ISSN 1996-0700

Asian Journal of Biotechnology

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

http://knowledgiascientific.com

Asian Journal of Biotechnology 3 (1): 1-21, 2011
ISSN 1996-0700 / DOI: 10.3923/ajbkr.2011.1.21
© 2011 Knowledgia Review, Malaysia

Wheat Triticin: A Potential Target for Nutritional Quality Improvement

[1]Dinesh Yadav and [2]Nagendra K. Singh

[1]Department of Biotechnology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur (UP) 273 009, India

[2]National Research Centre for Plant Biotechnology, Indian Agriculture Research Institute, Pusa Road, New Delhi-110012, India

Corresponding Author: Dinesh Yadav, Department of Biotechnology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur (UP) 273 009, India

ABSTRACT

Wheat is one of the major staple food sources of humanity consumed by majority of the world's population. It is the single largest source of protein and second only to rice in fulfilling the daily calorie need of Indian population. From nutritional point of view wheat protein is considered to be limiting in essential amino acids like lysine, tryptophan and threonine and hence there is a need for nutritional quality improvement using modern biotechnology tools. Attempts have been made to obtain high lysine mutants in maize, barley and sorghum by plant breeders though similar mutants could not be isolated in wheat possibly due to its polyploidy nature. With recent developments in recombinant DNA technology in plant molecular biology research, including plant transformation and regeneration, alternative molecular approaches can now be implemented to alter the amino-acids composition of seed proteins for improved nutritional quality. Various molecular approaches like protein sequence modification, synthetic genes, over expression of homologous genes and transfer and expression of heterologous genes can be targeted for nutritional quality improvement. Wheat seed storage proteins classified as gliadins, glutenins, globulins and albumins have been extensively studied. Triticin, a minor storage protein of wheat endosperm, accounting for only 5% of the total seed protein is considered to be nutritionally rich due to the presence of unique lysine-rich decapeptide repeat motif inserted in the hypervariable region of this gene. It belongs to the legumin super family (11-12S globulins) of storage proteins, which predominates in the seeds of legumes and some cereals such as rice and oat. There exists three possibilities for utilizing the triticin for higher lysine content namely by increasing the length of lysine-rich hypervariable region, enhancing the expression of the original/ modified triticin gene using more efficient prolamins promoter and increasing the gene copy number. Successful attempts have been made to clone full-length triticin cDNA, amplify hypervariable region from different wheat progenitors and clone strong seed storage protein promoters from wheat, oat and rice.

Key words: Triticin, seed storage protein, nutritional quality, lysine, legumin

INTRODUCTION

Plants are the primary source of all protein consumed by humans and livestock. Seeds, especially those of legumes and cereals, contain large quantities of protein and are a major source of dietary protein. Cereals contribute about 50% of the per capita energy intake worldwide and 65%

in developing countries and Asian centrally planned economics. Cereals account for about 45% of the daily per capita protein supply in the world and approximately 63% in developing countries. The protein content of cereals varies from 7 to 14% while that of legumes from 20 to 40%.

Seed storage proteins of grain crops meet the major dietary protein requirement of over half of the world population. However seed proteins in general are deficient in some essential amino-acids and are of poor nutritional quality. Lysine is the first nutritionally limiting essential amino acid in most cereals; tryptophan is the second limiting amino acid in maize and threonine in other cereals (Eggum and Beames, 1983). The high content of prolamins (alcohol-soluble fraction) in cereals is responsible for the low content of essential amino acids like lysine, threonine, valine and isoleucine. Rice and oats have a better balance of essential amino acids than other cereals due to a lower content of prolamins (Shewry and Meflin, 1984; Doll, 1984).

Plant geneticists and breeders have made significant efforts in the past to improve the quality of seed proteins. Thus mutations resulting in high-lysine corn and barley have been identified and developed in elite germplasm (Nelson, 1968; Bright *et al*., 1983). Unfortunately, there are undesirable traits associated with these mutations, such as lower yields and greater susceptibility to pests and diseases and this has prevented their agronomic utilization. Maize varieties containing improved lysine, tryptophan and methionine content have also been discovered (Mertz *et al*., 1964). Similarly high lysine mutants have been obtained in barley (Munk *et al*., 1969; Bansal *et al*., 1977) and sorghum (Singh and Axtell, 1973) similar mutants would not be isolated in wheat possibly due to its polyploidy nature. Seed storage proteins of cereals have been extensively reviewed (Mandal and Mandal, 2000; Shewry and Halford, 2002; Matta *et al*., 2009).

Wheat and its importance: Wheat (*Triticum aestivum* L.) with annual world production exceeding 500 million tones is a staple food for much of the world's population. It is the single largest source of protein and second only to rice in fulfilling the daily calorie need of Indian population. It is grown in all climatic zones apart from the Polar Regions, although it is generally restricted to higher elevations in the tropics and subtropics. There exist more than 2500 different cultivars of wheat differing in their climatic requirements and end use properties. Unlike maize and rice, the two other major cereal crops, virtually all of the wheat consumed by mankind is processed onto various foods. The range of wheat products is vast, including different kinds of breads, pasta, noodles, macaroni, chapattis, etc. Wheat is more widely used for the making of bread than any other cereal. This is because of the quality and quantity of its gluten protein. Wheat owes its wide popularity not only to its adaptability and high productivity but also due to the nutritional importance of the grain, particularly high calorific value (80-90% starch) and high protein content (10-17%), which is one of the highest among cereals (Altschul, 1965) Table 1. In addition to their nutritive value, cereals seed proteins also influence the utilization of the grain in food processing (Shewry and Halford, 2002).

Seed storage proteins and their classification: Seeds accumulate large quantity of storage protein during development, which serves as a source of nitrogen, sulfur and carbon for germinating seedlings. Storage proteins have six common characteristics:

- They are abundant proteins in the seed
- They have no enzymatic activity
- Their synthesis is under developmental control

- They are stored in membrane-enclosed structure (protein bodies)
- They are typically composed of a group of structurally related polypeptides and
- They are rapidly degraded during seed germination

Seed proteins can be broadly classified into two categories, viz. housekeeping proteins and storage proteins. The housekeeping proteins are responsible for maintaining normal cell metabolism. The seed storage proteins on the other hand, are non-enzymatic and have the sole purpose of supplying nutrition (nitrogen and sulphur source) to the growing seedling during germination.

Seed storage proteins were empirically classified by Osborne (1907) based on their solubility in to four classes: (1) albumines-water soluble (2) globulins-salt soluble (3) prolamines-alcohol soluble and (4) glutelins-acid or alkaline soluble. In general albumins are metabolically active proteins representing enzymes while globulins, prolamins and glutelins are non-enzymatic and are referred as storage proteins (Croy *et al*., 1984; Boulter and Croy, 1997). Globulins are the major storage proteins in the cotyledons of most dicot plants. These are frequently classified as 11S, 7S and 2S based on their sedimentation coefficient. In contrast prolamines are the predominant proteins in the endosperm of monocot species. They are named according to the species from which they are isolated, for example, *Zea mays*, Zein and *Avena sativa*, avenin. Oats and rice are unusual among cereals for they contain only small amounts of prolamins and their major storage protein is an 11S-type globulin (Table 2). Seed storage proteins are rich in the amide amino acids, glutamine and asparagine. These two amino acids often account for more than 40% of the total.

Table 1: Protein content of cereal grains

Cereal grains	Protein content (%)
Wheat	
Whole grain	12.2
White grain	10.9
Rice	
Brown	7.5
White	6.7
Parboiled	7.1
Corn	
Corn meal	9.5
Barley	
Whole seed	11.0
Oats	
Meal	13.0

FAO (1970)

Table 2: Proportions of different solubility groups of proteins in major cereals

Cereals	Albumin	Globulin	Prolamin	Glutelin
Wheat[a]	9	5	40	46
Maize[b]	4	2	55	39
Barley[b]	13	12	52	23
Oat[c]	11	56	9	23
Rice[b]	5	10	5	80
Sorghum[b]	6	10	46	38

Source: (a) Orth and Bushuk (1974), (b) Whitehouse (1973), (c) Peterson and Smith (1976)

Cereal storage proteins are poor in the essential amino acids lysine (1.5-4%), tryptophan (0.8-2%) and threonine (2.7-3.9%) (Bright *et al*., 1983). When compared with the WHO requirements of essential amino acids for humans, wheat, barley and rye are seen to be deficient in lysine, with threonine being the second limiting amino acids (Table 3).

