Seed Storage Proteins and Amino Acids Synthetic Pathways and Their Regulation in Cereals with Reference to Biologically and Nutritionally Important Proteins and Bioactive Peptides in Millets

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Abstract

Although the food systems in developing countries have changed dramatically since the green revolution, malnutrition still remains a challenge and is now known to include the concurrent dimensions of under-nourishment and micronutrient deficiency as a serious issue in developing as well as developed countries. An average cereal protein value of 10% will give us the total cereal protein production of approximately 17 million tons annually. The accumulation of seed protein is a complicated characteristic and seed storage proteins are proteins that considerably accumulate in developing seed, whose principal role is to behave as the nitrogen, carbon, and sulfur storage reserve. These proteins are mobilized quickly during the germination of seeds and are the principal cause of nitrogen reduction to increasing plantings. In particular, the enzymatic functions of seed storage proteins are not known although proteins are structurally distinct in storage from various crops, they all have certain prevalent features. Plant storage proteins may be categorized into two categories; proteins from seed storage

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(SSPs) and plant storage (VSPs). SSPs are a group of proteins that accumulate in seeds at high concentrations in the late stages of seed development, whereas VSPs are protein accumulation in vegetative tissues, such as roots and tubers, based on plant species. SSPs are depleted during germination, and the subsequent amino acids are used as a food source by the growing seedlings. The most popular proteins in crops are the SSPs and the most commonly consumed plant proteins by human beings are crop proteins. Millets are considered as an enriched source of many essential amino acids derived from many quality proteins. According to World Health Organization, the proteins harboring more than 40% essential amino acids are called quality proteins and upon digestion and hydrolytic cleavage, several bioactive peptides having multiple health attributes are generated. In this chapter, we have briefly described about the proteins and peptides and their role in nutritional improvement present in millet.

Keywords

Seed storage proteins · Amino acids · Metabolic pathway · Cereals · Nutrition · Bioactive peptides and millets

8.1 Introduction

Malnutrition imposes serious issues in developing as well as developed countries which includes concurrent dimensions of undernourishment and micronutrient deficiency. It has been a major challenge for scientists to solve the problem of malnutrition. Half of the hunger is caused by protein malnutrition, according to the World Health Organisation. Protein-energy malnutrition (PEM) is a common disorder and is mainly due to energy, protein, and micronutrient deficiency. PEM and mineral malnutrition are mainly responsible for the high mortality ratio due to the onset of various diseases/illnesses. Cereals are the primary sources of food for the rural population and fulfill over half of the world’s population’s high dietary protein needs (Mandal and Mandal 2000).

Proteins are one of the main macromolecules present in the system and their deficiency may lead to improper muscle functioning, muscle wasting, stunted growth in children, increased risk of bone fracture, poor immunity, and so on. Amino acids are the building blocks of all proteins. There are 22 amino acids usually present in the proteins which are classified as essential and nonessential amino acids (NEAA), based on their synthesis in human beings. Nonessential amino acids are synthesized within the human system and therefore their deficiencies usually do not occur. On the other hand, essential amino acids (EAA) are not synthesized within the system therefore they are required to take from other sources. The deficiency of EAA may cause severe health issues. Figure 8.1 is showing some important symptoms of EAA deficiency. Cereal grains that are consumed directly in human food and feed, supplying more than 70% of the global calorie intake, also serve as primary reservoir of protein in the human food at global level. The

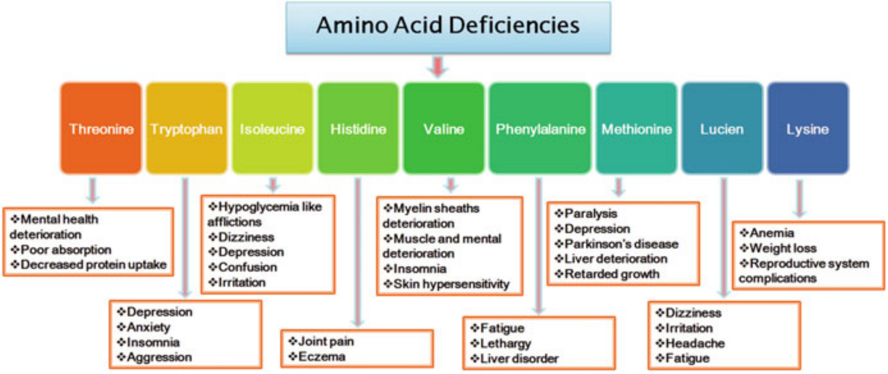

Fig. 8.1 Deficiency symptoms of nine EAA's showing their nutritional importance for human health

evergrowing demand for staple food crops for the increasing population is gradually being met by increasing production of grain seeds with an increased nutrient level (Martínez-Andújar et al. 2012). To meet this challenge, biofortification of cereals, i.e., wheat, rice, barley, and maize varieties and lines with high-quality proteins, high essential nutrients, and high yield is very necessary (Todorovska et al. 2005). In this chapter, we have discussed about the biosynthetic and regulatory pathways involved in the accumulation of seed storage proteins in cereals and also briefly touched on the importance of quality proteins stored in the seed endosperm of Poaceae family crops including millets.

8.2 Storage Proteins of Cereals and Millets

Cereals are the world's leading food crop and their seeds provide a perfect medium for manufacturing value-added food products, as they possess adequate and stable deposition of nutrients (Kawakatsu and Takaiwa 2010). As demand for food is growing worldwide, the attention paid to cereal proteins is increasing. The bulk of required human proteins are produced directly from livestock processing and from grain cereals. The most common protein foods are maize, rice, and wheat because of mainly synthezised and processed proteins in mature endosperm tissue. All of the proteins found in the mature endosperm are also known as the nonenzymatic storage protein for the exclusive purpose of supplying nutrition for germination and for development of a new plant (Doll 1977; Mandal and Mandal 2000). However, the endosperm features a wide variety of proteins different than formation, composition, and function (Doll 1977). Recently, seed proteins are classified into storage, biologically active, and structural proteins (Fukushima 1991). Storage proteins make up 50% of overall protein of mature cereal grains and hold a substantial effect on human and animal food nutritional quality and functionality in the production of food

