Chapter 6
Legumes and Pulses: Ways and Means to Enhance the Protein Quality

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Abstract Leguminous crops such as peas, beans, pigeon pea, lentils, and chickpea are also called as pulses. Pulses constitute one of the paramount sources of dietary proteins for major parts of the world's population, especially in those regions wherein consumption of animal protein is limited due to higher cost, nonaccessibility, and religious or cultural beliefs. In general, quality protein is mostly derived from animal source. The essential amino acids can also be obtained from plant proteins especially legume proteins by pairing them with cereal grains/proteins. Since, pulses have twofold high-protein content, compared with cereal grains, pulses can make an excellent complementary food source of protein for infants, children, and adults. In order to cater to the consumer's demand for plant proteins, various processing techniques have been employed to enhance the protein quality including protein digestibility. Thermal processing (cooking, autoclaving, heating, and microwave), germination, irradiation, fermentation, extrusion, and spray- and freeze-drying methods have been adopted for improving the protein quality, especially protein digestibility, in pulses. Biofortification and genetic engineering approaches could also contribute as viable options to enhance essential amino acids or quality protein in mature seeds. Adoption of economically feasible options like processing methods discussed in this chapter and genetic enhancement of essential amino acids as well as protein quality in pulses and legumes can greatly help in alleviating malnutrition.

Keywords Essential amino acids · Legumes · Malnutrition · Plant proteins · Protein quality

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R. S. V., S. Praveen (eds.), *Conceptualizing Plant-Based Nutrition*,
https://doi.org/10.1007/978-981-19-4590-8_6

6.1 Introduction

Leguminous crops such as beans, peas, lentils, pigeon pea, and chickpea are also called as pulses. Pulses constitute one of the principal sources of dietary proteins for major parts of the world, especially in those regions wherein utilization of animal protein is limited due to higher cost, non-accessibility, and religious or cultural beliefs. In general, pulses contain crude protein in the range of 21–40% by weight and are excellent source of protein than cereals like wheat, rice, barley, and quinoa (Nosworthy and House 2017). The amino acid composition of various pulses is presented in Table 6.1. Proteins are indispensable component of human nutrition and play an essential role in biological and cellular processes. These processes regulate the structural and functional role in living systems right from building tissues, muscles, immunity, synthesis of hormones, enzymes, and energy. Proteins are made of amino acids—among them, nine amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are considered as essential and are indispensable. Humans including other vertebrates cannot synthesize these amino acids in their body using metabolic intermediates and hence are required from daily dietary sources. Fundamentally, these essential amino acids can be obtained from single complete protein or quality protein which is generally derived from animal source. Protein quality is determined by taking into account of composition of essential amino acids, and its digestibility and bioavailability, and those proteins which satisfy the metabolic demands of these three components are called as quality food proteins. The essential amino acids can be obtained from incomplete proteins, especially plant-based proteins, wherein they are limited by one or two essential amino acids; for example, methionine and tryptophan are limited in pulses and lysine in cereals. In general, plant protein have digestibility rate of 75–80% compared to animal proteins (90–95%) (Sa et al. 2019). If pulses are paired with cereal grains/proteins, plant proteins can fulfill the daily requirement of essential amino acids at affordable price. Globally, plant-based proteins are in high demand not only because of its affordability, but most plant-based proteins contain a negligible amount of saturated fat, are cholesterol-free, and have heme iron and higher fiber; above all, they are an excellent source of antioxidants and other phytochemicals, which help in reducing risk of heart, and obesity related diseases. Several other studies have shown the advantages of daily intake of plant-based protein compared with animal proteins for minimizing the blood pressure (Elliott et al. 2006; Altorf-van der Kuil et al. 2012) and the risk of type 2 diabetes (Pounis et al. 2010; Sluijs et al. 2010). Pulses hold double the quantity of protein, compared with cereals, and hence, pulses can make an excellent complementary food source of protein for infants, children, and adults to meet their daily protein requirement in the diet.