In terms of their nutritional value to monogastric animals cereals and legumes reflect the amino profile of their major components, prolamine and globulin, respectively. The prolamines (e.g., Zein in corn, hordein in barley, gliadins and glutenins in wheat) are deficient in lysine and to a lesser extent have insufficient quantities of tryptophan and threonine. Similarly the grain legumes are generally deficient in the sulfur amino acids methionine and cysteine due to predominance of globulins.

The prolamine groups have been classified into three major sub-groups based on amino acid composition and sequence similarities (Shewry *et al*., 1986). These sub groups are:

- Sulphur- rich prolamins including α, β and γ gliadin and LMW subunit of glutenin
- Sulphur -poor prolamin including ω-gliadins
- HMW prolamin - HMW subunits of glutenin

The globulins are generally present as oligomers. The cereal prolamines are present as monomers or small aggregates, while glutelins form large disulphide bonded aggregates.

2S albumin storage proteins: These are widely distributed in dicot seeds especially in the cruciferae, notably oilseed rape (in which they are referred as napins) and Arabidopsis. The napins consist of two polypeptide chains linked by interchain disulfide bonds. They are synthesized as single precursor proteins that are proteolytically cleaved with the loss of a linker peptide and short peptides from both the N and C termini (Crouch *et al*., 1983; Ericson *et al*., 1986). This reflects the typical 2S albumin structure. The 2S albumins are compact globular protein with conserved cysteine residues.

The 2S albumins have been exploited in genetic engineering for nutritional improvements. The 2S albumin of Brazilnut has been used to increase the methionine content of tobacco seeds upto 30% (Altenbach *et al*., 1987, 1992; Youle and Huang, 1981). Similarly methionine-rich sunflower 2S albumin SFA8 has been used to increase the methionine content of forage grasses (Tabe *et al*., 1993).

Table 3: Contents of essential amino acids in grain of wheat, rye and barley compared with the WHO recommended levels (FAO, 1973)

Amino acids	Wheat	Rye	Barley	WHO recommended values
Cysteine	2.6	2.9	2.9	3.5
Methionine	1.3	1.7	1.7	3.5
Lysine	2.0	3.3	3.1	5.5
Isoleucine	3.6	3.6	3.6	4.0
Leucine	6.7	6.7	7.2	7.0
Phenylalanine	5.1	4.9	5.5	6.0
Tyrosine	2.6	2.1	2.7	6.0
Threonine	2.7	3.4	3.3	4.0
Tryptophan	1.1	1.8	2.0	1.0
Valine	3.7	4.4	4.6	5.0
Histidine	2.2	2.1	1.9	-

Prolamin storage proteins: Prolamins are traditionally recognized as a group on the basis of their solubility in aqueous alcohols (usually 60-70% [v/v] ethanol or 50% [v/v] propan-1-ol) and their high levels of glutamine and proline. Prolamin storage proteins are restricted to one family, grammineae. The grammineae family includes major cereal grains in which prolamins usually account for approximately half of the total grain nitrogen. Oats and rice are exceptions as in these two cereals the major storage proteins are 11S globulin-like and prolamine are present at a low level (5 to 10% of the total grain protein). The prolamines from wheat (*Triticum*), barley (*Hordeum vulgare*) and rye (*Secale cereale*) are called gliadins, hordeins and secalins, respectively. These are classified into three groups, namely sulfur- rich prolamins, sulfur- poor prolamins and high molecular weight (HMW) prolamins (Shewry and Tatham, 1990) (Table 4).

The sulfur-rich prolamins are quantitatively the most abundant group in all three species, accounting for approximately 80 to 90% of total prolamin fractions. They include polymeric (Those linked with interchain disulfide bonds) and monomeric (having only intra chain disulfide bonds) components and consists of at least two families in each species, the B- and γ-hordeins of barley; two types of γ-secalin of rye; and the α, β and γ-gliadins and Low Molecular Weight (LMW) glutenin subunits of wheat. Their amino acids sequences consist of two separate domains: an N-terminal domain composed of repeated sequences and a non-repetitive C-terminal domain. The repetitive domain consists of tandem or interspersed repeats based on one or two short peptide motifs rich in proline and glutamine.

The sulfur -poor prolamins include C-hordin of barley, ω-secalin of rye and ω-gliadin of wheat (Kasarda *et al*., 1983). These generally consist of repeats of the octapeptide motif Pro-Gln-Gln-Pro-Phe-Pro-Gln-Gln which are flanked at the N-terminal side by short unique sequences of 12 residues and at the C-terminal side by short unique sequences of either six (C hordeins) or four (ω-secalin) residues. The S-poor prolamins generally lack cystein residues and therefore cannot form oligomers or polymers.

The HMW prolamins are typified by the HMW subunits of wheat glutenin, D-hordeins and HMW secalins (rye). Extensive repeated sequences are present, flanked by non-repetitive N-and C-terminal domains. The repeated sequences are based on the motifs Gly-Tyr-Tyr-Pro-Thr-Ser-Pro or Leu-Gln-Gln., Pro-Gly-Gln-Gly-Gln-Gln and Gly-Gln in some subunits only. Differences in the number of repeated peptides are largely responsible for variations in the HMW subunit size.

Globulin storage proteins: Globulins are the most widely distributed group seed storage proteins present not only in dicots but also in monocots. These can be divided into two groups based on their sedimentation coefficient ($S_{20,w}$). The 7S vicilin- type globulins and the 11S legumin type globulins. The globulin storage proteins have been studied in most detail in legumes, notably peas, soybean, broad bean (*Vicia faba*) and french bean (*Phaseolus vulgaris).*

The 11S globulins: The 11S globulins are major storage proteins not only in most legumes but also in many other dicots (for example, brassicas, composits and cucurbits) and some cereals (oats

Table 4: Classification of seed prolamins in wheat, barley and rye

Group	Type	Wheat	Barley	Rye
Sulfur-rich prolamin (30-50 kDa)	Monomeric	α/β and γ- gliadin	γ-hordein	γ-secalin
	Aggregated	LMW glutenin sub-units	B-hordein	
S-poor prolamin (44 -80 kDa)	-	ω-gliadin	C-hordein	ω-secalin
HMW Prolamin (60-90 kDa)	-	HMW glutenin sub-unit	D-hordein	HMW secalin

and rice). The mature protein consists of six subunit pairs that interact non-covalently. Each of these subunit pairs consists in turn of an acidic subunit of M_r ~40,000 and a basic subunit of M_r ~20,000, linked by a single disulfide bond.

The typical 11S globulins are glycinin of soybean, legumins of pea and field bean and arachin of peanut (Pernollet and Mosse, 1983). These 11S globulins are widely distributed in seeds of non-leguminous plants also, such as sunflower (*Helianthes annus*), cotton (*Gossypium hirsutum*), rape (*Brassica napus*), pumpkin (*Cucurbita pepo*), sesame (*Sesamum indicum*) and oats (Shotwell and Larkins, 1989). The wheat triticin protein also belongs to this group which will be dealt in detail (Singh *et al*., 1988).

The 7S globulins: These are typically trimeric proteins of M_r ~150,000 to 190,000 that lack cystein residues and hence cannot form disulfide bonds. Their subunit compositions varies considerably, mainly because of differences in the extent of post-translational processing (proteolysis and glycosylation). The typical 7S globulins are **β**-conglycinin of soybean, vicilins of pea and field bean and phaseolin of french bean. Although the 7S and 11 S globulins show no obvious sequence similarities, they do have similar properties, including the ability to form both trimeric and hexameric structures suggesting their common origin.

Nutritional aspects of seed storage proteins: There are a total of twenty amino acids found in all living organisms. The monogastric animals including human can synthesize only 12 of these amino acids. The remaining eight amino acids namely lysine, valine, leucine, isoleucine tryptophan, phenylalanine, methionine and threonine which cannot be synthesized by human beings are referred as essential amino acids. These amino acids must be incorporated in diet in a correct proportion. Seeds, especially those of legumes and cereals, contain large quantities of protein and are a major source of dietary protein. However usually they are deficient in one essential amino acid or other. In general, cereal seed storage proteins are low in lysine and tryptophan while legume seed storage proteins are deficient in the sulfur amino acids, methionine and cysteine (FAO, 1970; Yamaguchi, 1980). Consequently, seed proteins are nutritionally incomplete.

In developing countries like India, majority of the population depends on cereal-based diet. This is one of the reasons for the prevalence of protein energy malnutrition in these countries. There are various strategies for nutritional improvement of dietary proteins including (1) complementation (2) supplementation (3) conventional breeding approach and (4) molecular genetic approach.