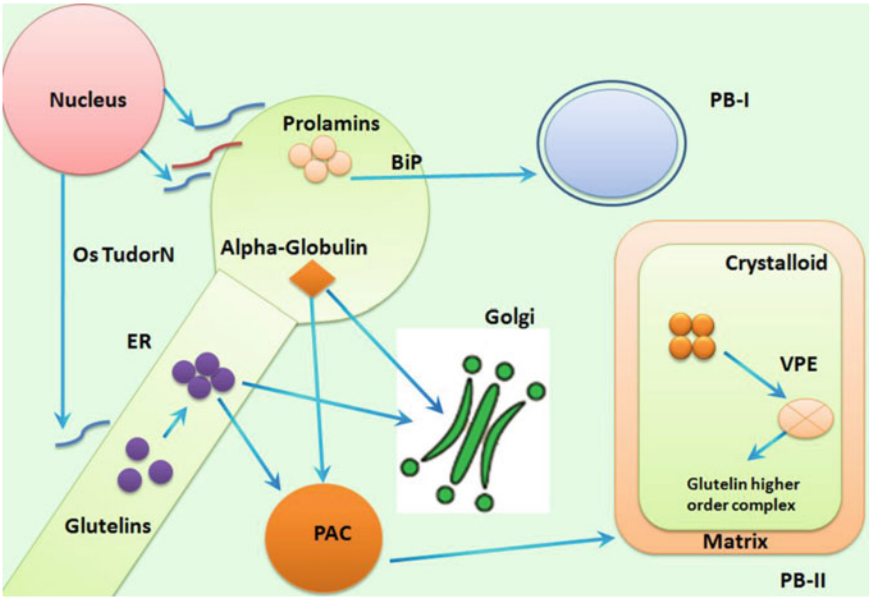

Fig. 8.2 Synthesis, trafficking, and deposition of different SSPs in rice

(Shewry and Halford 2002). These also acted as innovative products and balanced feeds in the processing of conventional local foodstuffs from ancient times. The typical cereal SSP classifications are based on their solubility are albumins (water-soluble), globulins (soluble salt), prolamins (alcohol-soluble) as well as glutelins (diluted acid/base soluble) (Landry et al. 2000; Osborne and Mendel 1914). The SSP structures generally based on DNA and protein sequencing are isolated and discovered by Shewry and Halford (2002). Though all grains commonly contain Albumins, prolamins and glutelins are frequently found in monocotyledonous and dicotyledonous grains, respectively. SSPs are of slightly different molecular masses between 10 and 100 kDa (Shewry et al. 1999). The SSPs are synthesized on the raw endoplasmic reticulum (ER) (Fig. 8.2), but the time the protein biosynthesis is at differing levels of seed processing. Studies of protein synthesis in maturing grains have shown that in the early stages of seed growth the fraction of albumin and globulin protein is synthesized and deposited in aleuronic tissue surfaces in cotyledon, while in later stages of seed ripening prolamin and glutelin are synthesized and located in protein body and matrix deposits. The use of available nitrogen for protein production by the highly productive cereals is usually very successful experiments that have persisted for so many years with the complicated aspects of their packaging, assembly, and partitioning (Doll 1977). The existence of retaining motifs, for example, for the zein motif, the cupin motif for globulin, and the intern series, followed by the hexapeptide repeat for albumin, characterize certain seed storing proteins.

8.2.1 Albumin

The most popular study of albumins in dicot seeds has been found in the Cruciferae and Arabidopsis. They have initially identified as 2s albums based on their sedimentation coefficients (S20wo) (Youle and Huang 1978). They are synthesized as single proteins and protected by the removal of both connection and short peptide from Aminoas well as Carboxyl terminus (Crouch et al. 1983). Both 2s albumins are compact globular proteins, with retained cysteine residues, despite their variations in structure and synthesis. In barley, the sum of albumins in general (3–5% of the total protein) is comparatively poor (Helm et al. 2004).

8.2.2 Globulin

Globulins are the most commonly distributed category of storage proteins, which can be classified into two groups according to their sedimentation coefficiency (S20. w); (a) vicilin-like globulars 7S (Templeman et al. 1987), (b) legumes 11S globulin type. Due to the post-translation process, both classes exhibit significant variance in their form and both types have cysteine and methionine lacking amino acids. In legumes, particularly peas, soybean, broad beans (We/afaba), and french beans were examined in greater detail for globulin storage proteins (*Phaseolus vulgaris*). The embryo and the outer layer of the endosperm contain Globulin proteins which have been more precisely characterized in maize embryo (Kriz 1989, 1999).

8.2.3 Prolamin

Prolamins serve as vital source of dietary protein for both livestock and human, besides being the main storage protein in most cereal seeds. These proteins are known as alcoholic-soluble (Osborne and Mendel 1914) proteins and have been named prolamin because of their high content of proline and glutamine (amid nitrogen). Cereal prolamins are found in the form of monomers or small compounds (Coleman and Larkins 1999) and these are different size and charge heterogeneous protein group (Esen et al. 1985). There are two typical structural features in most prolamins; firstly, there are different regions and domains which follow different structures and origins. Secondly, amino acid chains comprise of blocks reproducing or enriching them into Methionine-like amino acid residues, based on one or shorter peptide motif. These features arise due to increased concentrations of proline, glutamine, prolamine groups and some special amino acids including histidine, phenylalanine, glycine, and methionine (Shewry and Halford 2002). Because of its complexity and unique nomenclature, the nonexpert may have confusion on the prolamine structure and the properties. The existence of full amino acid sequences from all major prolamin groups made it possible for their structural and evolutionary relationship classification to be redefined (Shewry and Tatham 1990). The prolamin family and subfamily are named after each grass species. Table 8.1 provides a set of

Table 8.1 Classification of prolamins present in different species based on their evolution and homology among other crops

Subfamily	Tribe	Species	Gene family	Sub family	Prolamin group	References
Panicoideae	Andropogoneae	Maize (*Zea mays*)	Zein	α, δ	I	Lawton (2002)
				β, γ	II	
		Sorghum (*Sorghum bicolor*)	Kafirin	α, δ	I	Bietz (1982)
				β, γ	II	
	Paniceae	Foxtail millet (*Setaria italica*)	Setarin	α, δ	I	Monterio et al. (1982, 1987), Parameswaran and Thayumanavan (1995)
				β, γ	II	
Ehrhartoideae	Oryzeae	Rice (*Oryza sativa*)	Oryzein	10kd	I	
				13kd, 16kd	II	
Pooideae	Brachypodieae	Brachypodium (*Brachypodium distachyon*)	Brachypodin	Bra1, Bra2	II	
				Bra3	III	
	Triticeae	Wheat (*Triticum aestivum*)	Glutenin, gliadin	S-rich, S-poor	II	Shewry and Tatham (1990), Qi et al. (2012)
				HMW	III	
		Barley (*Hordeum vulgare*)	Hordein	S-rich, S-poor	II	Bietz (1982)
				HMW	III	

examples of prolamin and its names in various crops and even SSPs encoded by various sized multigene families (Feng et al. 2009).

8.3 Transcriptional Regulation of Seed Storage Proteins Mediated by Transcription Factors

Transcription factors are essential gene expression regulators comprising at least four discreet dominants, DNA-binding domain, nuclear localization signals (NLS), and oligomerization sites that function together to control a broad variety of physiological and biochemical processes in order to modulate the rates of transcription of the target genes. Transcription variables are also categorized according to their structural characteristics (a) Helix-turn helix (HTH), (b) Leucine zipper, (c) Zinc finger binds DNA, (d) helicopter-loop-helix (HLH), and (e) high mobility group (HMG) box motifs are the major transcription factors classified. DNA binds helix, leucine-pinching helix as a dimer while zinc finger can bind DNA both as a dimer and as monomers. Of these, the largest family of zinc finger proteins in eukaryotes can bind RNA, DNA, or nither. The term "zinc finger" refers to the sequence motifs in which zinc atom(s) are controlled by cysteines or histidines to form local peptide structures that are necessary for their particular functions. In several transcription factors that play a crucial role in interactions with other molecules involved in gene expression, the sometimes classified Zinc finger motifs dependent on the zinc-binding amino acids are frequently found (Yesudhas et al. 2017).

