies; these are the most abundant proteins in rice.
In terms of their biological function, albumins are largely known as metabolically active proteins representing enzymes. The remaining three protein classes are non-enzymatic and have been called as storage proteins. These have been named so because these are synthesized at one stage of life cycle that is during seed development and are stored for utilization at another stage for supply of nitrogen and sulphur during seed germination (Croy et al., 1984, Boulter and Croy, 1997). Some of the albumin polypeptides, as in pea (Croy et al., 1984) , Brazil nut (Altenbach et al., 1992) and sunflower (Kortt et al., 1991), have been known to be degraded at the time of seed germination and thus, have also been ascribed the storage function.

Two major groups of crop plants that is cereals and legumes together support the per capita food supply of about 70% of the world population (Shewry et al., 1981). The protein content of leguminous seeds varies between 20 - 25% and is relatively much higher than that of 8 - 13% in cereals (Boulter and Derbyshire, 1978). These food crops are known for an imbalanced proportion of amino acids in their seed proteins. Whereas cereals lack primarily in basic amino acids and secondarily in tryptophan, leguminous seeds are deficient in sulphur-containing amino acids. Plant scientists have been constantly striving towards combating the menace of malnutrition by achieving qualitatively better and higher amount of proteins in different crops. The earlier strategies for this involved screening of lines with higher protein content and the lines with higher essential amino acids. Later on, with the development of highly efficient techniques of protein purification and characterization, focus shifted to the identification of genotypes having protein fractions and polypeptides richer in limiting amino acids. Better understanding of the structure and expression of genes for seed storage proteins has facilitated the application of techniques of modern biotechnology towards isolation of genes for nutritionally superior polypeptides followed by their use in genetic modifications aimed at enhanced nutritional quality in different crops. Efforts based on manipulation of genes for various enzymes which regulate the metabolic pathways for synthesis and degradation of certain essential amino acids, have also represented another fascinating strategy towards improving the level of these amino acids in different food crops (Zhu and Galili, 2004; Wakasa et al., 2006; Houmard et al., 2007). In view of the importance of cereal proteins in our nutrition and in industry, an attempt is being made to briefly review the status of our knowledge about seed storage proteins in cereals and their manipulation as one of the strategies towards improvement of grain quality using genetic engineering.

SEED STORAGE PROTEINS OF MAJOR CEREALS

Since the period when human ancestors started earliest cultivation by gathering and saving seeds of various crops of interest, cereals have evolved as the most important group of food crops through selection of desirable traits over the long period of domestication; these have provided an indispensable source of energy and dietary protein to a large part of the world population. Cereal seed proteins were among the first to be studied by Italian scientist Beccari (1745) who is known for isolation of gluten from the wheat flour. As mentioned earlier, the major reserve proteins in cereals are represented by prolamins. However, in rice and oats, these are represented by glutelins and globulins respectively. A brief description of the composition, nutritional and functional characteristics of seed storage proteins in major cereals is given below.

Maize

The most abundant proteins in maize which are represented by prolamins constitute 60% of the endosperm protein (Nelson, 1966) and have been named as zeins. Known as one of the extensively studied cereal proteins, zeins are subdivided into four types α, β, γ and δ fractions (Peterson et al, 1982) . The α-zeins contribute about 75% of total zeins and are constituted by polypeptides of mol. wt. 19 kDa and 22 kDa. The β -zeins consist of polypeptides of mol. wt. 14 kDa and 16 kDa, and account for 10 - 15% of total zeins whereas γ -zein and δ zein are represented by polypeptides of mol. wt. 27 kDa and 10 kDa respectively (Esen, 1986). The low content of lysine and tryptophan in all the zein fractions makes the maize proteins inferior in nutritional quality. The α-zeins, due to one or two cysteine residues per molecule are present either as monomers or oligomers, while the β, γ and δ zeins have higher levels of cysteine and /or methionine and form alcohol insoluble polymers that can be extracted only under reducing conditions. In this way, due to very low cysteine and methionine, α- zeins have lower nutritional value as compared to β, γ and δ zeins.