The overall protein quality of cereal-legume mixtures is better than that of either protein source alone due to the complementary nature of their amino acid profiles. Cereals are low in lysine and relatively high in methionine and cysteine, while legumes are high in lysine and low in methionine and cysteine.

Supplementation of cereals and legumes with crystalline amino acids, improves their value to support growth of animals and increases the efficiency of utilization of dietary protein (Bressani and Elias, 1968). Both complementation and supplementation have major drawbacks. Firstly, in supplemented foods and feeds, leaching of free methionine or lysine during processing leads to losses and subsequent bacterial fermentation resulting in objectionable odours and flavours (Nakai and Powrie, 1981). Secondly there is poor consumer acceptability of supplemented foods and thirdly it is not always economically feasible.

Conventional breeding approach by plant-geneticists and breeders led to the development of many mutants with improved lysine content in maize, barley and sorghum. High lysine mutants

have been developed in maize namely *opaque*-2 and *Floury*-2 (Mertz *et al*., 1964; Nelson *et al*., 1965), in barley (Munk *et al*., 1969) and sorghum (Singh and Axtell, 1973).

Unfortunately, there are undesirable traits associated with these mutations, such as lower yields; inferior grain texture and greater susceptibility to pests and diseases and these have prevented their agronomic utilization (Nelson, 1968; Bright *et al*., 1983). Efforts in breeding legumes containing proteins with increased levels of sulfur amino acids have not met with significant success (Payne *et al*., 1983). Due to the hexaploid nature, the efforts to isolate high lysine mutants in wheat have met with no success (Siddique, 1990).

Molecular genetic approaches for improving seed storage protein quality include (1) modification of existing protein sequences to include required essential amino acids (Wallace *et al*., 1988) (2) Synthetic high essential amino acids encoding genes (HEAAE) (Yang *et al*., 1989) (3) over expression of homologous genes (Blechl and Anderson, 1996; Altpeter *et al*., 1996; Barro *et al*., 2003; Blechl *et al*., 2007) and (4) transfer and expression of heterologous genes with improved essential amino acids contents (Altenbach *et al*., 1989; Guerche *et al*., 1990; Zheng *et al*., 1995; Sindhu *et al*., 1997; Katsube *et al*., 1999; Stoger *et al*., 2001; Hagan *et al*., 2003; Lee *et al*., 2003; Zhao *et al*., 2003; Yu *et al*., 2004; Rasco *et al*., 2004; Forsyth *et al*., 2005; Tamas *et al*., 2009).

With advancement in the methods of gene transfer and development of successful transgenic in various crops it is now feasible to incorporate desired genes for improved nutritional quality into any of the major crop plants.

Wheat seed storage proteins: Wheat occupies a unique position in cereal grains owing to its ability to form a dough that exhibits the rheological properties required for the production of leavened bread and other products. On a dry weight basis, protein comprises about 8-16% of the wheat grains produced under normal conditions. These proteins generally play a dominant role in determining the processing quality of dough for breadmaking.

The amino acid composition of gliadins and glutenins of wheat are reasonably similar. Gliadins certainly have more proline and less glycine than do glutenins. However, the main difference between them is that gliadins consist of single polypeptide chains whereas the glutenin is made up of multiple polypeptide chains bonded together by disulfide bonds. The resulting high molecular weight of the glutenins is responsible for their comparative insolubility.

Ewart (1990) has criticized the classification of wheat prolamins on the basis of sulfur amino acid content as proposed by Shewry *et al*. (1986), arguing that the polymeric glutenins are fundamentally different from the monomeric gliadins because of their inter-molecular disulfide bonding capacity. The property of the glutenins that results in its contribution to the dough functionality is very different from those of the gliadins.

Based on molecular size wheat protein can be categorized into two major groups namely polymeric (i.e., intermolecular disulfide bonded proteins) and monomeric (Gliadins and albumins/globulins).

Polymeric proteins: It includes glutenin, high molecular weight (HMW) albumin (mostly **β**-amylase) and triticin (or HMW globulins).

Glutenin: It is a large, heterogeneous protein complex ranging in molecular weight from several hundred thousands to several millions (Huebner and Wall, 1976). It is built up of 15 or more different subunits (Payne and Corfield, 1979) which are probably connected together by a

combination of disulfide bonds and hydrophobic interactions. Glutenin play an important part in the breadmaking process, adding strength and elasticity to the dough (Bietz and Wall, 1973). Three groups of subunits are identified by SDS-PAGE after the reduction of glutenin by a disulfide bond-breaking reagent such as β-mercaptoethanol (ME) or dithiothreitol (DTT). One set of glutenin subunits with slowest mobility form the high molecular weight (or A group) glutenin subunits (Mr = 80,000 to 120,000). The other two groups; B subunits (Mr 40,000-55,000) and C subunits (30,000 to 40,000) have electrophoretic mobilities similar to many of the gliadin components and together referred to as the Low Molecular Weight (LMW) glutenin subunits.

HMW albumins: A group of protein bands (69, 63, 60 and 45 kDa) appears in SDS-PAGE after reduction of disulfide bonds. The proteins corresponding to these bands are insoluble in aqueous ethanol showing them to be non-prolamins. However, they are soluble in water and can be classified as albumins (Gupta *et al*., 1991). They occur in both polymeric and monomeric forms in their native state. There is evidence to suggest that a large proportion of the albumin subunits are mutually linked by disulfide bonds and not linked to glutenin subunits. Immunological analysis of HMW albumins has confirmed that 69, 63 and 60 kDa proteins are β-amylases but not the 45 kDa protein (Gupta *et al*., 1991).

Triticins (HMW globulins): SDS-PAGE of unreduced total protein extracts from hexaploid wheat endosperm shows three slow moving bands (denoted triplet bonds) in a zone of heavy background streaking. These proteins have been studied in detail by Singh and Shepherd (1985) and have been named triticins. The amino acid compositions and solubility properties of these proteins resemble those of rice glutelin and the storage globulins of oats and legumins (Singh *et al*., 1988).

By using a non-reducing/reducing form of two dimensional electrophoresis, the triticin protein were shown to be disulfide-linked and to be heterotetramers made up of four subunits namely D (Mr 58,000), δ (Mr 22,000), A (Mr 52,000) and α (Mr 23,000). The three triplet bands in decreasing order of size corresponded to T1 (DδDδ), T2 (DδAα) and T3 (AαAα). On partial reduction, the tetramers dissociate into dimers and, on complete reduction, are broken down to monomers as shown in (Table 5).

Monomeric proteins

Gliadins: Gliadins are the major storage protein of wheat endosperm and may account for as much as 75% of the total seed proteins in some genotypes (Bietz and Wall, 1972). They comprise of single

Table 5: Subunit composition of triticins as indicated by non-reducing/reducing two-dimensional electrophoresis procedure

Condition	Subunit composition	Molecular weight (Dalton)
No-reduction	DδDδ	160,000
	DδAα	155,000
	AαAα	150,000
Partial reduction	Dδ	80,000
	Aα	75,000
Complete reduction	D	58,000
	A	52,000
	δ	22,000
	α	23,000

Singh and Shepherd (1985)

polypeptide chains stabilized by intrachain disulfide bonds. They have unusual amino acid composition with large amounts of glutamine (38-50% of all amino acid residues) and proline (15-30%) and a small amount of lysine (Nelson, 1968). Four main groups of gliadins are usually distinguished in electrophoresis. These α, β, γ and ω are in decreasing order of mobility and therefore, increasing molecular size. Molecular weight of α, β and γ-gliadins ranges from 30,000 to 45,000 daltons whereas that of the ω gliadins ranges from 65,000 to 80,000 daltons (Bietz *et al*., 1977).

The ω-gliadins, the highest molecular weight group, are the sulphur-poor prolamins. They are clearly separated from other gliadins in SDS electrophoresis whereas there is often some overlap of the α-β- and γ-gliadins. Gliadins do have disulfide bonds but all are apparently intramolecular. All gliadins are soluble in aqueous ethanol in their native state and are thus true prolamins.

Albumins/Globulins: These are a mixture of low-molecular weight compounds, many of which are enzyme. They are usually called albumins (water-soluble and globulins (salt-soluble) in accordance with Osborn classification (Osborne 1907). Like the HMW albumins and triticins, their amino acid compositions are quite different from those of the major gluten proteins, glutenins and gliadins.