One of the most important zinc finger TF is Dof TF which highly plants specific and regulates the expression of SSPs. Dof TFs show specific binding with P-box and are identified as PBF (Prolamin Box Binding Factor). These proteins are found to be involved in activation of zein protein (prolamin) expression in maize seeds, in coordination with another basic leucine zipper transcription factor Opaque 2. Indeed the binding sites of these two transcriptional factors are just 20 bp apart on the Zein promoter suggesting their interaction in activating the zein gene expression. Opaque 2 (O2) is a transcriptional regulator for SSP gene expression (Schmidt et al. 1990) that encrypts basic bZIP (leucine zipper) transcription factor analogous to GCN4 of yeast has been shown to include amino acid metabolism. Orthologous genes from different crop plants like (SPA)/putative ESBF-II from wheat, BLZ2 from Barley (a bZIP TF 2) and RISBZ1 from rice (bZIP TF 1) were cloned (Onate et al. 1999). Typically the Opaque 2 resembling TFs bind to the GCN4 motif to trans-active the expression of SSP gene while in the α-globulin gene and the RISBZ2/REB binds with the ACGT motif for its activation (Nakase et al. 1996). GAMYB is another TF that shows interaction with DOF TFs including BPBF and SAD of Barley, RPBF/OsDOF3 of rice (Diaz et al. 2002, 2005). GAMYB expressed coordinately with DOF during seed maturation in both barley and rice. During seed germination GAMYB is coordinately expressed with SAD, while it acts antagonistically with BPBF in barley (Moreno-Risueno et al. 2007). Figure 8.3 is showing the TFs and their binding sites involved directly or indirectly in the regulation of SSP synthesis in cereals.

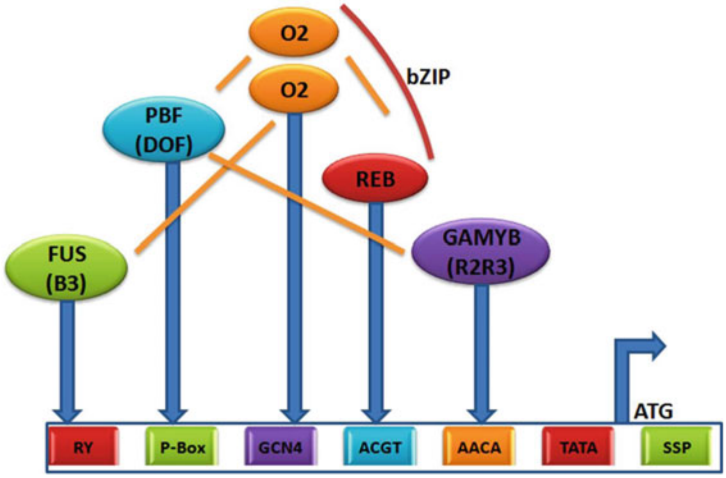

Fig. 8.3 TFs and their cis-regulatory binding sites involved in the synthesis and regulation of SSPs in cereals

8.3.1 DOF: A Zinc Finger Transcription Factor

The expression of plant genes comprises classes of transcription factors that have developed specifically to regulate plant genes and/or mediate a number of plant-specific signals. A typical example for Zinc finger transcription factors is the DOF (DNA link to one finger) family (Yanagisawa 2002, 2004). The DOF family is one of the most well-known transcription factors for plants with various roles (Fig. 8.3). The DOF (One Finger DNA-binding) proteins are defined as DNA-binding proteins, consisting of typically 200–400 amino acids with a strongly conserved Dof domain (Yanagisawa 2002). In maize, there was identified first protein MNB1a with Dof domain, which may connect with the CaMV(35S) promoter (Yanagisawa and Izui 1993). Every identified Dof transcription domain consists of 52 residues of amino acids which are homologous in different domains; however, there has been reported no homology outside these domains in these transcription factors. The lucid molecular description of different Dof domains indicates that it independently functions at structural and functional level (Yanagisawa 1996) (Fig. 8.3).

8.3.2 Dof TFs Involved in C:N Metabolism

Transcriptome studies confirmed that exogenous application of N regulates uptake and assimilation of N, and expression of regulatory genes coordinating C and N metabolism (Kumar et al. 2013). Photosynthesis stimulates the uptake and assimilation of N (Foyer and Noctor 2002) which ensures its correlation with C status. This linkage shows the importance of the study to explore the molecular mechanism and regulatory factors involved in C:N metabolism. There are so many regulatory factors and/or enzymes involved in C and N metabolism among them Dof TFs are the first plant species in its class to be identified as a master regulator to orchestra the C:N metabolism. Dof TFs also affect the NUE, by regulating the expression of multiple genes involved in C and N metabolic pathways and other plant developmental processes that are interrelated and influence the biomass, yield, and quality of a crop. Identification of cis-elements responsible for C and N signaling interactions (Palenchar et al. 2004) provides new avenues for manipulating the pathway to enhance NUE. Thus, alteration in the expression of Dof TFs might be a powerful approach for the generation of crops with enhanced NUE combined with superior agronomic traits and improved photosynthetic performance to achieve nutritionally superior crop with high yield.

The low protein content in most cereal grains with a low nutritional quality stimulated scientific efforts to genetically improve the protein at quantitative as well as qualitative level. Nutritional value for cereal protein has higher possibility for improving the low-prolamine, usually called high-lysine types. However, the observation that most high-lysine type cereals have low grain yield suggests that inhibition of prolamin synthesis also inhibits storing up of carbohydrates in the endosperm (Doll 1977). Although the improvement of quality is very important and necessary for the production of starch should not be compromised as it decreases the production of grain. Therefore, it is important that important nutritional traits of cereals should not be perturbed while improving the protein quality (Doll 1977).

8.4 Regulatory Elements/Genes and Proteins Involved in the Regulation of SSP Synthesis and Accumulation

In endosperm cells, Sulfur and Nitrogen are mainly deposited, in the form of SSPs, during cereal seed growth (Shewry and Tatham 1995). The encoding genes for SSPs are coordinated expressed in the development of endosperm, where spatial and temporal transcription regulation is carried out, by binding of transcription factors containing various cis and trans-acting motifs which bind to promoters of SSP genes. In the conferral of endosperm specificity on cereals, multiple consensus sequences in gene promoters are included (Albani et al. 1997; Mena et al. 1998; Carbajosa et al. 1997). The prolamin box (P-box) in the prolamin gene in barley, wheat, and other members of the Pooideae subfamily (Poaceae) is the most prominent of these cis-motifs. The P-box is based both on the highly conserved nucleotide sequence (5′-TGTAaAG-3′) and on the position (−300 region) in relation to the prolamin genes start translation codon (Fig. 8.4) (Forde et al. 1985).