In order to cater the consumer's demand for plant-based proteins, several processing techniques have been employed to enhance the protein quality, especially the trait protein digestibility. Thermal processing (cooking, autoclaving, heating, and

Table 6.1 Amino acid content in the various legumes reported in g/100 g protein

Amino acids (g/100 g)	Peas[a]	Faba beans[a]	Sweet lupines[a]	Soybeans[a]	Chickpea[b,c,d]	Pigeonpea[e]	Lentils[c]	Mung bean[d,e,f]
Lysine	1.68	1.67	1.54	2.39	6.68	6.99	1.720	6.95
Methionine	0.23	0.22	0.22	0.53	1.30	1.12	0.210	1.20
Cysteine	0.32	0.35	0.45	0.57	1.34	1.15	0.322	0.87
Threonine	0.97	0.95	1.18	1.44	3.71	3.53	0.882	3.28
Leucine	1.6	2.00	2.27	2.89	7.12	7.13	1.786	7.75
Isoleucine	0.97	1.13	1.38	1.63	4.30	3.60	1.06	4.23
Valine	1.04	1.22	1.28	1.82	4.19	4.31	1.22	5.18
Phenylalanine + tyrosine	1.80	2.03	2.53	2.26	7.83	11.04	1.88	9.02
Tryptophan	0.21	0.24	0.26	0.49	0.96	0.97	0.22	1.90
Arginine	2.02	2.40	3.26	2.85	9.42	5.98	1.90	6.99
Histidine	0.56	0.73	0.83	0.99	2.75	3.57	0.69	2.93
Sum of indispensable amino acids	11.40	12.94	15.20	17.86	49.6	49.39	11.89	50.33
Alanine	0.95	1.05	1.14	1.59	4.29	4.47	1.03	4.39
Aspartic acid	2.56	2.80	3.26	3.89	11.65	9.88	2.72	11.52
Glutamic acid	3.87	4.40	7.00	6.05	17.48	23.15	3.18	17.83
Glycine	0.95	1.09	1.38	1.32	4.14	3.70	1.00	3.98
Proline	0.94	0.99	1.37	1.65	4.13	4.38	1.03	4.57
Serine	1.05	1.22	1.61	1.67	5.04	4.71	1.14	4.92
Sum of dispensable amino acids	10.32	11.55	15.76	16.17	46.73	50.29	10.1	47.21
AA score	100	91	91	100	137	–	–	135
True digestibility (%)	95.9	90.8	89.4	90.7	85.2	57	71	80.2
PDCAA score	95.9	82.6	81.4	90.7	83.6	–	63	–

[a]From Erbersdobler and Jahreis (2017)
[b]From Alajaji and El-Adawy (2006)
[c]https://nutritiondata.self.com/facts/legumes-and-legume-products/4326/2
[d]From Han et al. (2020)
[e]From https://www.medindia.net/nutrition-data/pigeon-peas-red-gram-mature-seeds-raw.htm and https://www.medindia.net/nutrition-data/mung-beans-mature-seeds-raw.htm
[f]From Mubarak (2005)

microwave) is adopted for improving the protein quality though other processing techniques like germination, irradiation, fermentation, extrusion, spray-drying and freeze-drying methods are in vogue. The major challenge in utilizing plant proteins is to develop novel food processing techniques that could successfully blend two or more plant proteins to yield a complete protein and simultaneously enhances the protein quality and functional properties. Furthermore, the developed plant protein products must satisfy consumer's acceptance in terms of taste, texture, flavor, nutrition, and affordability. Protein quality enhancement is also been addressed by genetic manipulation of seed storage proteins and engineering of synthetic proteins which are tailored to meet the market specifications and consumer's demand (Young and Pellet 1994; Jiang et al. 2016). This chapter majorly focuses on strategies like supplementation, processing techniques, and genetic tools that help in improving the quality of plant proteins.

6.2 Protein Quality Assessment

The protein quality depends on the types of amino acids particularly the dietary indispensable amino acids, the physiological utilization of specific amino acids after digestion (or protein digestibility), as well as their bioavailability (Friedman 1996; Butts et al. 2012). The digestibility of protein starts with hydration and solubilization in the mouth. Once the food reaches the stomach, HCl denatures the proteins and enables proteolytic digestive enzymes to act upon the peptide bond. The pepsinogen produced in the stomach is converted into the pepsin (a type of protease enzyme) by HCl, which helps in cleavage of food-derived proteins into peptides. The proteolytic enzymes such as trypsin, chymotrypsin, carboxypeptidases, collagenase, and elastase secreted by pancreas enter into the duodenum and further catalyze cleavage of peptides into smaller peptides. The aminopeptidases and tripeptidases present in the small intestine further cleave shorter peptides into amino acids or dipeptides and tripeptides, which facilitates its absorption by the mucosal cells of intestine. Unabsorbed or undigested peptide/nitrogenous residues are transported to the large intestine (colon), wherein possibility of exposure of these substances into microbial modifications within the large intestine can occur, prior to fecal excretion.