Wheat

The term gluten has been used for the water insoluble proteinaceous mass left after removal of bulk of starch and other components from the wheat dough. It mainly consists of glutelin and prolamin protein fractions which have been named as glutenins and gliadins respectively

in wheat. Gliadins are monomeric, soluble in 70% ethanol and constitute about 50% of the seed protein; due to their extensive polymorphism, these have been widely used for identification of wheat cultivars. On the other hand, glutenins are polymeric and require the presence of a reducing agent for breaking disulphide bonds during extraction (Shewry et al., 1989).

Gliadins have been classified into four groups as α–, β–, γ– and ω- gliadins on the basis of their mobility. These have also been described on the basis of their amino acid composition as sulphur-rich prolamins (α, β and γ gliadins) and sulphur- poor prolamins (ω gliadins). With their molecular weights in the range of 30 to 45 kDa, α, β and γ gliadins are poor in lysine, arginine and histidine, and hence are responsible for the poor nutritional quality of wheat; the ω-gliadins, in contrast, are resolved in the range of higher mol. wt. of 44 - 80 kDa (Charbonnier, 1974). The glutenin polymers held together by disulphide linkages may occur as aggregates of very high mol. wt. upto 20,000 kDa – the largest in the plant kingdom. Based on their mobilities on SDS-gels under reducing coditions, glutenin subunits are classified into two groups of bands called as high molecular weight glutenin subunits (HMW-GS) with mol. wt. 95 to 140 kDa and low molecular weight glutenin subunits (LMW-GS) of mol. wt. 30 to 50 kDa (Payne and Corfield, 1979). The HMW glutenin subunits have been further divided into x- and y-type on the basis of their slower and faster electrophoretic mobility respectively. The glutenin polymers, especially the HMW subunits, are largely responsible for dough strength and possess a highly elastic structure similar to that of elastin and titin (Shewry et al., 1989); it is due to this unique viscoelastic property that wheat dough can be made into different foods like bread, biscuits, noodles and pasta etc. It is mentioned that due their similarities such as solubility in alcohol, higher proline and glutamine content and structural homology, glutenins and gliadins both have been considered as prolamins (Shewry et al., 1981).

Rice

Unlike the alcohol-soluble prolamins dominating in grains of most of the cereals, glutelins represent the major protein fraction in rice. Juliano (1972) reported these as constituting 80% of the total seed protein; however, using a different extraction protocol, Krishnan and White (1995) reported a lower proportion of 53% for glutelins. These are formed by polypeptide pairs of mol.wt. 57 kDa, each consisting of a large acidic (37 - 39 kDa) and a small basic (22 - 23 kDa) subunit (Yamagata et al., 1982). With respect to the molecular weights of subunit pairs and their subunits, rice glutelins show similarity with the legumin-like proteins of pea and soybean; these proteins have also been considered homologous due to similarity in their biosynthesis and amino acid sequences (Yamagata et al., 1982; Takaiwa et al., 1986). Based on the primary sequence comparisons, glutelins have been classified into A and B types (Takaiwa et al., 1991), the B-type glutelin having more of lysine is suggested as a good genetic resource to improve rice protein quality. In contrast to only one band of glutelin subunit pairs (Yamagata et al., 1982), as many as five glutelin subunit pairs over a range of mol. wt. 25 - 60 kDa have been observed by Singh (2006) . The alcohol soluble prolamins which are present in PB-I type of protein bodies, account for approx. 35% of rice protein (Krishnan and White, 1995) whereas Juliano (1972) reported prolamins as representing less than 5% of the grain protein. The 13 kDa prolamin polypeptide has a higher content of glutamic acid, aspartic acid and leucine and a low content of lysine and sulphur-containing amino acids. In contrast, 10 kDa and 16 kDa polypeptides have a higher content of sulphur -containing amino acids (Mitsukawa et al., 1999). On the other hand, globulins represent approx. 10% of the seed proteins and are rich in basic amino acids (Padhye and Salunkhe, 1979).