Studies show the interrelation of these TF’s with SSP’s synthesis and accumulation and consequence of nitrogen availability on SSP’s synthesis and accumulation (Schmidt et al. 1990; Albani et al. 1997; Mena et al. 1998; Yanagisawa and Schmidt 1999; Yanagisawa 2002; Yanagisawa 2004; Diaz et al. 2002; Kumar et al. 2018a, b). The availability of Zn may also affect the expression of these TFs and therefore the synthesis of SSPs (Riechmann et al. 2000). The SSPs are nutritionally more balanced in comparison to other proteins of cereals and millet as they have a high percentage

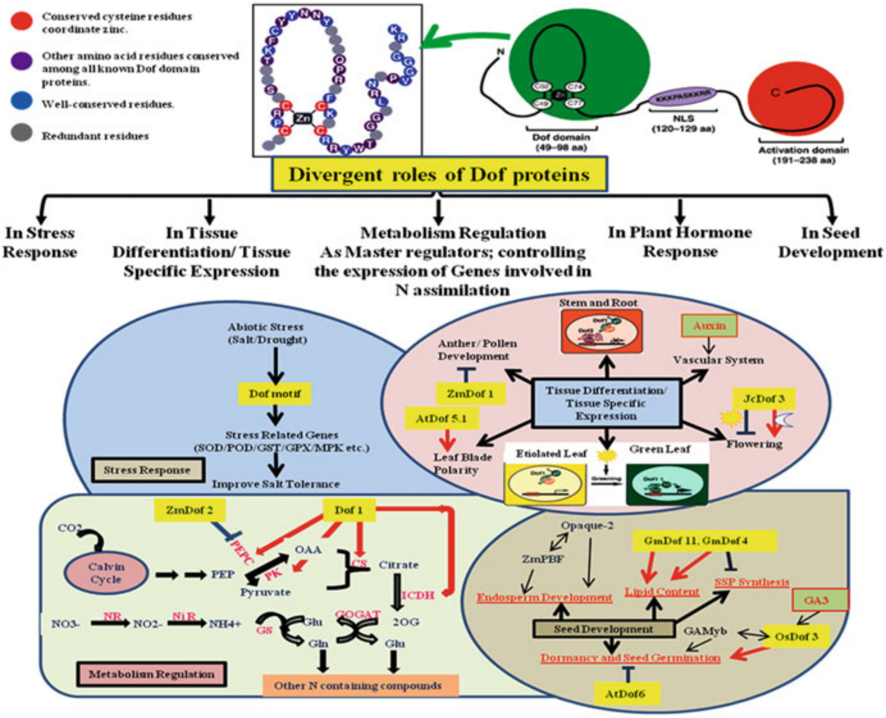

Fig. 8.4 A proposed structure of the Dof domain with N-Terminal amino acid sequence, representing divergent roles of Dof proteins

of essential amino acids which can be further exploited to develop value-added products to solve the problem of malnutrition and health problems.

The conserved P-box motif contains the recognition core for Dof proteins, i.e., (A/T AAAG) (Yanagisawa and Schmidt 1999), which regulates the expression of prolamin protein in coordination with Opaque 2. GCN4 motif provides the binding site to Opaque 2. Mutation analysis of P-box in zein promoter suggested that both PBF and Opaque 2 binding sites are necessary for zein protein accumulation in maize seeds (Carbajosa et al. 1997) and horde in protein accumulation in barley seeds (Diaz et al. 2002). This P-box is found to be conserved in all the prolamin gene promoters among cereals, including maize, rice, wheat, sorghum, barley, oat, and rye activate the transcription of prolamins by binding of PBF. So, the protein content can be improved by over-expression of PBF which in turn will increase the expression of the prolamin gene in cereal seeds and thus we can improve the protein content in cereal seeds (Diaz et al. 2002; Carbajosa et al. 1997).

The three storage proteins of millets are albumin, globulin, and prolamin among which prolamin is predominant. During cereal seed development, the genes encoding these seed storage proteins are simultaneously expressed in developing endosperm (Shewry and Tatham 1995). They are under transcriptional control, involving cis-motifs in their promoters and TFs. Few consensus sequences in gene promoters play an important role in conferring endosperm specificity in cereals (Albani et al. 1997; Mena et al. 1998). In the crops belonging to the Graminae (Poaceae) family, the most well-known cis-motifs is the prolamin box (P-box). The conserved P-box motif contains the recognition core for Dof proteins, i.e., A/T AAAG (Yanagisawa and Schmidt 1999). The Dof protein binds with P-box is identified as PBF (Prolamin Box Binding Factor) and found to activate the expression of prolamin protein in maize and barley (Carbajosa et al. 1997; Diaz et al. 2002; Kumar et al. 2018a, b). These PBF proteins which share a high degree of homology in their protein sequences with each other are specifically expressed during the grain filling stage. Recently 48 Dof genes have been reported in finger millet (Gupta et al. 2018). DOF TFs having DOF (DNA-binding with One Finger) domain is specific to higher plants. These have been shown to interact with both DNA and proteins, and enhance the expression of multiple genes implicated in C4 photosynthetic pathway or nitrogen assimilation (Yanagisawa 2002, 2004). Opaque 2 (O2) is a transcriptional SSP gene regulator that encodes abZIP (leucine zipper) transcription factor close to GCN4 yeast and has also been shown to be involved in amino acid metabolism. These O2-like TFs link to the GCN4 motif and switch SSP genes on expression.

8.5 Synthesis of Essential Amino Acids Determining the Accumulation of Quality Proteins

Nutritional quality of food is a crucial factor in maintaining human health and optimizing human genetic potential. In order to address the issue of deep-rooted food insecurity and starvation, the nutritional content of proteins should be improved (Fanzo 2015). Inherent factors are impacting the value of dietary protein in terms of amino acids affecting the dietary supply and the digestibility of the protein. Protein obtained from plant matter is limited to essential amino acids.

8.5.1 Regulatory Genes Involved in EAA's Metabolism

The crop or genotypes itself have high or low protein content and may be lacking in one or more amino acids. This amino acid deficiency can be resolved by combining two or three protein sources; however, antinutritive factors will still be present, limiting digestibility and bioavailability. Extensive attempts have been made to improve crops with these important amino acids by means of conventional breeding and mutagenesis. Additional attempts were made using genetic engineering methods to improve the production and reduce the metabolism of essential amino acids, as well as the expression of the recombinant proteins enriched with them, which were not sufficient and ineffective. It is therefore important to carry out a thorough analysis of the fundamental biological processes which are associated with the synthesis and aggregation of these amino acids in plants along with different factors linked with the biofortification of crop plants with these essential amino acids (Galili and Amir 2013).

Some regulatory enzymes of the EAA's biosynthetic pathways can alter the concentration of these EAA's by controlling their biosynthesis through a feedback regulatory mechanism. These enzymes are regulated by feedback regulatory mechanism in which the end products (intrinsic) of biosynthetic pathways inhibit the enzyme, e.g., Threonine is the −ve regulator of Homoserine Dehydrogenase (HD) and Aspartate Kinase (AK), which inhibit the biosynthesis of Threonine itself and Lysine, respectively. Besides feedback regulation other regulatory mechanisms are also operative during amino acid biosynthesis (Pratelli and Pilot 2014). It has been proved in different studies that external factors can also modulate the expression of these regulatory enzymes and therefore the synthesis of EAAs (Catala et al. 2007; Devoto et al. 2002). Results from the previous studies also show the possibility that the synthesis of EAA can be improved by altering the expression of these regulatory genes in response to exogenous application of fertilizers.