The nutritional quality of the dietary proteins can be assessed using a variety of different markers and approaches such as amino acid score (AAS), nitrogen balance (NB), protein efficiency ratio (PER), net protein ratio (or retention) (NPR), net protein utilization (NPU), protein digestibility, biological value (BV), and protein digestibility corrected amino acid score (PDCAAS) (Boye et al. 2012). These bioassays (PER, NPR, NPU), mostly performed on animal models such as laboratory mice, are questionable as the amino acid requirements of these animals are quite different from humans and also entail time and are expensive (Schaafsma 2012). The in vitro protein digestibility (IVPD) methodology can be adopted to quickly evaluate

protein digestibility as compared to in vivo methods. The in vitro assays are simple, inexpensive, reliable, and rapid. In vitro methods also provide information on protein stability and quality and can be used for quick profiling of large number of samples (Coda et al. 2017). The PDCAAS method, which was approved and recommended by Food and Agriculture Organization (FAO) in 1991 to determine protein quality, is most widely used method which considers two factors into account such as availability of essential (indispensable) amino acids and protein digestibility (Butts et al. 2012; Tavano et al. 2016). In PDCAAS, amino acid profile of a food protein is compared to a reference value, and an amino acid score is determined by the ratio of the limiting amino acid content in the test protein to that of the reference protein. The amino acid score is then corrected by multiplying with digestibility (true digestibility, fecal digestibility or in vitro digestibility) of the protein to generate a PDCAAS value (Schaafsma 2012; Hughes et al. 2011). There are different recommended patterns given by FAO or World Health Organization (WHO). For infants under 1 year of age, the recommended reference protein is human milk protein and the reference pattern used for other age groups is the amino acid scoring pattern recommended by FAO/WHO (1991) for children in the age group of 2–5 years, since at this age the requirement of the essential amino acids by human body is the maximum, and further, it decreases slightly with advancing age (Schaafsma 2012).

6.3 Processing Techniques to Improve the Protein Digestibility

6.3.1 Germination

Germination is also called as a bioprocess involving a series of biochemical reactions that facilitates changes in protein content by activating the transcriptional machinery of seeds (Ohanenye et al. 2020; Weitbrecht et al. 2011). Seed protein quality is determined by seed structure, primary structure of proteins, and antinutritional compounds (phytic acids, polyphenols, trypsin–chymotrypsin inhibitors, and tannins). Therefore, germination helps in improving protein digestibility in legume seeds by disintegrating cell wall, stored proteins in the seed, and antinutrients. Germination of lentil and faba bean favored protein digestibility causing reduced content of antinutrients (phytic acid and tannins) (Expósito et al. 2021). Enhanced true protein digestibility (TPD) in cooked black bean products was reported by Kannan et al. (2011), and they observed no correlation between TDS and PDCAAS due to limiting amino acid score. However, highest TPD and PDCAAS values were observed for cooked germinated beans in combination with rice. Little effect of germination on amino acid profile of cowpea was documented; however, increased in vitro starch and protein digestibility with higher PDCAAS score were documented

in germinated cowpea flour-based weaning food (Jirapa et al. 2001). Germinated mung bean, chickpea, and cowpea not only showed increased IVPD in the order of 15–25, 6–17, and 6–17%, but also influenced the higher protein content in the order of 9–11, 11–16, and 8–11% (Uppal and Bains 2012). A sixth day after germination caused increased protein content in chickpea (*Cicer aretinium* L.), lentil (*Lens culinaris* Merr.), and yellow pea (*Pisum sativum* L.) (Xu et al. 2019). Indian bean (*Dolichos lablab* var. *lignosus*) was found to have increased IVPD and PER after 32 h of germination and was comparable with that of reference protein casein (Ramakrishna et al. 2008). In addition to protein, germination of green gram (*Vigna radiata* (L.) R. Wilczek), cowpea (*Vigna unguiculata* (L.) Walp.), lentil (*Lens culinaris* Medik.), and chickpea (*Cicer arietinum* L.) caused a significant increase in in vitro iron, calcium bioavailability, thiamine content, and in vitro protein digestibility (Ghavide and Prakash 2007). Increased methionine content was reported in germinated soybean and lupin seeds (Escobedo et al. 2014; Chilomer et al. 2010; Martínez-Villaluenga et al. 2010). Enhanced digestibility of protein was due to germination and is majorly attributed to increased enzymatic hydrolysis of protein that causes compositional changes in the constituents like phytic acid, polyphenols, and protease inhibitors (Chitra et al. 1996; Mbithi-Mwikya et al. 2000; Bau et al. 1997).