Barley

Prolamin fraction called as hordein in barley, accounts for 50% of the total protein and is of poor nutritional quality due to its low lysine content The hordein polypeptides have been variously classified as B-hordeins (35 - 46 kDa), C-hordeins (55 - 70 kDa) and D-hordeins (105 kDa) on the basis of their molecular weights (Shewry and Miflin, 1983). The B- and D- hordeins are polymeric in nature and are stabilized by intermolecular disulphide-bonds. On the basis of their amino acid composition and sequences, barley prolamins have also been classified as sulphur-rich (S-rich), sulphur-poor (S- poor) and high molecular weight (HMW) prolamins. The S-rich prolamins include B-hordeins and account for about 80% of the total barley prolamins. These have very high glutamine-proline content and relatively higher cysteine content. The S-poor prolamins which include C-hordeins are known for lacking cysteine and have very low methionine and lysine. The HMW prolamins represented by polypeptides of mol. wt. 105 kDa include D-hordeins and are closely related to the HMW- glutenin subunits of wheat (Shewry et al., 1988); these are rich in lysine, glutamine and proline.

Others

The storage protein composition of oats is quite different from other cereals in having globulins as the most abundant proteins (70 - 80%) followed by albumins, prolamins and glutelins (Peterson and Smith, 1976). The oat globulins further consist of three subfractions - α, γ and globulins with sedimentation coefficient as 3S, 7S and 12S respectively. As described by Shotwell et al. (1988), the predominant 12S fraction is hexameric with

subunit pairs of mol. wt. 53 - 58 kDa each further consisting of one large subunit (mol. wt. 32 - 37 kDa) disulphide-bonded to a small subunit (mol. wt. 22 - 24 kDa). Thus, this globulin fraction resembles that of the legumes in its structure and also in deficiency of sulphur-containing amino acids. The prolamins of oats are designated as avenins and have been divided into three subgroups as α, β and γ-avenins. Like other prolamins, avenins also have higher proportion of glutamine and proline residues and are deficient in lysine (Kim et al., 1978). In this way, on account of having a combination of relatively higher globulins and lower prolamins along with a high protein content of 15 percent, oat seeds provide a better source of nutritional quality as compared to other cereals.

In sorghum, prolamins called as kafirins represent 70 - 80% of the total endosperm proteins (Hamaker et al., 1995). On the basis of their structural properties and solubility characteristics, kafirin polypeptides have been classified as α-kafirins 23, 25 kDa), β-kafirins (16, 18 and 20 kDa) and γ-kafirins (28 kDa). The α-kafirins representing 80% of the total prolamins are located in the interior of protein bodies, and β- and γ-kafirins which have high cysteine content are stored at the periphery (Shull et al., 1992). The kafirins may occur in monomeric or polymeric forms and their composition is known to be responsible for poor digestibility.

IMPROVEMENT OF GRAIN QUALITY

The storage protein genes are similar to other eukaryotic genes in their plan of organization and characteristics. Structural genes coding for specific polypeptides in eukaryotes have a number of sequences located proximally to the transcriptional initiation site. These upstream promoter elements are known to be responsible for basal transcription and accuracy of initiation. These *cis*-elements along with other DNA elements interact with proteins known as *trans*-acting factors for regulation of gene expression. The eukaryotic genes also have coding sequences interrupted by the non-coding sequences called introns. At the downstream end, untranslated region has the sequences signaling polyadenylation, another characteristic of eukaryotic genes. Investigations on cloned genes for different storage proteins have revealed the occurrence of various such sequences in different regions. Sequences in the 5' flanking regions are reported to act in different ways to determine the time and place of expression of storage protein genes. As reported for other eukaryotes, different *trans*-acting factors are also known to interact with various *cis*-acting elements for the expression of different storage protein genes (Casey and Domoney, 1987).

The information generated through characterization studies of seed storage proteins and their genes has been used towards improvement of grain quality using modern biotechnology in the last two decades. As is known, polypeptides show variation in their amino acid composition and as per standard WHO recommendations for human nutrition, the nutritional value of different proteins is determined by the amount of essential amino acids present in these. In addition to their importance in nutrition, grain proteins are also known to determine and influence the industrial uses like baking quality and malting quality of wheat and barley respectively. With the advancement in genetic engineering and transformation technology, genes for different polypeptides have been transgenically expressed in cereals with an aim to enhance the nutritive and processing value of their grains. Different approaches which have been followed with successful results in different cereals include, a) altering the composition of storage protein fractions by manipulating the expression levels of genes for nutriationally superior and inferior homologous polypeptides, b) transgenes coding for heterologous proteins and c) modifying the gene sequences by insertion of codons for limiting essential amino acids for which the polypeptides have a lower content. Various achievements towards enhancing the grain quality of major cereals (Table 1) are described here in brief.

