

Seed proteins maneuver to improve cereals grain quality: A review

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Abstract

Protein plays an important role in human nutrition. Cereals, the major type of our food crops have provided the main source of energy and dietary protein. The 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 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. This paper reviews information on various achievements by different scientists through initial attempts in this direction.

Keywords: Seed storage proteins; grain quality; transgenic crops; cereals

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Introduction

Protein was 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 hormones and in cell repair, defence 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 configurations and sizes. Generally, carbohydrates and fats are used as a source of energy but under certain situations 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. Therefore, 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 technical 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 lactating women of poor populations.

Seed storage proteins of major cereals

Since the period when human ancestors started earliest cultivation by gathering and saving seeds of

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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 of 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

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 molecular weight 19 kDa and 22 kDa. The β -zeins consist of polypeptides of molecular weight 14 and 16 kDa, and account for 10-15% of total zeins whereas γ -zein and δ -zein are represented by polypeptides of molecular weight 27 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 Dgliadins). 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 molecular weight of 44-80 kDa (Charbonnier, 1974). The glutenin polymers held together by disulphide linkages may occur as aggregates of very high molecular weight upto 20,000 kDa, the largest in the plant kingdom. 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 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 molecular weight 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

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 a-zein of maize	RNAi , antisense RNA technology	Reduced level of zeins, increased Lys, Trp in maize	Segal et al. (2003)
19 kDa a-zein of maize	Gene modified by Lys, Trp codons insertion	Increased Lys and Trp in maize	Wallace et al. (1988)
y-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)
Sb401 of 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	Transformation using homologous protein gene	Better dough quality in wheat	Altpeter et al. (1996)
1Ax1 HMW-GS of wheat	Transformation using homologous protein gene	Better dough quality in wheat	Barro et 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 homologous protein gene	Increased Lys in wheat	Tamas et al. (2009)
Legumin of pea	Transformation using homologous protein gene	Increased Lys in wheat	Stoger et al. (2001)
Glycinin of soybean	Transformation using homologous protein gene	Increased Lys in rice	Katsube et al. (1999)
3-phaseolin of french bean	Transformation using homologous protein gene	Increased Lys in rice	Zheng et al. (1995)
Legumin of pea	Transformation using homologous protein gene	Increased Lys in rice	Sindhu et al. (1997)
Sunflower seed albumin	Transformation using homologous protein gene	Increased Met in rice	Hagan 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 gene	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)

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).

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 δ 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 molecular weight

53-58 kDa each further consisting of one large subunit (molecular weight 32-37 kDa) disulphide-bonded to a small subunit (molecular weight 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.

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