6.3.2 Infrared Heating and Wet/Heat Moisture Treatment

Depending on the degree of processing and its types, protein digestibility and compositions of amino acid can be modified to improve the protein quality or nutritional value of the food grains/flours. Significant increase in the PDCAAS value from 0.65 to 0.71 was only found in *desi* chickpea when the sample was subjected to tempering (20% moisture) and heating at 135 °C (Bai et al. 2018). Enrichment of protein quality and digestibility of starch in navy bean and chickpea seeds was reported utilizing infrared heating with tempering (Guldiken et al. 2022). Total essential amino acid content increased in cowpea, pea, and kidney beans following soaking, boiling, microwave cooking, and autoclaving treatments (Khattab et al. 2009). The sulfur-containing amino acids were also increased, and the autoclaving was found to be highly efficient in improving the protein quality in terms of PER and amino acid scores. IVPD and PER improved by all cooking treatments from 84 to 90% and 2.3 to approximately 2.5, respectively. The improved IVPD was due to decreased antinutritional compounds (phytic acid, tannins, trypsin inhibitor, hemagglutinin activity, and saponins) in chickpea (Saleh and El-Adawy 2006). Soaking followed by cooking was found to be highly effective in reducing trypsin inhibitor activity in *Dolichos lablab* beans, and improved IVPD was observed in mung bean, chickpea, and cowpea sprouts upon pressure cooking and microwaving (Osman 2007; Uppal and Bains 2012). These processing treatments cause partial denaturation of protein, thereby providing easy

accessibility to the action of protease digestive enzymes. It also destroys protease inhibitors at different levels of seed moisture and surface temperatures which in turn affects the protein quality, functional, physicochemical, and nutritional properties of the flours.

6.3.3 Extrusion

Extrusion is thermomechanical process which involves subjecting the food ingredient to high-temperature and high-shearing processes and is predominantly used in food texturization, commercially being used for the production of various snacks. It is effective in reducing the activity of antinutritive compounds (Cotacallapa-Sucapuca et al. 2021). The high temperature and shear pressure in the process induce chemical and structural changes and cause denaturation of protein which in turn affects protein digestibility. The impact of extrusion process on the protein or amino acid content of various pulses was documented (Batista et al. 2010; Kelkar et al. 2012; Simons et al. 2015). Although the process of extrusion does not facilitate alteration in protein content in beans (Batista et al. 2010; Simons et al. 2015), reduced sulfur-containing amino acid like cysteine and methionine was observed due to high temperatures and disruptive forces of the extruding process (Arija et al. 2006). Increased IVPD was reported in faba bean, pea, chickpea, and kidney beans at extrusion conditions of 140–180 °C (El-Hady and Habiba 2003; Nosworthy et al. 2018; Batista et al. 2010). Slight increase in protein recovery with decreased trypsin inhibitory activity was observed in extruded pea seeds. Significant decrease in valine, phenylalanine, and lysine content was reported at 129 °C, and decreased trypsin content was observed at 142 °C (Frias et al. 2011). Enhanced protein digestibility by 56% was observed in cooked cowpea flour subjected to extrusion at 120 °C (Batista et al. 2010). It was reported that extrusion did not impact the protein digestibility of soybean; however, it helped to reduce trypsin inhibitors (Bertipaglia et al. 2008).

6.3.4 Irradiation

Food irradiation is a kind of processing technique, wherein food is exposed to ionizing radiation (alpha, beta, gamma, X-ray, or energetic electrons), to destroy pathogens of microbial origin and insects which influences food quality as well as safety. Depending upon the purpose, different doses of irradiation are being used; for instance, <1 kGy is used to achieve insect disinfection, delayed sprouting, and ripening; 1–10 kGy is being employed to kill microorganisms and to change the

functional properties of food, whereas 10–50 kGy is utilized for commercial sterilization like virus elimination (Ehlermann 2016; Lima et al. 2019). Food irradiation is not allowed to employ for nutritional profile modification; nevertheless, its impact on protein quality has been evaluated. The dry common beans showed improved protein digestibility when irradiated with 1, 5, and 10 kGy doses of γ-radiation using ^{60}Co as source (Lima et al. 2019). Electron beam-irradiated lotus seeds showed higher content of essential amino acid like threonine, valine, leucine, tyrosine, tryptophan, and lysine; however, decreased PDCAAS from 43 to 24% was reported when the samples were subjected to 30 kGy irradiation (Bhat and Sridhar 2008). Irradiation at 0.5–1 kGy improved protein digestibility (79.9%) and decreased phytic acid in Sudanese faba bean (Osman et al. 2014).