8.5.2 Millets: Source of Quality Proteins for Combating Malnutrition

Millets being rich in terms of proteins with essential amino acids, minerals like calcium, and phosphorus, vitamins of B group, and antioxidants have higher nutritional value than the common cereals like wheat, rice, and maize and are considered as nutri-cereals. Millets, minor small-seeded annual cereals, hold sixth rank in the world in terms of cereal grain production. They are climate-resilient crops because they grow in tropical and arid climates. Among the millets, Finger millet (FM) is minor millet and an ethnic crop of Uttarakhand which ranks fourth in importance among millets. Commonly known as Madua or Ragi, Finger millet (*Eleusine coracana*) (ragi) is highly nitrogen use efficient crop. It is a high nutritious value crop that has many valuable nutritional characteristics such as high amounts of minerals (Ca, Fe, Zn, etc.), vitamins, polyphenols, fiber, and high-quality grain proteins, which are a good source of essential amino acids. Despite growing under low or minimal nitrogen conditions, the protein content of finger millet grain is as good as that of major cereals such as rice and wheat which consume significant quantities of nitrogen fertilizers. Perhaps the finger millet has evolved special pathways for protein aggregation under the condition of limiting nitrogen conditions as compared to rice and wheat.

8.5.3 Nutritional Quality of Seed Storage Protein

Nitrogen is a crucial factor for the growth and production of plants used by young seedlings during germination and stored in the seed in the form of storage proteins. Roughly 16–50% protein is present in the seeds which include 1/3 of all dietary protein nitrogen (Graham and Vance 2003). Grain crop SSPs fulfill the main dietary protein needs of more than 50% of the world's population. Differences in synthesis and structure are of considerable significance for recognizing the capacity for greater nutritional benefit of SSPs in the endosperm. FM seeds grown under natural conditions produce 7–13% protein content of 100 g dry weight. Oat grain can have a high nutritional value relative to other cereals such as Barley and Wheat due to the high content of globulin storage proteins which implies a significant consideration for using it in livestock feed (Lockhart and Hurt 1986; Cuddeford 1995). Wheat SSPs include gliadins, glutenins, globulins, and albumins. "Legumin" type globulins found in wheat are called triticin, which accounts for around 5% of the total protein in wheat endosperm. It is recognized as nutritionally rich because a special decapeptide repeat pattern motif which is rich in lysine remains present in hyper-variable portion of this gene. Glutelin and globulin and protein fractions of barley possess high lysine content (about 5%) (Shewry et al. 1980). However, the horde in protein fraction of barley is especially rich in glutamine and proline and poor in lysine, i.e., approximately 0.8% (Shewry et al. 1980). Protein profiling of 52 FM genotypes was performed in our lab, which revealed no substantial variations in banding pattern between genotypes, whereas quantitative estimation of SSP fractions using Lowry method showed that glutelin was the highest followed by prolamin, globulin, albumin, and a significant difference was found based on comparative quantitative analysis of total seed protein. The content, yield, and composition determined the nutritional value and usefulness of SSPs (Chen et al. 2018). The essential amino acid content of SSPs of different cereals and millets has also been compared (Table 8.2) which reveals finger millet to be a nutritionally rich crop.

Table 8.2 Composition of essential amino acids in different cereals (mg/100 g)

Nutrients	Finger millet	Barnyard millet	Foxtail millet	Proso millet	Kodo millet	Rice	Wheat
Isoleucine	4.4	8.8	7.6	8.1	3.0	3.3	3.8
Leucine	9.5	16.6	16.7	12.2	6.7	6.7	8.2
Lysine	2.9	2.9	2.2	3.0	3.0	2.8	3.8
Methionine	3.1	1.9	2.8	2.6	1.5	1.5	2.3
Cystine	2.2	2.8	1.6	1.0	2.6	2.2	1.4
Phenylalanine	5.2	2.2	6.7	4.9	6.0	4.5	5.2
Tyrosine	3.6	2.4	2.2	4.0	3.5	3.0	3.9
Threonine	3.8	2.2	2.7	3.0	3.2	2.8	4.1
Tryptophan	1.6	1.0	1.0	0.8	0.8	1.5	1.4
Valine	6.6	3.8	6.9	6.5	3.8	4.4	5.5
Histidine	2.2	1.5	2.1	1.9	1.5	2.3	2.4

Adapted Gopalan et al. (1989)

8.5.4 Factors Affecting the Nutritional Quality of Seed and Proteins

The nutritional content of crops can directly or indirectly affected by several factors. These are soil influences, such as pH, abundance of nutrients, organic matter content, and soil-water relations; environmental and climatic conditions; crops and cultivars; and pesticide applications and cultural practices. Among all, the availability of nutrients and fertilizer applications and management affect the nutritional quality of field crops more directly. Some other factors regulate the nutritional quality of crops at the molecular level. These factors play a key role at transcriptional (promoters, transcription factors), translational, and post-translational levels (modification, refining, and deposition) (Kawakatsu and Takaiwa 2010) and influence the efficiency of seed w.r.t. yield, protein, and nutrient accumulation. Earlier studies into the nutritional status of crops produced using fertilizers are conflicting findings on seed yields and nutritional consistency (Leesawatwong et al. 2005). The supply of nutrients is an environmental variable while modern crop species are optimized for growth in soils with high abundance of nutrients and the genotype portion of G × E interaction is relatively stable, each cultivar has a different degree of efficiency in the acquisition and use of nutrients. It is therefore necessary to maximize the efficiency in which fertilizers are used in the production of crops. Studies also show that the applications of nutrients and fertilizers may alter the expression of genes involved in the acquisition of micro and macronutrients (Hammond et al. 2004).

Bogard et al. (2011) found that the increased absorption of N after synthesis was a significant factor in increasing the protein content of grain (GPC). They have shown that the synchronization of Nitrogen demand and supply in plants and the interaction between N supply and other environmental variables can affect GPC. However, generally, a negative correlation between grain yield and GPC has been observed and that may be an obstacle for improvement in protein accumulation. Since grain protein cannot adequately form without available N; therefore, a supply of N is a prerequisite for high protein yield. It has also been seen that changes to the availability of N to the plant at critical developmental stages can also improve the NUE (Kumar et al. 2013). Grain protein is directly related to the availability of soil nitrogen but the mechanism of Zn accumulation upon nitrogen application is not known. Therefore, the effect of Zn with nitrogen fertilizers/doses on grain protein quality and protein accumulation along with Zn is needed to be studied. Collecting knowledge on how N fertilization influences the aggregation of Zn in the shoot and the grain would further lead to a deeper understanding of the physiological and molecular processes underlying the relation between grain Zn and protein.

Millets are grown throughout the world due to the low farming costs, their biodiversity, such as finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), and proso millet (*Panicum miliaceum*) and the high biological characteristics. Low fat (1.5–5%) is the one character of millet grains but is rich in carbohydrates (60–70%), proteins (7–12%), and fibers (2–7%). They are a good source of magnesium, iron, and calcium. Proteins in millets are rich in essential amino acids that in other cereals and legumes are usually limited. Millet proteins are relatively rich in amino acids that contain sulfur which include methionine and cysteine. Millets are essential ingredients of a nutritionally balanced and healthy diet. The presence of proteins and BPs in millets helps improve the quality of their food protein, adding "functionality" to everyday consumption. It is well-known to be associated with reduction of cancer, cardiovascular disease, obesity, and diabetes and chronic disorders by taking diets rich in full-grain and related products. Because of their hypoglycemic property, their antioxidant activities millets can be used in the management of type 2 diabetes.