Maize with maximum global production amongst cereals has poor nutritional quality due to low lysine content of its major grain protein fraction – the zeins. Therefore, reduced proportion of zeins accompanied by higher lysine content has been targeted for improved quality of maize proteins employing different strategies. The 19 kDa and 22 kDa -zein polypeptides lack lysine and tryptophan residues. A reduction in these polypeptides was achieved in transgenic maize plants using RNA-interference and antisense RNA technology; significant decline in accumulation of these polypeptides was achieved by suppressing gene expression for these polypeptides, singly (Segal et al., 2003; Huang et al., 2004) or both together (Huang et al., 2006). The reduction in accumulation of zeins was also accompanied by increased lysine and tryptophan content of transgenic lines. It was suggested that the transgenic lines had increased synthesis of certain non-zein proteins in place of reduced zeins (Huang et al., 2006). This alteration of polypeptide composition was comparable with that observed in the high lysine maize mutants *opaque-2* (*o2*) and *floury-2* (*fl2*). Whereas zein reduction in *opaque-2* mutant has been reported to be the result of non-availability of a *trans*-acting factor O2 (Schmidt et al., 1987), the *floury -2* mutation had the defective 22 kDa zein polypeptide with a signal peptide that cannot be cleaved and is thus, not sequestered within protein bodies (Coleman et al., 1996). The *opaque-2* mutant was also shown to accumulate higher γ-zeins and non- zein proteins such as globulin 1 and EFI- (Habben et al., 1995). In another attempt, the gene for methionine-rich 10 kDa zein was over-expressed in maize with enhanced mRNA stability through post-transcriptional regulation

Table 1. Seed storage proteins and various genetic engineering approaches for improvement of grain quality in different cereals.

Protein manipulated	Genetic engineering approach followed	Improvement targeted	Reference
19 kDa and 22 kDa -zein of maize	RNAi , antisense RNA technology	Reduced level of zeins, increased Lys, Trp in maize	Segal et al (2003); Huang et al (2004, 2006)
10 kDa zein of maize	Enhanced stability of mRNA	Increased Met in maize	Lai and Messing (2002)
19 kDa -zein of maize	Gene modified by Lys, Trp codons insertion	Increased Lys and Trp in maize	Wallace et al (1988)
-zein of maize	Gene modified by Lys codons insertion	Increased Lys in maize	Torrent et al (1997)
22 kDa kafirin of sorghum	Transformation using heterologous protein gene	Kafirin synthesis in maize	Song et al (2004)
Sb401of *Solanum berthaultii*	Transformation using heterologous protein gene	Increased Lys and protein content in maize	Yu et al (2004)
Amarantin of *Amaranthus hypochondriacus*	Transformation using heterologous protein gene	Improved protein and essential amino acids in maize	Rascon-Cruz et al (2004)
1Dx5:1Dy10 HMW-GS subunits construct	Transformation using homologous protein gene	Better dough quality in wheat	Blechl and Anderson (1996);
1Ax1 HMW-GS of wheat 1Ax1 HMW-GS	Transformation using homologous protein gene	Better dough quality in wheat	Altpeter et al (1996) Barro et
of wheat	Transformation using homologous protein gene	Better dough quality in wheat	al (2003)
1Dx5 and 1Dy10 HMW-GS of wheat	Transformation using homologous protein gene	Better dough quality in wheat	Blechl et al (2007)
Lys-rich Ama1 of *Amaranthus hypochondriacus*	Transformation using heterologous protein gene	Increased Lys in wheat	Tamas et al (2009)
Legumin of pea	Transformation using heterologous protein gene	Increased Lys in wheat	Stoger et al (2001)
Glycinin of soybean	Transformation using heterologous protein gene	Increased Lys in rice	Katsube et al (1999)
-phaseolin of french bean	Transformation using heterologous protein gene	Increased Lys in rice	Zheng et al (1995)
Legumin of pea	Transformation using heterologous protein gene	Increased Lys in rice	Sindhu et al (1997)
Lysine rich protein of winged bean	Transformation using heterologous protein gene	Increased Lys in rice	Liu (2002)
Sunflower seed albumin	Transformation using heterologous protein gene	Increased Met in rice	Hagan et al (2003)
Sesame 2S albumin	Transformation using heterologous protein gene	Increased Met and Cys in rice	Lee et al (2003)
Glutelin A of rice	Antisense RNA technology	Glutelin decreased, Met-rich prolamin increased in rice	Maruta et al (2001)
1Dx5 HMW-GS of wheat	Transformation using heterologous protein gene	Rice flour with dough quality proteins	Oszvald et al (2007)
Glycinin of soybean	Gene modified by Met. codon insertion	Glycinin accumulation in rice	Katsube et al (1999)
Hordothionin of barley	Transformation using heterologous protein gen	Improved Lys in sorghum	Zhao et al (2003)
Chymotrypsin inhibitor 2 of barley	Transformation using heterologous protein gene	Increased Lys in sorghum	Forsyth et al (2005)