Genetics of wheat seed storage proteins: The common bread wheat *Triticum aestivum* is an allohexaploid (2n = 6x = 42) comprising of three genomes, A, B and D, each having seven pairs of chromosomes and each having been derived from a different ancestral diploid species. In their turn, these donor species have evolved from a common ancestral diploid (Riley, 1965). Due to this ancestral origin, each genome carries genes that are present in each of the other genomes. This triplication of genetic material enables wheat to tolerate the loss of or increased dose of individual chromosomes. This aneuploidy has been used extensively in the genetical analysis of wheat and in the transfer to wheat of chromosomes and parts of chromosome from its wild relatives (Riley *et al*., 1968; Sears, 1972).

The three genomes are denoted by letters A, B and D and the seven pairs of chromosomes in each genome are numbered from 1-7. Tetraploid wheat, *Triticum turgidum* var. durum, used extensively in pasta production have only two genomes (A and B) and thus 28 chromosomes. Diploid wheat has only genome and therefore 14 chromosomes and represents most primitive forms of wheat.

The chromosomal location of genes coding for particular proteins has been achieved with cytogenetic stocks in combination with electrophoresis. Aneuploid lines that are deficient in a single chromosome or chromosome arm have been used for such studies. Sears (1969) conducted much of the pioneering work in this area. In simple terms, when a particular chromosome is missing, this may coincide with the disappearance of a certain protein band or bands in the electrophoretic pattern of the wheat protein, thus identifying the chromosome that carries the gene(s) responsible for the synthesis of the missing protein(s).

HMW glutenin subunits (A subunits): The HMW glutenin subunits are coded by genes on the long arm of chromosomes 1A, 1B and 1D and are designated *Glu*-A1, *Glu*-B1 and *Glu*-D1 respectively. Electrophoretic studies have revealed extensive polymorphism in the number and types of HMW glutenin subunits in different wheat cultivars. That is, the *glu*-1 genes on the chromosome 1 long arms shows multiple allelism. The different subunits have been studied for a

large number of varieties (Lawrence and Shepherd, 1980; Galili and Feldman, 1985). It has been shown that chromosome 1D carries genes for two HMW glutenin subunits which form different types of sub-patterns. Two of these (namely 5+10 and 2+12) were common in most varieties studied (Lawrence and Shepherd, 1980; Payne *et al*., 1981a, b, 1982).

LMW (B and C) glutenin subunits: These are encoded by genes on the short arms of chromosomes 1A, 1B and 1D and are designated as *Glu*-A3, *Glu*-B3 and *Glu*-D3 (Singh and Shepherd, 1988). Allelic variation in the LMW glutenin subunits has been studied for more than 200 cultivars by Gupta and Shepherd (1990) using two-step one-dimensional SDS-PAGE. The least number of subunits was controlled by chromosome 1A and about 40% of the cultivar examined contained no band controlled by this chromosome. The greatest polymorphism is shown by chromosome 1B. There is also evidence that some LMW subunits are controlled by gene on the group 6 chromosomes, particularly 6DS (Gupta, 1989).

Triticins: Singh and Shepherd (1985) using nullisomic-tetrasomic and ditelocentric lines of the variety Chinese spring have studied the genetic control of triticin. These proteins are encoded by genes on the short arm of chromosomes 1A (*Tri*-A1 locus) and 1D (*Tri*-D1 locus). The slowest moving band of the triticin is controlled by gene(s) on chromosome arm 1DS and the fastest moving band is controlled by gene(s) on 1AS. The band with intermediate mobility is a hybrid aggregate of the subunits controlled by 1DS and 1AS. However, the genes on chromosome 1B are not expressed and those on chromosome 1D are expressed with twice the intensity of those on chromosome 1A (Singh and Shepherd, 1985).

HMW albumins: Analysis of aneuploid stocks of the wheat variety Chinese Spring has revealed that the HMW albumins of 69, 63, 60 and 45 kDa are controlled by genes on the chromosome arms 4 DL, 4 AL, 5 AL and 5 DL, respectively (Gupta *et al*., 1991).

Gliadins: Genes coding for the gliadins are located on the short arms of groups 1 and 6 chromosomes (Wrigley and Shepherd, 1973; Brown *et al*., 1981). Gliadins are simple monomeric proteins, the synthesis of which is controlled by one or several related genes of a limited number of multigene families (Payne *et al*., 1982). At least 50 different genes control the synthesis of gliadins (Kreis *et al*., 1985). The group 1 chromosome control all the ω-gliadins, most of the γ-gliadins and few of the β-gliadins, whereas genes on the group 6 chromosomes code for all the α-gliadins, most of the β-gliadins and some of the γ-gliadins. Six gliadin coding loci *Gli*-A1, *Gli*-B1, *Gli*-D1, *Gli*-A2, *Gli*-B2 and *Gli*-D2 are located on the short arms of chromosomes 1A, 1B, 1D, 6A, 6B and 6D, respectively. The genes coding for gliadins proteins occur as a single complex locus on each of the short arms of groups 1 and 6 chromosomes rather than at two or more loci.

Monomeric albumins/globulins: The genetic location of genes that controls the many types of non-gluten proteins that occur in the wheat endosperm has been reviewed (Garcia-Olmeda *et al*., 1982). Genes for the major albumins and globulins of wheat have been assigned to chromosomes group of 3, 4, 5, 6 and 7.

Wheat Triticin: a legumin-like protein (11S globulins): The legumin-like proteins are predominant in the seeds of many dicotyledonous plants and have a sedimentation coefficient (S_{20w})

of 11-12S and are composed of six subunit pairs (Mr~60 kD) of disulphide-linked acidic (~40 kD) and basic (~20 kD) polypeptides (Casey and Domoney, 1987; Derbyshire *et al*., 1976). The acidic and basic polypeptides arise from post-translational cleavage of a precursor protein of approximately ~60 kD (Croy *et al*., 1980; Spencer and Higgins, 1980). There is now conclusive evidence, based on amino acid and nucleic acid sequence data, that rice glutelins, oat 12S globulins and wheat triticin are homologous to the legumin proteins (Takaiwa *et al*., 1987; Walburg and Larkins, 1986; Singh *et al*., 1993).

Legumin like proteins (11-12S globulins) are one of the most widely occurring classes of storage proteins accumulated in the seeds of angiosperms (Boroto and Dure III, 1987). However, their proportion in the total seed storage protein varies considerably, ranging from almost 100% in some pumpkin seeds to an apparent absence from barley and rye seeds (Danielsson, 1949; Derbyshire *et al*., 1976). In general, these proteins are much more prevalent in the legume seeds than in cereals and represent one of the two most abundant classes of seed storage proteins in the dicotyledonous plants, the other being vicilin-like proteins (7S globulins).

The amino acid sequence data supported by immunochemical analysis using anti-oat 12S globulin antibodies, provide definitive evidence that the triticin protein is homologous to the pea legumin and related seed storage proteins of oats, rice and several dicotyledonous species. Triticin proteins like other legumins have large (A, D) and small (α, δ) polypeptides linked by disulphide bonds to form dimers, tetramers and other higher oligomers (Singh and Shepherd, 1985). Triticins have many other similarities with legumin-like proteins, including similar solubility, subunit composition, biological function, isoelectric points and amino acid compositions (Singh and Shepherd, 1987).

Structure and biochemical features of 11S globulins: Legumin- like proteins (11S globulins) are predominant in the seeds of many dicotyledonous plants though also reported in cereals like rice, oat and wheat have been described in detail in various species. The triticin of wheat belong to the same class of storage protein (Singh and Shepherd, 1985).

These proteins, like legumins, have large acidic (Tri-A, 52 kDa; Tri-D, 58 kDa) and small basic (α 23 kDa and δ 22 kDa) polypeptides linked by disulphide bonds to form dimers, tetramers and other higher oligomers (Singh and Shepherd, 1985). Triticin have many other similarities with legumin-like proteins, including similar solubility, subunit composition biological function, isoelectric points and amino acid composition (Singh and Shepherd, 1987). The maximum overall amino acid sequence homology was found with oat protein (61%) followed by rice (53%), pea (49%), cotton seed (48%), soybean (45%) and rapeseed (36%) (Singh *et al*., 1988).