8.6 Bioactive Peptides

Peptides may have natural existance or they can be derived from native proteins' cryptic sequences, and are protein molecules smaller than 10 kDa. Bioactive Peptides (BPs) are low molecular weight organic substances composed of amino acids combined with the so-called covalent bonds of amide or peptide bonds. BPs are defined to promote heath with high tissue specificity and effectiveness. Peptides with physiological activity in the body consisted of 2–20 amino acids, which allows them to cross the intestinal barrier to have an effect on the level of tissue.

BPs have been detected in the hydrolysates and fermented milk products of enzymatic protein but gastrointestinal proteins digestion can also be responsible for their release (Gobbetti et al. 2007; Hartmann and Meisel 2007). BPs are mostly found by the digestion of microbial, plant proteolytic enzyme, and also by food processing through hydrolysis (cooking, fermentation, and ripening) BPs are considered as the next generation organic regulatory body because they not only preclude oxidation and microbial degradation in foodstuff but also improve treatment for different diseases and disorders (Lemes et al. 2016). The bioactive peptides display different biological effects of drugs, for example, antiproliferative, antimicrobial, ACE (inhibitory) effects, lowering cholesterol capacity, and antioxidant activities, improved absorption and bioavailability of minerals, and opioid-like activities. There is a dire need to identify various bioactive peptides that are present in various quality proteins of millets. Identification of such bioactive peptides in intact proteins having positive impact on body functions and influence health are given below:

Cardiovascular system: antihypertensive, antithrombotic, antioxidative, hypocholesterolemic activity.
Nervous system: antagonist activity and opioid agonist activity.
Gastrointestinal system: anti-appetizing, mineral binding, antimicrobial activity.
Immune system: immunomodulatory, cytomodulatory, antimicrobial activity.

8.6.1 Millet Proteins and Bioactive Peptides

Albumin, Globulin, cross-linking of prolamin, b-prolamin, and glutein are important constitutional fractions of Millet seed protein. However, these proteins relatively vary in their levels in different millets (Virupaksha et al. 1975). Millet protein amino acid analysis has found the presence of high amounts of amino acids which contain sulfur such as cysteine and methionine and various essential amino acids (Geervani and Eggum 1989). In our lab, efforts were made to focus on understanding the molecular basis of accumulation of quality proteins and accordingly several nutritionally important genes/proteins and promoters were identified. Besides, we are also interested to identify the EF-hand calcium-binding proteins for enhancing calcium accumulation and uptake in human gut.

Recently in our lab, few seed storage proteins have been characterized from finger millet that are nutritionally important as they contain elevated levels of essential amino acids like *Fima 1* lysine-rich gene (upto 9.0%), *Fimp2* methionine rich (upto 15.90%) besides two other Leu-Lys-Met-Pro and Leu-Lys-Val rich proteins having more than 40% EAAs (two patents filed). In order to understand the mechanism by which finger millet stores high calcium in seed, SEM-EDX analysis carried out in different finger millet genotypes revealed that maximum Ca is present in the aleurone layer, followed by seed coat and embryo (Goyal et al. 2005; Nath et al. 2013). It was therefore thought that chelators such as phytate, oxalate, pectate, and calcium-binding proteins (CBPs) may associate with calcium in these components during grain filling process (Goel et al. 2012). Although, phytates and oxalates are reported to be antinutrients, consuming finger millet has never been reported to cause negative health effects. Hence, research endeavors were mainly focused to identify genes encoding calcium-binding proteins involved in calcium sensing, transportation, and sequestration into cellular organelles. Subsequently, using conserved primer approaches, 3′ and 5′ RACE technique and developing seed transcriptome data, several genes encoding calcium-binding proteins such as calcium exchangers, calcium channel, calmodulin, Ca^{2+} ATPase and CaM dependent protein kinase (CDPK), calreticulin and calcinurin were identified in finger millet and their expressions studied in contrasting finger millet genotypes (Mirza et al. 2014; Singh et al. 2014, 2015; Chinchole et al. 2017).

These traditional functional genomics approaches have mainly investigated the changes in mRNA abundance, which due to transcriptional regulation and complex post-translational modifications of proteins, do not provide a true indication of protein expression and activity levels, respectively (Hittalmani et al. 2017). Only a few proteomics studies have so far been attempted to decipher the molecular basis of higher calcium build up in seeds of finger millet. This includes the identification of a 48 kDa Calreticulin as a CBP from finger millet using nano liquid chromatography–tandem mass spectrometry (Nano LC-MS) (Hirschi 2004). With influx of abundant genomic information and improvements in analytical technologies, proteomics has emerged as a powerful tool for studying different aspects of plant properties and growth regulations. Hence, the exploration of the seed proteome of finger millet not only appears to be essential to compliment the transcriptomics studies but also for the identification of new protein(s) with desirable functional characteristics such as nutraceuticals. Alternatively, the characteristic genetic traits of high nutritional value and stress tolerance make finger millet a particularly attractive system for proteomics study to analyze the proteins associated with nutritional quality and stress tolerance. In recent studies on seed storage protein in our lab, about 18 sequences of SSPs were recognized in the transcriptome of finger millet developing spikes namely LTP_DIR1, Germin-like_protein_1–3, Storage_protein, 11S_Globulin_seed_storage_protein, Glycine-rich_Protein-5, NSLTP_A, NSLTP_GPI-anchored_1, LTP_VAS, 19_kDa_globulin, NSLTP-3_like, Alpha-amylase_inhibitor-5, Vicilin-like_SSP, Glutelin_type-D-1, 10_kDa_prolamin, NSLTP-2, NSLTP-1, NSLTP-3, and NSLTP-4.

8.6.2 Characterization of Proteins Specifically Rich in Lysine, Arginine, and Methionine

BPs derived from millet seeds such as finger millet, Sorghum bicolor, buckwheat foxtail, pearl millet, quinoa and chia are described in Table 8.3 (Majid and Priyadarshini 2019).

8.6.3 Antimicrobial Peptides

A wide range of bacteria, fungus, and viruses is affected directly by peptides. The lengths (12–50 amino acids), compositions, charge, and presence of disulfide bonds vary from antimicrobial peptides. Antimicrobial peptides can kill bacteria directly by making pores through the membrane of the bacterial cord or by interacting in the microbial cells with macromolecules. There have been extensive studies on cereal and millet antimicrobial activity.

Camargo et al. (2008) reported antiviral peptides (2 kDa) which strongly inhibited replication of BHV (bovine herpes virus), HSV-1 (herpes simplex virus type 1), and weakly acted against polio virus. A 4 kDa buckwheat peptide is inhibiting the reverse HIV-1 transcriptase activity of *Mycosphaerella arachidicola* and *Fusarium oxysporum* in vitro as well. The antifungal activity in these peptides was caused by glycine and cysteine-rich amino acids (Egorov et al. 2005; Fujimura et al. 2003; Leung and Ng 2007). Fa-AMP1 and Fa-AMP2 are two antibiotical peptides of Buckwheat which have shown a broad range of antimicrobial activity against various gram-positive, gram-negative bacteria, and plant pathogens (Fujimura et al. 2003).