and this resulted in an increased methionine content of grains (Lai and Messing, 2002). Another strategy for enhancing the essential amino acids involves modification of protein structure by inserting codons for these amino acids into gene sequences using site- directed mutagenesis. Such modifications need to be made in protein regions identified as variable regions in such a way that the stability and function of the protein are not affected. Thus, for improvement of maize protein quality, codons for lysine and tryptophan were inserted in the cDNA for 19 kDa –zein at specific positions (Wallace et al., 1988) for initial transformation experiments. Similarly, lysine codons were added to the γ-zein gene (Torrent et al., 1997) and

transgenic maize plants showed higher accumulation of Lys-rich γ-zeins in protein bodies; however, later studies reported that the muntant proteins were not stable and showed post-translational modifications. Genetic modifications expressing the genes for heterologous proteins have also provided promising results towards improvement of nutritional quality in maize. Using particle bombardment, a cluster of 22 kDa kafirin gene when introduced and expressed in maize genome yielded significant kafirin synthesis (Song et al., 2004). Another heterologous protein sb401 expressed in the pollens of *Solanum berthaultii* and known for its high lysine content, was transferred into maize and expressed using maize seed specific promoter; the resultant transgenic plants showed increased levels of lysine (by 16-50%) and protein content (Yu et al., 2004) . *Amaranthus hypochondriacus* is known for the high nutritional value of its grains. The gene for its globulin fraction amarantin, when expressed in maize resulted in the increased protein and essential amino acids content (Rascon-Cruz et al., 2004). The zein polypeptides are rich in sulphur-containing amino acids. Also, the transfer and expression of genes coding for polypeptides of different zein fractions like -zeins (Ohtani et al., 1991), -zeins (Bellucci et al., 2005), δ-zeins (Bagga et al., 1997) and γ- zein (Zhang et al., 2003) has been attempted in different plants.

The bread making quality of wheat dough is determined by the number and the types of HMW glutenin subunits found in different wheat genotypes. The genes for these subunits are located on long arm of chromosome 1 of the three wheat genomes A, B and D; each locus represents the tightly linked pair of genes for x- and y-type subunits (Payne, 1987). With y-allele for HMW-GS of 'A' genome being silent and unexpressed, the number of HMW-GS subunits is reported as varying from 3 - 5 in a given wheat genotype. Depending on the number and type of HMW glutenin subunits, wheat cultivars have been assigned different quality scores such as 4 for subunit 1Dx5+1Dy10, 3 for 1Ax1 and 1Ax2*, 2 for 1Dx2+1Dy12 etc. Certain subunits being responsible for stronger and others for weaker doughs, quality scores of various wheat cultivars from different countries have been worked out by different workers (Singh, 2004). Earlier reports of wheat transformations using genes for these subunits for improved bread making quality came from the works of Blechl and Anderson (1996) and of Altpeter et al. (1996) who introduced genes for hybrid 1Dx5: 1Dy10 construct and for 1Ax1 subunit respectively. Further transformation experiments on expression of HMW glutenin subunits showed that expression of 1Ax1 subunit improved the dough properties and that of 1Dx5 decreased dough quality drastically (Barro et al., 2003). In a study involving expression of 1Dx5 and 1Dy10 subunit genes in transgenic wheat, Blechl et al. (2007) reported that increasing these subunits affected the dough making strength and tolerance differently, 1Dx5 having comparatively a larger effect. The nutritional value of wheat is known to be limited due to the lower levels of lysine and threonine in prolamin fraction. Using a wheat glutenin promoter, gene *ama1* encoding the lysine- rich albumin of *Amaranthus hypochondriacus* has been expressed in wheat leading to a higher content of essential amino acids (Tamas et al., 2009). In addition, the functional properties of dough of transgenic wheat lines were also improved (Oszvald, 2009). The gene for another high lysine heterologous protein that is *legA* for pea legumin was also expressed successfully in wheat under the control of an LMW glutenin subunit promoter (Stoger et al., 2001).