The nucleotide sequence of a triticin cDNA has revealed homology with legumins. It has several common features:

- A hydrophobic N-terminal signal peptide which is involved in transport of the nascent polypeptide across the ER membrane and is subsequently removed
- A highly conserved post-translational proteolytic cleavage site between the large-acidic and small basic polypeptide
- Two highly conserved cysteine residues at positions 121 and 387 which are thought to link the large and small polypeptides by a disulphide bond before the post-translational proteolytic cleavage. The typical structure of triticin gene and protein is shown in Fig. 1

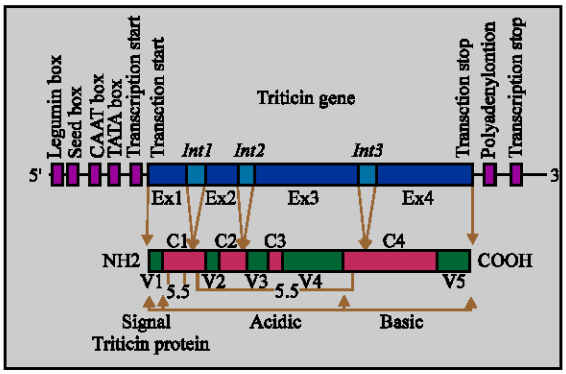

Fig. 1: Structure of a typical triticin gene and protein

Triticin is a minor storage protein of wheat endosperm with good essential amino acids balance but it contributes less than 5% of the total seed proteins. This proteins has been characterized well at genetic and biochemical levels (Singh and Shepherd, 1985, 1987, 1988; Singh *et al*., 1988).

Triticin cDNA clones have also been isolated from a lambda gt11 cDNA library using anti-triticin polyclonal antibodies (Singh *et al*., 1993). These clones have been characterized at the DNA sequence level, but no full-length triticin cDNA clone could be isolated so far. The largest clone Tri 25 has the N-terminal translational start signal including a 59 bp 5' leader sequence but surprisingly it lacks the sequence corresponding to 80 amino acids at the C-terminal end of the protein.

The cDNA λ Tri-25 has a single long open reading frame of 502 amino acids starting at the nucleotide position 60. However, the protein sequence did not terminate in this cDNA, suggesting that a part of the C-terminus was missing.

One interesting feature of the Tri-25 sequence is that its untranslated 5' leader sequence has an in-frame translational initiation codon ATG (nucleotide 21-23) and a stop codon TGA (nucleotide 33-35), situated upstream to the proposed initiation site at nucleotide 60.

Although both the initiator codons conform to the consensus translation start site for eukaryotic genes (A/GXXATGG) proposed by Kozak (1984). The second site was preferred as (i) the first initiation site would lead to a termination of the polypeptide chain after only 4 amino acid residues and (ii) the second site matches strongly with the start site of the closely related 125 oats globulin sequence.

The triticin sequences also reveal some interesting facts like:

- Presence of a hydrophobic N-terminal signal peptide
- Presence of highly conserved post-translational proteolytic cleavage site between the large acidic and small basic polypeptides (between Asn and Gly residues at 380 and 381)
- Presence of 2 highly conserved cysteine residues at 121 and 387, which are thought to link the large and small polypeptides by a disulphide bond before the post-translational proteolytic cleavage.

A unique feature of the triticin sequence is that it contains a lysine- rich repetitive domain, inserted in the hypervariable region (between amino acid positions 270 and 370) of the typical legumin- like genes.

From the nutritional point of view, lysine is the first limiting amino acid in many cereal seeds, including wheat. Triticin gene offers new opportunities to increase the number of these decapeptide

repeat units and/or to increase the proportion of triticin in wheat seeds to improve its nutritional quality. The HVR, which is over 100 amino acids long and tolerate a wide range of sequences, can accommodate more than ten of these decapeptide repeat units. Since this repeat motif is naturally present in the wheat triticin, it is unlikely to adversely affect the biological stability of triticin in the seeds, or the normal kernel development (Singh *et al*., 1993).

Genetic manipulation of wheat triticin gene for high lysine content: The development of efficient regeneration and transformation protocols in wheat has led to the production of number of transgenic plants with different traits. The target for enhancing grain quality of wheat includes increasing the protein content, increasing the proportion of essential amino acids like lysine, improving bread-making quality by manipulating high molecular glutenin proteins and modifying starch composition (Patnaik and Khurana, 2001). Successful efforts have been made to improve the bread making quality exploiting high molecular weight glutenin subunits (HMW-GS) of wheat seed proteins (Vasil and Anderson, 1997; Blechl and Anderson, 1996; Altpeter *et al*., 1996; Barro *et al*., 1997; Shimoni *et al*., 1997; He *et al*., 1999; Rooke *et al*., 1999; Alvarez *et al*., 2000). Efforts have also been made for modifying wheat starch for different end uses (Baga *et al*., 1999, 2000; Li *et al*., 1999; Gao and Chibbar, 2000). Increasing the lysine content, which is one of the most limiting amino acids in wheat, is still a challenge.

The genetic manipulation of triticin gene in increasing lysine content could be possible either by enhancing the expression using more efficient seed storage protein promoters or manipulating the hypervariable region (Singh *et al*., 1988). Triticin accounts for only five per cent of total seed proteins in wheat but it has a much superior amino acid balance with lysine content of four moles present. Presence of a lysine-rich repetitive domain in the hyper variable region of triticin gene offers new opportunities for the genetic engineering of triticin for even higher lysine content (Singh *et al*., 1993). Various strategies can be made in this direction. First is to increase the lysine content of triticin to at least ten moles per cent by increasing the number of lysine-containing repeat units (Table 6).

The maximum number of repeat units possible is ten in the present in hyper variable region of the gene, this modification is less likely to affect the biological stability of the protein Secondly, the proportion of triticin in wheat seed protein can be increased from the present 5% to about 15% (Table 6). The low amount of triticin in wheat may be due to either a low copy number of the gene or their inefficient expression due to weak promoters.

Isolation and characterization of full-length triticin cDNA: Full-length cDNA clones are a prerequisite for any meaningful genetic manipulation work. A probe created by serial deletion and representing 500 bp coding for the N-terminal region of the triticin gene was used to screen a wheat seed cDNA library in lambda gt 10 to get full-length triticin cDNAs. Fourteen cDNA clones were isolated and purified by three repeated cycles of plaque purification. However, only eleven clones showed presence of cDNA inserts when digested with *EcoR1*. In Southern blot analysis, all the eleven cDNA inserts, but not the lambda arms hybridized with the triticin probe. These clones were characterized by restriction analysis, PCR amplification using triticin specific internal primers designed from published Tri-25 sequence (Srinivas *et al*., 2001). Full-length cDNA and genes for triticin protein were cloned and characterized from wheat varieties K-68 and Chinese Spring differing considerably in grain colour, total protein content, grain hardness, milling behavior and baking characteristics (Singh *et al*., 2009).

PCR amplification of hypervariable region (HVR) from different wheat progenitors: The presence of unique lysine-rich decapeptide repeat motif inserted in the hypervariable region of triticin gene (Singh *et al*., 1993) provides an opportunity for its further enhancement using genetic engineering techniques. The hypervariable region, which is over 100 amino acid long, can tolerate a wide range of sequence and accommodate more than 10 of these repeats units. Since these repeat motifs are naturally present in the wheat triticin, it is unlikely to adversely affect the biological stability of triticin in the seeds or the normal kernel development. Attempts have been made to analyze different wheat progenitors like *Aegilops speltoides*, *A. longissima*, *A. squarrosa*, *Triticum urartu* along with bread varieties HD 2329, UP-262 and Kalyansona for natural variation in the hypervariable region for variable lysine content by PCR amplification using different sets of primers (Shailja *et al*., 2002; Yadav and Singh, 2008).

Cloning of seed specific promoters: A total of 24 promoter sequences with assigned accession number EF393165 to EF393188 and representing major seed storage proteins of wheat namely High molecular weight glutenin subunit (HMW-GS), low molecular weight glutenin subunits (LMW-GS) alpha/beta gliadins, triticin along with rice glutelins and oat 12S globulins were cloned from indigenous cultivars of wheat, rice and oat (Table 7). These cloned promoters were analyzed using various bioinformatics tools (Yadav *et al*., 2007; Yadav *et al*., 2008).

The presence of additional motifs like RY repeats, ABRE, AC-11, CAAT box, LTR, UTR, CCGTCC box, G box, GARE, MBS along with the common motifs present in seed storage promoters like Prolamin-box, TATA, CAAT indicates the potential of utilizing for higher expression. *In silico* analysis of one of cloned wheat HMW glutenin seed storage protein promoter is shown in Fig. 2 (Yadav *et al*., 2008).