Peptides derived from foxtail millet had a different range of antimicrobial and antifungal activity (Xu et al. 2011). FFMp4, FFMp6, and FFMp10 three peptides derived from foxtail millet were strong antibacterial in *E. coli* ATCC 8099. Also peptides, as isolated from barnyard millet, finger millet, and proso millet reveal activity against *Pseudomonas areuginosa* (Bisht et al. 2016). Millet-derived BPs have high antivirus, bacterial, and fungal activity.

Table 8.3 Biologically active peptides in different millets and cereals crops

Source	Peptide sequence/ hydrolysates	Precursor protein	Bioactivity/against	References
Jower	Protein hydrolysates	Acid-soluble protein	Antiviral activity/herpes simplex virus type 1 (HSV-1), bovine herpes virus (BHV), Poliovirustype 1	Camargo et al. (2008)
Jower	VAITLTMK and VSKSVLVK	Total protein	Antioxidant activity	Agrawal et al. (2017)
Buckwheat	Protein hydrolysates	Total protein	Antifungal activity/ *Mycosphaerella arachidicola* and *Fusarium oxysporum*	Leung and Ng (2007)
Buckwheat	Protein hydrolysates	Total protein	Antiviral activity/HIV-1 reverse transcriptase	Leung and Ng (2007)
Foxtail	Protein hydrolysates	Total protein	Antifungal activity/ *Alternaria alternate*	Xu et al. (2011)
Finger millet	TSSSLNM VRGGLTR and STTVGLGISMRSASVR	Total protein	Antioxidant	Agrawal et al. (2019)
Buckwheat	Protein hydrolysates	Total protein	Antimicrobial activity/ *Agrobacterium rhizogenes* MAFF 210265, *Agrobacterium radiobacter* MAFF 520028, *Clavibacter michiganensis* subsp. *michiganensis* MAFF 301044, *Fusarium oxysporum* IFO 6384, *Geotrichum candidum* and *Curtobacterium flaccumfaciens* pv. *oortii* MAFF 301203	Fujimura et al. (2003)
Foxtail	Protein hydrolysates	Total protein	Antioxidant and antibacterial activity/ *E. coli* ATCC 8099	Mohamed et al. (2012), Amadou et al. (2013)
Pearl millet	SDRDLLGPNNQYLPK	Total protein	Antioxidant effect	Agrawal et al. (2016)
Foxtail	Protein hydrolysates	Total protein	Antihypertensive effect	Chen et al. (2017)
Buckwheat	WPL, VPW, VFPW	Total protein	Antioxidant effect	Ma et al. (2010)
Buckwheat	DVWY, FQ, VVG, VAE, GPP, DTPF, and WTFR	Total protein	Antihypertensive effect	Ma et al. (2006), Koyama et al. (2013)

(continued)

Table 8.3 (continued)

Source	Peptide sequence/ hydrolysates	Precursor protein	Bioactivity/against	References
Finger millet	Protein hydrolysates	Total protein	Antibacterial effect/ *Pseudomonas aeruginosa* (MTCC 424), and *Salmonella entrica* (MTCC 739)	Bisht et al. (2016)
Quinoa	Protein hydrolysates	Total protein	ACE-inhibitory activity and radical scavenging activity	Aluko and Monu (2003)
Buckwheat	Protein hydrolysates	Total protein	Anticancer/ antiproliferative against Hep G2 (hepatoma) cells, L1210 (leukemia) cells, breast cancer (MCF-7) cells, liver embryonic WRL 68 cells	Leung and Ng (2007)
Chia	Protein hydrolysates	Total protein	Antihypertensive effect	Segura Campos et al. (2013)
Quinoa	IQAEGGLT, DKDYPK, GEHGSDGNV	Globulin B	Antidiabetic effect	Vilcacundo et al. (2017)
Buckwheat	LQAFEPLR and EFLLAGN	Globulin protein	Antidiabetic effect	Wang et al. (2015)

8.6.4 Antioxidant Peptides

Oxidative stress causes the occurrence and onset spread of several diseases. Reactive oxygen species (ROS) are widely known to damage all macromolecules as well as lipids, protein, and DNA. Protein hydrolysis and digestion were used to produce antioxidant peptides from millets. Antioxidant properties are found in certain amino acids including Trptophan, Histidine, Lysine, and Tyrosine. Millets contribute significantly to the delivery of antioxidants such as tannins and phenolic acids which counteract in vitro and in vivo oxidative stress (Subba Rao and Muralikrishna 2002; Sudhakar et al. 2015; Han et al. 2018). An excellent antioxidant effect of a 14-Mer peptide (SDRLLPNNQYLPK) was demonstrated which was isolated from pearl millet (Agrawal et al. 2016). The antioxidant effect of 28 Leu-Leu-Pro-His-His synthetic peptides has been reported in soybean protein digestion. Among these peptides maximum antioxidant activity was reported in P-H-H tripeptide. Sorghum-isolating seven peptides, BPs, F2A, F2B, F2D, F2E, F3A, and F3B, of which 74.19% and 78.27% of free radical inhibition were F2B (VAITKTMK) and F3A (VSKSVLVC) higher in antioxidants compared with other peptides (Agrawal et al. 2017). In addition, antioxidant activity of finger millet BPs has also been reported by the same group. Studies related to molecular docking of the peptides (TSSLNMAVRGGLTR and STTVGLGISMRSASVR) showed that they interact with free radicals through the serine and threonine residue (Agrawal et al. 2019). Also, bioactive peptides derived from foxtail millet are enriched with Tyrosin/ Leucine and di, tri, and tetrameric peptides of buckwheat containing proline and tryptophane, and quinoa protein hydrolysates showed radical scavenging activities (Aluko and Monu 2003; Amadou et al. 2013). Together these studies show the application, and may assist in development of functional nutrients or functional foods, of millet-derived peptides to reduce oxidation stress and associated diseases.

8.6.5 Anticancer Peptides

Uncontrolled proliferation of cells is an important characteristic for cancer growth and development. Several BPs extracted from different sources demonstrated activity in anticancer (Wu et al. 2014; Wang et al. 2007). The proliferation of breast cancer (MCF-7), Hep G2 (hepatoma), and WRL 68 cells were inhibited by 4 kDa peptide derivative buckwheat with IC_{50} of 33 mM, 25 mM, and 37 mM respectively (Leung and Ng 2007). In addition, peptides such as LWREGM (F-1), DKDYPK (F-2), RELGEWGI (F-3), DVYSPEAG, and IFQEYI extracted from Quinoa have been suggested as potential anticancer agents due to their antioxidant and chemical protective activities. More studies are needed to identify potential BPs in millets and food products.

8.6.6 Antidiabetic Peptides

Blood sugar caused by inadequate insulin secretion is a metabolic disease. Type 1 insulin-dependent and type 2 noninsulin-dependent diabetes are the two types of diabetes. Millets have low rates of starch and protein digestibility in comparison to other cereals. BPs are isolated from plant source hydrolysates and have a key role to play in energy balance, insulin signaling, and resistance with a significant potential for development of suitable diabetes therapies (Chakrabarti et al. 2018). The hypoglycemic property of Millets has been attributed to the diverse factors, including polyphenols, fiber, interactions among starch, protein, and lipid and the intrinsic structural properties of starch (Kumar et al. 2018a, b).