Efforts towards improving rice grain quality have also been made employing different strategies. Genes for a number of heterologous proteins expressed under the promoter of rice proteins have been used to achieve higher amount of deficient essential amino acids in transgenic rice plants. A number of such examples targeting increased level of lysine include the use of genes for lysine- rich leguminous proteins such as hexameric glycinin protein of soybean (Katsube et al., 1999), –phaseolin gene of French bean (Zheng et al., 1995) and legumin gene of pea (Sindhu et al., 1997). Similarly, a cDNA encoding the lysine rich protein (LRP) of winged bean was cloned and when transferred into rice, resulted in about 20% increase in lysine content (Liu, 2002). Genetic transformations have also been attempted for increased levels of methionine and cysteine in rice proteins using the genes for heterologous proteins like sunflower seed albumin (Hagan et al., 2003) and sesame 2S albumin (Lee et al., 2003). By employing the antisense RNA technology, Maruta et al. (2001) could reduce the level of glutelin A with the aim of increasing the proportion of methionine-rich prolamins. Similarly, the over- production of glutelin at the cost of reduced prolamins led to an increased content of lysine in transgenic rice plants (in Sun and Liu, 2004). Following another approach, rice plants transformed with transcription factors of maize O2 and the prolamin box binding factor showed transactivation of glutelin and globulin promoters (Hwang et al., 2004). A glycinin gene modified by inserting four methionine residues was used for rice transformations. The experiments successfully led to enriched accumulation of glycinin as hybrid hexameric molecules containing glycinin and glutelin subunit pairs (Katsube et al., 1999). As rice lacks the dough quality proteins of wheat, efforts have been made to introduce 1Dx5 subunit of wheat HMW glutenin into rice. This was done with a view to partially substitute the wheat flour with rice flour in bread and bakery products. This would be particularly useful in countries having more rice production as compared to wheat and also for patients suffering from gluten intolerance (Oszvald et al., 2007). For improvement of nutritional quality of sorghum, genes for heterologous proteins have been used for transformation. A seed protein called hordothionin occurring in barley and having 5 lysine residues was mutated to have

7 additional lysine residues. Sorghum plants transformed with this modified heterologus protein gene were shown to contain 50% more lysine (Zhao et al., 2003). Similarly, the gene for lysine rich chymotrypsin inhibitor 2 of barley was also modified to contain three additional lysine codons, and the sorghum transgenics exhibited an increased content of lysine (Forsyth et al., 2005).

In view of the emphasis on better living standards and to meet the rightful dietary requirements of populations of different geographic regions, plant scientists have been constantly engaged towards achieving increased yield and enhanced quality of different food crops. A large number of achievements for improvement of protein quality as documented in the present review promise a bright future for efforts towards fighting the menace of malnutrition. The modern approaches not only require information about storage proteins genes and efficient transformation technology but also about various factors underlying the stable integration and expression of transgenes such as transcription rate, mRNA stability, rate of translation and stability of the accumulated products. Various transgenic lines raised for enhanced grain quality after successful *in vitro* assay, greenhouse and small scale field trials are rigorously assessed to meet the requirements for biosafety approval and public acceptance. Also, care should be taken to ensure that attempts to produce changes in the grain quality do not affect other important characteristics like seed functionality, processing, grain yield, agronomic performance etc. Domestication accompanied by constant selection in favour of certain traits is known to have led to the loss of some other useful genes and traits in different crops. Therefore, screening of germplasm for quality proteins will need to be extended to wider sources by undertaking characterization studies on seed proteins of different minor and non-conventional food plants. It may be emphatically stated that success in achieving the goal of enhanced grain quality in food crops will depend on active collaborative efforts of the academia having expertise in breeding, molecular biology, biotechnology, food technology and the industry.

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