Table 6: Existing and projected protein fraction and lysine content in wheat using proposed transgenic approach

Protein fraction	Prolamin	Triticin	Albumin/Globulin	Total
Existing (%)				
Protein fraction	85.0	5.0	10.0	100
Lysine in protein	1.5	4.0	5.0	2.0
Projected (%)				
Protein fraction	75.0	15.0	10.0	100
Lysine in protein	1.5	15.0	5.0	3.85

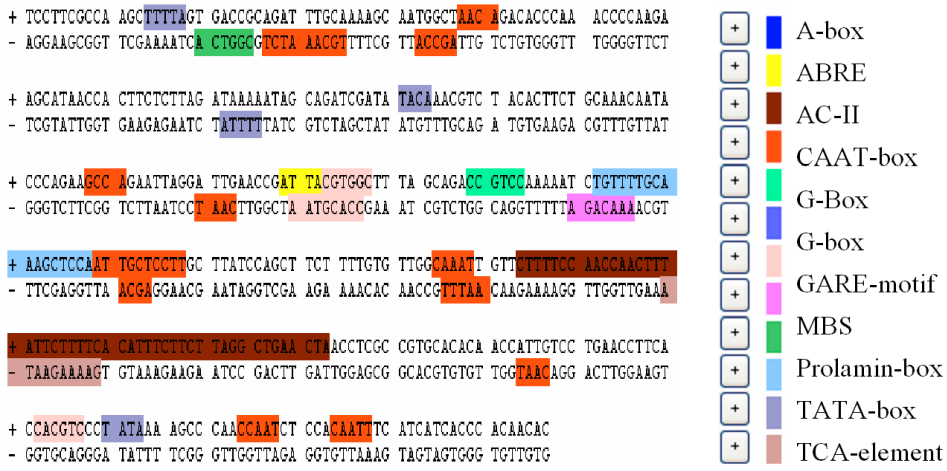

Fig. 2: *In silico* analysis of cloned wheat HMW glutenin seed storage protein promoter

Table 7: List of seed storage protein promoters cloned from different cultivars of wheat with assigned accession numbers

Seed storage protein promoters	Accession No.	Cultivars	Length (bp)
HMW Glutenin	EF396165	UP-262	402
	EF396184	UP-262	487
	EF396166	UP-262	397
	EF396167	UP-262	412
	EF396168	UP-262	385
	EF396169	UP-301	385
	EF396170	UP-301	393
	EF396171	UP-301	398
	EF396172	UP-301	392
	EF396173	UP-301	393
LMW Glutenin	EF396187	HD-2329	551
α/β gliadin	EF396174	Kalyansona	520
	EF396175	Kalyansona	564
	EF396177	UP-262	591
	EF396178	UP-262	521
	EF396176	UP-262	563
	EF396182	UP-301	548
Triticin	EF396181	HD-2329	428
	EF396183	HD-2329	370
	EF396185	HD-2329	452
		EF396186	
		Kalyansona	343

CONCLUSIONS

The advancement in the transformation and regeneration technology has led to the development of transgenic crops and the potential of transgenic technology for crop improvement has been witnessed by several countries in the recent years. The nutritional quality improvement is one of the major issues which needs to be addressed by the current advanced technology like biotechnology aided with the genomic revolution. The seed storage proteins have been studied extensively in different crops and knowledge about its regulation has also been elucidated to some extent. The wheat seed storage proteins have been targeted for quality improvement mainly bread making quality though nutritional quality improvement is also underway. Wheat triticin could be one such target for enhancing the lysine content of wheat seed protein which is one of the major limiting essential amino acid in wheat followed by threonine. Since the amount of triticin in total seed storage protein of wheat is quite less, different strategy has to be adopted either to enhance the expression of triticin or manipulate the hypervariable region of triticin or by increasing the copy number.

REFERENCES

Altenbach, S.B., C.C. Kuo, L.C. Staraci, K.W. Pearson, C. Wainwright, A. Georagescu and J. Townsend, 1992. Accumulation of a Brazilnut albumin in seeds of transgenic canaola results in enhanced levels of seed protein methionine. Plant Mol. Biol., 18: 235-245.

Altenbach, S.B., K.W. Pearson, F.W. Leung and S.M. Sun, 1987. Cloning and sequence analysis of a cDNA encoding Brazilnut protein exceptionally rich in methionine. Plant Mol. Biol., 8: 239-250.

Altenbach, S.B., K.W. Pearson, G. Meeker, L.C. Staracci and S.M. Sun, 1989. Enhancement of the methionine content of seed proteins by the expression of a chimeric gene encoding a methionine rich protein in transgenic plants. Plant Mol. Biol., 13: 513-522.

Altpeter, F., V. Vasil, V. Srivastava and I.K. Vasil, 1996. Integration and expression of the high molecular weight glutenin subunit 1Ax1 gene into wheat. Nature Biotechnol., 14: 1155-1159.

Altschul, A.M., 1965. Protein: Their Chemistry and Politics. Chapman and Hall, London.

Alvarez, M.L., S. Guelman, N.G. Halford, S. Lustig and M.I. Reggiardo *et al.*, 2000. Silencing of HMW glutenins in transgenic wheat expressing extra HMW subunits. Theor. Applied Genet., 100: 319-327.

Baga, M., R.B. Nair, A. Repellin, G.J. Scoles and R.N. Chibbar, 2000. Isolation of a cDNA encoding a granule-bound 152-kilodalton starch-branching enzyme in wheat. Plant Physiol., 124: 253-264.

Baga, M., R.N. Chibbar and K.K. Kartha, 1999. Expression and Regulation of Transgenes for Selection of Transformants and Modification of Traits in Cereals. In: Advances in Cellular and Molecular Biology of Plants, Molecular Improvement of Cereal Crops, Vasil, I.K. (Ed.). Vol. 5. Kluwer Publication, Dordrecht, The Netherlands, pp: 8-13.

Bansal, H.C., K.N. Srivastava, B.O. Eggum and S.L. Mentu, 1977. Nutritional evaluation of high protein genotypes of barley. J. Sci. Food. Agric., 28: 157-160.

Barro, F., L. Rooke, F. Bekes, P. Gras and A.S. Tatham *et al.*, 1997. Transformation of wheat with high molecular weight subunit genes results in improved functional properties. Nat. Biotechnol., 15: 1295-1299.

Barro, F., P. Barcelo, P.A. Lazzeri, P.R. Shewry, J. Ballesteros and N.A. Martin, 2003. Functional properties of flours from field grown transgenic wheat lines expressing the HMW glutenin subunit 1AX1 and 1DX5 genes. Mol. Breeding, 12: 223-229.

Bietz, J.A. and J.S. Wall, 1972. Wheat gluten subunits: Molecular weights determined by sodium sulfate-polyacrylamide gel electrophoresis. Cereal Chem., 49: 416-430.

Bietz, J.A. and J.S. Wall, 1973. Isolation and characterization of gliadin-like subunits from glutenins. Cereal Chem., 50: 537-547.

Bietz, J.A., F.R. Huebner, J.E. Sanderson and J.S. Wall, 1977. Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. Cereal Chem., 54: 1070-1083.

Blechl, A., J. Lin, S. Nguyen, R. Chan, O.D. Anderson and F.M. Dupont, 2007. Transgenic wheats with elevated levels of Dx5 and /or Dy10 highmolecular-weight glutenin subunits yield doughs with increased mixing strength and tolerance. J. Cereal Sci., 45: 172-183.

Blechl, A.E. and O.D. Anderson, 1996. Expression of a novel high molecularweight-glutenin subunit gene in transgenic wheat. Nat. Biotechnol., 14: 875-879.

Boroto, K. and L. Dure III, 1987. The globulin seed storage proteins of flowering plants are derived from two ancestral genes. Plant Mol. Biol., 8: 113-131.

Boulter, D. and R.R.D. Croy, 1997. The structure and biosynthesis of legume seed storage proteins: A biological solution to the storage of nitrogen in seeds. Adv. Bot. Res., 27: 2-84.

Bressani, R. and L.G. Elias, 1968. Processed vegetable protein mixtures for human consumption in developing countries. Adv. Food. Res., 16: 1-103.

Bright, S.W.J., P.R. Shewry and D.D. Kasarda, 1983. Improvement of protein quality in cereals. CRC Critical Rev. Plant Sci., 1: 49-93.

Brown, J.W.S., C.N. Law, A.J. Worland and R.B. Feavell, 1981. Genetic variation: An analysis by two-dimensional electrophoresis using intervarietal chromosomal substitution lines in wheat endosperm proteins. Theor. Applied Genet., 59: 361-371.

Casey, R. and C. Domoney, 1987. The structure of plant storage protein genes. Plant Mol. Biol. Report., 5: 261-281.

Crouch, M.L., K.M. Tenbarge, A.E. Simon and R. Ferl, 1983. CDNA clones for *Brassica napus* seed storage proteins: Evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide. J. Mol. Applied Genet., 2: 273-283.