The inhibitory activities of DPP4 showed peptides identified by oat, buckwheat, and barley proteins have varying IC (50) values. In addition to hydrolysates derived from millet and BPs, DPP4 displayed inhibitory effect in vitro (Vilcacundo et al. 2017). Millets-derived BP are therefore a significant reservoir of diabetes-targeting inhibitory DPP4 peptides.

8.6.7 Antihypertensive Peptides

Prolonged systolic blood pressure is a definite feature of high blood pressure. The renin-angiotensin pathway and kallikrein-kinnine system (KKS) have a vital role to play in regulation of blood pressure and maintenance of sodium homeostasis. The renin and ACE (angiotensin-converting enzymes) are the main enzymes which are involved in the renin-angiotensin system (RAS). ACE also degrades bradykinin, the central molecule of the KKS system, which is generated through the cleavage of kininogen kallikrein. Liberated BK stimulates nitric oxide (NO) and superoxide formation that leads to vasodilation (Erdos et al. 2010; Ceravolo et al. 2014). Different organic peptides, such as wheat, oat, barley, and rice, have been extracted from different cereals (Majumder and Wu 2014; Fan et al. 2019; Ganguly et al. 2019). Six BPs, viz., FQ, VAE, VVG, DVWY, WTFR, and FDART have decreased blood pressure from the lactic buckwheat sprouts (Koyama et al. 2013). The Quinoa-derived BPs and Chia derived from controlled protein hydrolysis significantly inhibited activity of ACE (Aluko and Monu 2003; Segura Campos et al. 2013). These findings demonstrate that RAS as well as KKS pathways are affected by ACE-inhibitory activity of millet BP and that it can be used to treat hypertension and related diseases.

8.6.8 Production and Processing of Bioactive Peptides

The BPs are dormant in the parent protein sequence and become active when released. The release of peptides can occur through digestive enzymes, microorganisms with proteolytic action and microbe or plant-derived enzymes derived in various ways (Wang et al. 2018). The functionality, efficiency of BPs and their absorption through the small intestine, and bioavailability in target tissues depend mainly on their amino acid composition, size, sequence and other parameters including charge, hydrophobicity, and hydrolysis rate (Shen and Matsui 2017; Ganguly et al. 2019; Wang et al. 2019). Therefore, their production is an important step, and research and attention need to be enhanced.

Fermentation using *Lactobacillus* spp. is one of the commonly used methods to release BPs from millet protein hydrolysate. The advantage of this method is that the main amino acid and environmental stability are not lost (Hajfathalian et al. 2018; Raveschot et al. 2018). Millet BPs was derived using enzymes (trypsin and pepsin) to imitate normal human digestion. The production of BPs from millets is challenging, as millets contain lower amount of protein than that from other sources (Agrawal et al. 2016; Bisht et al. 2016).

The sonication and hydrostatic pressure treatment have been shown to enhance protein hydrolysis and release of effective BPs (Li and Aluko 2010). After production, these bioactive peptides have high yield along with greater efficacy and are therefore important for downstream processing. Ultrafiltration techniques are also used to produce small molecular BPs in millets by using varying cutoff membranes of low molecular weight and chromatography with size exclusion. Reverse-phase HPLC is used to fractionate peptides based on hydrophobic properties of peptides (Pownall et al. 2010). A combination of different methods including conventional electrical dialysis, electrophoresis, and an ultrafiltration membrane with separation helps to achieve high efficiency in terms of concentration and desalination of peptides. This procedure was used to divide low molecular weight BPs in the range from 300 to 700 Da from flaxseed hydrolysate protein (Doyen et al. 2013; Firdaous et al. 2009).

Moreover, some affinity adsorbents, such as activated carbon, are also utilized to produce peptide fractions high in amino acids but low in aromatic amino acids (Adachi et al. 1992; Stone and Kozlov 2014; Dias et al. 2016). Pulse electric field was used to improve the ability of antioxidant properties in pine nut (KCHQP) and soybean peptides (SHCMN). The conditions in which BPs are processed to maintain their bioactivity and bioavailability are therefore important to optimize.

8.6.9 In Silico Approach to Identify Bioactive Peptides

Food-based peptides are potential functional food-ingredients for the incorporation of health-promoting diets targeted in the prevention of many chronic diseases. Conventional in vivo methods are tedious and time-consuming to produce, identifying and validating BPs. Due to the focused approach, lower time consumption, rapid results acquisition rate, and the resultant cost-effectiveness, bioinformatics/in silico analysis could be a powerful means for discovering BPs (Dziuba and Dziuba 2010). In order to identify potential BPs in food protectants, bioinformatics tools offer a great opportunity. Elucidating protein sequences is an essential step from BPs and molecular docking in the observation of protein–ligand interactions to determine the therapeutical potentials of novel BPs derived from foods. Many databases such as PepBank, PeptideDB, BIOPEP with antimicrobial peptide, APD, CAMP, and FeptideDB recorded the ample amount of bioactive peptides case already exist. The Web application includes tools to generate all possible peptides cleaved by different available enzymes from the input protein. FeptideDB is ultimately a computer aid for the evaluation of peptide bioactivities is freely available at http://www4g.biotec.or.th/FeptideDB/ (Panyayai et al. 2019).

8.7 Conclusion

Grains are the real basic food and frequently the main source of protein in several developing nations of Africa, Latin America, and Asia. Grains typically supply half of the people with dietary protein and can provide 70% of people's food in developing countries with protein intake. The seed protein ratios switch from 10% (in grains) of dry weight to ~40% (in certain plants and oilseeds) for an exceptional origin of dietary protein. The lack of protein contributes to protein exhaustion and a number of other issues impacting typical organic ability. Among minor grains, finger millet is recognized for its healthy quality (*Eleusine coracana* L. Gaertn). Finger millet calcium is 5–30 times higher than different oats. Finger millet has second main component in the form of grain protein content (GPC), which is 7% natural and ranges from 4.88% to 15.5%, supplying 44.7% of the essential amino acids. It also has various medical advantages including hypoglycemia, hypocholesterol, and ulceration. The yield is adapted to a wide range of situations, can resist critical saltiness, dry spell is moderately impermeable to waterlogging, and has a few true effects. Due to its high dietary benefit and storage features, the finger millet is developed primarily by subsistence farms and is filled as a food safety plant. In yield changes at molecular level, the enhancement of grain quality for food and feed is an important goal. Transcriptional and translational control has been shown to take a significant role in regulating seed development articulation (Curtis and Halford 2014).

At transcriptional level, genetic differences can be seen in the amalgamation of power proteins. Opaque 2 (o2) is an important translation factor (TF) of the leucine zipper (bZIP), binding to and regulating GCN4 value, cytosolic pyruvate cypd kinase (cyPDK) protein b32, and ribosomal inactivating properties. Several systems for DNA markers are actually used for respectable crop analysis. RFLP, AFLP, and Microsatellites or Single Sequence Repeat (SSRs) are the most widely employed marker structures (Gupta et al. 2017). The advantages of finger millet, such as hypoglycemia, hypocholesterol, and ulcerative behavior, are many. Grain is used as flour for cookies, bread, and other baked goods and is packed to provide good baby food (Ramakrishnan et al. 2016). In the coming very long period, the late released finger millet WGS will be helpful for all these assets to be examined for characterization of seed storage proteins, their amino acid composition, and also bioactive peptides present in the nutritionally and biologically important proteins can also be identified.

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