Croy, R.R.D., J. A. Gatehouse, I. M. Evans and D. Boulter, 1980. Characterization of the storage protein subunits synthesized *in vitro* by polyribosomes and RNA from developing pea (*Pisum sativum* L.) Legumin. Planta, 148: 49-56.

Croy, R.R.D., M.S Hogue, J.A. Gatehouse and D. Boulter, 1984. The major albumin proteins from pea (*Pisum sativum* L.) Purification and some properties. Biochem. J., 218: 795-803.

Danielsson, C.E., 1949. Seed globulin of gramineae and leguminosea. Biochem. J., 44: 387-400.

Derbyshire, E., D.J. Wright and D. Boulter, 1976. Legumin and vicilin storage protein of legume seeds. Phytochemistry, 15: 3-24.

Doll, H., 1984. Nutritional aspects of cereal proteins and approaches to overcome their deficiencies. Philos. Trans. R. Soc. London B, 304: 373-380.

Eggum, B.O. and R.M. Beame, 1983. The Nutritive Value of Seed Proteins. In: Seed Proteins Biochemistry, Genetics and Nutritive Values, Gotteschalk, W. and P.H. Muller (Eds.). Junk J.N. Publishers, The Hague.

Ericson, M.L., J. Rodin, M. Lenman, K. Glimellus, L.G. Josefsson and L. Rask, 1986. Structure of the rapeseed 7S storage protein, napin and its precursor. J. Mol. Biol., 17: 1217-1231.

Ewart, J.A.D., 1990. Comments on recent hypotheses for glutenin. Food Chem., 38: 159-169.

FAO, 1970. FAO Nutritional Studies No. 24. FAO UN, Rome.

FAO, 1973. In FAO Nutritional Meet Rep. Ser. No. 52. WHO, Rome.

Forsyth, J.L., F. Beaudoin, N.G. Halford, R. Sessions, A.R. Clark and P.R. Shewry, 2005. Design, expression and characterisation of lysine rich forms of the barley seed protein CI-2. Biochim. Biophys. Acta, 1747: 221-227.

Galili, G. and M. Feldman, 1985. Structural homology of endosperm high molecular weight glutenin subunits of common wheat (*Triticum aestivum*). Theor. Applied Genet., 70: 634-642.

Gao, M. and R.N. Chibbar, 2000. Isolation charcterization and expression analysis of starch synthase IIa cDNA from wheat. Genome, 43: 768-775.

Garcia-Olmeda, F., P. Carbonero and B.L. Jones, 1982. Chromosomal location of genes that control wheat endosperm proteins. Adv. Cereal. Sci. Technol., 5: 1-47.

Guerche, P., E.R.P. DeAlmeida and M.A. Schwarztein, 1990. Expression of the 2S albumin from bertholletia excelsa in brassica napus. Mol. Gen. Genet., 221: 306-314.

Gupta, R.B. and K.W. Shepherd, 1990. Two-step one dimensional SDS-PAGE analysis of LMW subunits of glutenin. Theor. Applied Genet., 80: 65-74.

Gupta, R.B., 1989. Low molecular weight glutelins in wheat and related species. Ph.D. Thesis, University of Adelaide.

Gupta, R.B., K.W. Shepherd and F. MacRitchie, 1991. Genetic control and biochemical properties of some high molecular weight albumins in bread wheat. J. Cereal. Sci., 13: 221-235.

Hagan, N.D., N. Upadhyaya, L.M. Tabe and T.J.V. Higgins, 2003. The redistribution of protein sulfur in transgenic rice expressing a gene for a foreign, sulfur-rich protein. Plant J., 34: 1-11.

He, G.Y., L. Rooke, S. Steele, F. Bekes and P. Gras *et al.*, 1999. Transformation of pasta wheat (*Triticum turgidum* L. var. *durum*) with high-molecular-weight glutenin subunit genes and modification of dough functionality. Mol. Breeding, 5: 377-386.

Huebner, F.R. and J.S. Wall, 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem., 53: 258-269.

Kasarda, D.D., J.C. Autran, E.J.L. Lew, C.C. Nimmo and P.R. Shewry, 1983. N-terminal amino acid sequences of ù-gliadins and ù-secalins; Implications for the evolution of prolamin genes. Biochim. Biophys. Acta, 747: 138-150.

Katsube, T., N. Kurisaka, M. Ogawa, N. Maruyama, R. Ohtsuka, S. Utsumi and F. Takaiwa, 1999. Accumulation of soybean glycinin and its assembly with the glutelins in rice. Plant Physiol., 120: 1063-1074.

Kozak, M., 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. Nucleic Acid Res., 12: 857-872.

Kreis, M., P.R. Shewry, B.G. Forde, J. Forde and B.J. Miflin, 1985. Structure and Evolution of Seed Storage Proteins and their Genes with Particular References to Those of Wheat, Barley and Rye. In: Oxford Surveys of Plant and Molecular Cell Biology, Miflin, B.J. (Ed.). Oxford University Press, London, pp: 253-317.

Lawrence, G.J. and K.W. Shepherd, 1980. Variation in glutenin protein Subunits of wheat. Aust. J. Biol. Sci., 33: 221-233.

Lee, T.T.T., M.M.C. Wang, R.C.W Hou, L.J. Chen, R.C. Su, C.S. Wang and J.T.C. Tzen, 2003. Accumulation of a sesame 2S albumin enhances methionine and cysteine levels of transgenic rice seeds. Biosc. Biotech. Biochem., 67: 1699-1705.

Li, Z., X. Chu, G. Mouille, L. Yan and B. Kosar-Hashemi *et al*., 1999. The localization and expression of the class II starch synthases of wheat. Plant Physiol., 120: 1147-1156.

Mandal, S. and R.K. Mandal, 2000. Seed storage protein and approaches for improvement of their nutritional quality by genetic engineering. Curr. Sci., 79: 576-589.

Matta, N.K., A. Singh and Y. Kumar, 2009. Manipulating seed storage proteins for enhanced grain quality in cereals. Afr. J. Food Sci., 3: 439-446.

Mertz, E.T., L.S Bates and O.E. Nelson, 1964. Mutant gene that change protein composition and increase lysine of maize endosperm. Science, 145: 279-280.

Munk, L., K.E. Karlson and A. Hagberg, 1969. Comparing the strategy of breeding for protein quantity and quality in barley and broad bean. Proceedings of the 2nd International Barley Genetics Symposium, (IBGS'69), Washington State University Press, Pullman, Washington, pp: 544-548.

Nakai, S. and W.D. Powrie, 1981. Modification of Proteins for for Functional and Nutritional Improvements in Cereals. In: A Renewable Resources Theory and Practice, Pomeranz, Y. and L.M.N. Munk (Eds.). Academic Press, New York, pp: 217-242.

Nelson, O.E., 1968. In new approaches to breeding for improved plant protein. A Panel Meeting Organized by FAO/IAEA, Sweden.

Nelson, O.E., E.T. Merz and L.S. Bates, 1965. Second mutant gene amino acid and pattern of maize endosperm protein. Science, 150: 1469-1470.

Orth, R.A. and W. Bushuk, 1974. Studies on glutenin VI Chromosomal location of genes coding for submits of glutenine of common wheat. Cereal Chem., 51: 118-126.

Osborne, T.B., 1907. The Protein of the Wheat Kernel. Publication No. 84. Carnegie Institute, Washington, DC.

Patnaik, D. and P. Khurana, 2001. Wheat biotechnology. Electronic J. Biotechnol., 4: 2-2.

Payne, P.I. and K.G. Corfield, 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociation mechanism. Planta, 145: 83-88.

Payne, P.I., K.G. Corfield and J.A. Blackman, 1981a. Correlation between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J. Sci. Food Agric., 32: 51-60.

Payne, P.I., L.M. Holt and C.N. Law, 1981b. Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. I. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor. Applied Genet., 60: 229-236.

Payne, P.I., L.M. Holt, G.J. Lawrence and C.N. Law, 1982. The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. Qual. Plant. Plant Foods Hum. Nutr., 31: 229-241.

Payne, P.I., L.M. Holt and G.J. Lawrence, 1983. Detection of a novel high molecular weight subunit of glutenin in some Japanese hexaploid wheats. J. Cereal Sci., 1: 3-8.

Pernollet, J.C. and J. Mosse, 1983. Structure and Location of Legume and Cereal Seed Storage, Proteins. In: Seed Protein, Daussant, J., J. Mosse and J. Vaugham (Eds.). Academic Press, London, pp: 151-191.

Peterson, D.M. and D. Smith, 1976. Changes in nitrogen and carbohydrate fractions in developing oat groats. Crop Sci., 16: 67-71.

Rasco, C.Q., G.S. Sinagawa, J.A. Osuna-Castro, N. Bohorova and O. Paredes-Lopez, 2004. Accumulation, assembly and digestibility of amarantin expressed in transgenic tropical maize. Theor. Applied Genet., 108: 335-342.

Riley, R., 1965. In Essays on Crop Plant Evolution. Cambridge University Press, Cambridge, London and New York, pp: 103-122.

Riley, R., V. Chapman and R. Johnson, 1968. Introduction of yellow rust resistance of Aegiolops comorsa into wheat by genetically induced homoeologous recombination. Nature, 217: 383-384.

Rooke, L., F. Bekes, R. Fido, F. Barro and P. Gras *et al.*, 1999. Overexpression of a gluten protein in transgenic wheat results in greatly increased dough strength. J. Cereal Sci., 30: 115-120.

Sears, E.R., 1969. Wheat cytogenetics. Ann. Rev. Genet., 3: 451-458.

Sears, E.R., 1972. Chromosome engineering in wheat. Proc. 2nd Int. Stadler Genet. Symp., 4: 23-38.

Shailja, K., M. Rathore, N. Puri, D. Yadav and N.K. Singh, 2002. PCR amplification of hypervariable region of wheat triticin gene. J. Cereal Sci., 35: 129-134.

Shewry, P.R. and B.J. Meflin, 1984. Seed storage proteins of economically important cereals. Adv. Cereal Sci. Technol., 7: 1-83.

Shewry, P.R., A.S. Tatham, J. Forde, M. Kreis and B.J. Miflin, 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. J. Cereal Sci., 4: 97-106.

Shewry, P.R. and A.S. Tatham, 1990. The prolamin storage proteins of cereal seeds: Structure and evolution. Biochem. J., 267: 1-12.

Shewry, P.R. and N.G. Halford, 2002. Cereal seed storage proteins: Structures, properties and role in grain utilization. J. Exp. Bot., 53: 947-958.

Shimoni, Y., A.E. Blechl, O.D. Anderson and G. Galili, 1997. A recombinant protein of two high molecular weight glutenins alters gluten polymer formation in transgenic wheat. J. Biol. Chem., 272: 15488-15495.

Shotwell, M.A.Y. and B.A. Larkins, 1989. The Biochemistry and Molecular Biology of Seed Storage Protein. In: The Biochemistry of Plants, Marcus, A. (Ed.). Vol. 15. Academic Press, San Diego, California, pp: 297-345.

Siddique, K.A., 1990. Mutation in Wheat Future Possibilities. In: Biotechnology in Agriculture and Forestry Wheat, Bajaj, Y.P.S. (Ed.). Narosa Publishing House, New Delhi, pp: 549-571.

Sindhu, A.S., Z.W. Zheng and N. Murai, 1997. The pea seed storage protein legumin was synthesized, processed and accumulated stably in transgenic rice endosperm. Plant Sci., 130: 189-196.

Singh, R. and J.D. Axtell, 1973. High lysine mutant gene (bl) that improves protein quality and biochemical value of grain sorghum. Crop Sci., 13: 535-539.

Singh, N.K. and K.W. Shepherd, 1985. The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor. Applied Genet., 71: 79-92.

Singh, N.K. and K.W. Shepherd, 1987. Solubility behavior, synthesis, degradation and subcellular location of new class of disulfide-linked proteins in wheat endosperm. Aust. J. Plant Physiol., 114: 245-252.

Singh, N.K. and K.W. Shepherd, 1988. The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor. Applied Genet., 71: 79-92.

Singh, N.K., K.W. Shepherd, P. Langridge, L.C. Gruen, J.H. Skerritt and C.W. Wrigley, 1988. Identification of legumin like protein in wheat. Plant Mol. Biol., 11: 633-639.

Singh, N.K., G.R. Donovan, H.C. Carpenter, J.H. Skerritt and P. Langridge, 1993. Isolation and characterization of wheat triticin cDNA revealing a unique lysine rich repetitive domain. Plant Mol. Biol., 22: 227-237.

Singh, B.K., A. Singh, K. Gaikwad and N.K. Singh, 2009. Cloning and characterization of full-length triticin cDNA and genes from wheat varieties K-68 and Chinese spring. J. Plant Biochem. Biotechnol., Vol. 18.

Spencer, D. and I.J.Y. Higgins, 1980. The biosynthesis of legumin in maturing pea seeds. Biochem. Int., 1: 502-509.

Srinivas, B., D. Yadav and N.K. Singh, 2001. Cloning and Charactrization of full-length cDNAs of wheat triticin. Physiol. Mol. Biol. Plants, 7: 23-32.

Stoger, E., M. Parker, P. Christou and R. Casey, 2001. Pea legumin overexpressed in wheat endosperm assembles into an ordered paracrystalline matrix. Plant Physiol., 125: 1732-1742.

Tabe, L.M., C.M. Higgins, W.C. McNabb and T.J.V. Higgins, 1993. Genetic engineering of grain and pasture legumes for improved nutritive value. Genetika, 90: 181-200.

Takaiwa, F., S. Kilkuchi and K. Oone, 1987. A rice glutelin family: A major type of glutetin m RNA can be divided in two classes. Mol. Gen. Genet., 208: 15-22.

Tamas, C., B.N. Kisgyorgy, M. Rakszegi, M.D. Wilkinson and M.S. Yang *et al*., 2009. Transgenic approach to improve wheat (*Triticum aestivum* L.) nutritional quality. Plant Cell Rep., 28: 1085-1094.

Vasil, I.K. and O.D. Anderson, 1997. Genetic engineering of wheat gluten. Trends Plant Sci., 2: 292-297.

Walburg, G. and B.A. Larkins, 1986. Isolation and Characterization of cDNA encoding Oat 12s globulin m RNA. Plant Mol. Biol., 6: 161-169.

Wallace, J.C., G. Galili, E.E. Kawata, R.E. Cuellar, B.A. Shotwell and B.A. Larkins, 1988. Aggregation of lysine-containing zeins into protein bodies in Xenopus oocytes. Science, 240: 662-664.

Whitehouse, R.N.H., 1973. The Potential of Cereal Grain Crops for Protein Production. In: The Biological Efficiency of Protein Production, Jones, J.G.W. (Ed.). Cambridge University Press, UK., pp: 83-99.

Wrigley, C.W. and K.W. Shepherd, 1973. Electrofocusing of grain proteins from wheat genotypes. Ann. N. Y. Acad. Sci., 209: 154-162.

Yadav, D., V.K. Singh and N.K. Singh, 2007. *In silico cis*-regulatory elements analysis of seed storage protein promoters cloned from different cultivars of wheat, rice and oat. Online J. Bioinformatics, 8: 75-83.

Yadav, D. and N.K. Singh, 2008. PCR amplification of hypervariable region of wheat triticin gene from different wheat progenitors. Icfai J. Biotechnol., 2: 27-33.

Yadav, D., V.K. Singh, Sarita, N.C. Bisht and N.K. Singh, 2008. PCR based cloning of seed storage protein promoters of wheat, oat and rice and their *in silico* analysis for *cis*-regulatory elements. Bioinformatics Trends, 3: 67-74.

Yamaguchi, M., 1980. In World Vegetables. AVI Publishing Co., Connecticut, Westport, New York, pp: 395.

Yang, M.S., N.O. Espinoza and P.G. Nagpala, 1989. Expression of a synthetic gene for improved protein quality in transformed potato plants. Plant Sci., 64: 99-111.

Youle, R.J. and A.H.C. Huang, 1981. Occurrence of low molecular weight and high cysteine containing albumin storage protein in oilseeds of diverse specs. Am. J. Bot., 68: 44-48.

Yu, J., P. Peng, X. Zhang, Q. Zhao and D. Zhy *et al*., 2004. Seed-specific expression of a lysine rich protein sb401 gene significantly increases both lysine and total protein content in maize seeds. Mol. Breed., 14: 1-7.

Zhao, Z.Y., K. Glassman, V. Sewalt, N. Wang and M. Miller *et al*., 2003. Nutritionally Improved Transgenic Sorghum. In: Plant Biotechnology 2002 and Beyond, Vasil, I.K. (Ed.). Kluwer Academic Publishers, The Netherlands, ISBN: 1-4020-1126-1, pp: 413-416.

Zheng, Z.W., S. Kazuhiko, T. Kunisuke and N. Murai, 1995. The bean seed storage protein phaseolin is synthesized, processed and accumulated in the vacuolar type-II protein bodies of transgenic rice endosperm. Plant Physiol., 109: 777-786.