6.3.5 Fermentation

Fermentation is a type of processing technique, wherein desirable biochemical changes in food matrix are brought by microorganisms (bacteria, molds, and yeasts) particularly through enzymatic action (Kahajdova and Karovicova 2007). It is being adopted to improve the nutrient bioaccessibility as well as bioavailability from various food sources (Hotz and Gibson 2007) and improves shelf life and organoleptic properties of food (Chaves-Lopez et al. 2014). Fermentation aids in hydrolysis process which could contribute to decreased antinutritional compounds and thus helps in nutritional quality enhancement in the food. Fermented chickpea seeds (soaked chickpeas subjected to cooking at 90 °C for 30 min and inoculated with *Rhizopus oligosporus* and allowed for fermentation at 34.9 °C for 51.3 h followed by drying at 52 °C for 12 h followed by milling) showed improved IVPD of 83% compared to unfermented seeds (72%). Further, improved PER, NPR, and PDCAAS from 1.6 to 2.3, 2.7 to 3, and 73 to 92%, respectively, were reported (Angulo-Bejarano et al. 2008). A fermented product of common beans (tempeh-type) prepared using *R. oligosporus* showed decreased trypsin inhibitor, phytic acid content, and improved protein quality (Paredes-López and Harry 1989). Increased protein digestibility was attributed to increased proteolytic enzymes, wherein fermentation process not only degrades antinutritional compounds but also helps in breaking down complex proteins into simpler and smaller peptides, thereby facilitating release of peptides and amino acids (Nkhata et al. 2018). However, Kannan et al. (2011) found no significant increase in PDCAAS scores in fermented black bean products. The effects of physical processing methods on protein quality of pulses are summarized in a Fig. 6.1.

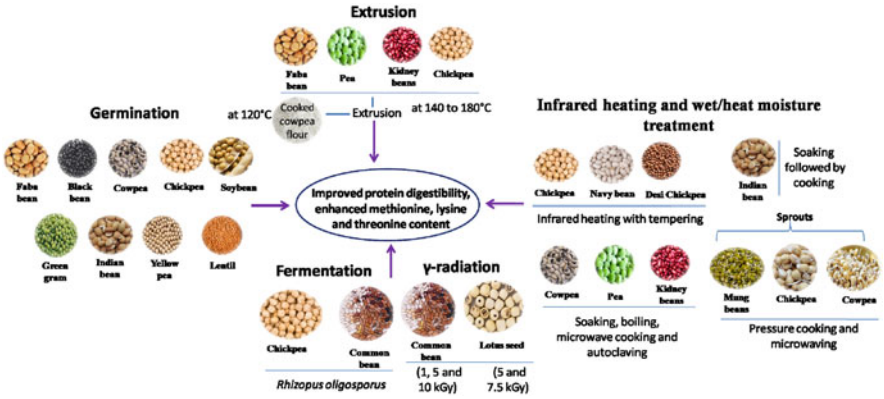

Fig. 6.1 Graphical representation showing the effect of physical processing methods on protein quality of pulses

6.4 Genetic Approaches to Enhance Protein Quality of Pulses

Biofortification of crops with quality protein or essential amino acids through genetic engineering approach has both economic and nutritional significance. Application of genetic approaches to improve protein quality of crop is predominantly restricted to model plants with enhanced essential amino acid (like Lys, Trp, and Met) synthesis. Due to lack of annotated crop genome sequences, this approach has witnessed limited success in crop species. Nevertheless, enhanced methionine content (11.11 mg/g protein) in soybean was reported by overexpressing de novo synthetic protein—MB16 (Zhang et al. 2014). Overexpression of endogenous Met-rich protein is one of the strategies to address the deficiency of sulfur-containing amino acids (SAA) in plants. This approach was used to enhance total methionine content in seeds by 6.8% in transgenic soybean (Yamada et al. 2008). Enrichment of free SAA as well as increased SAA-rich seed proteins was successful in narbon bean (*Vicia narbonensis*)—a close relative of faba bean (Demidov et al. 2003). Overexpression of 2S albumin storage protein and bacterial aspartate kinase gene resulted in enhanced seed methionine content up to 2.4-fold compared to that of wild type. The impact of starch biosynthesis on seed protein content especially albumin fraction (rich in SAA) was reported, wherein mutation in genetic loci of pea plants showed lower levels of starch content and enhanced protein content with relatively high content of the albumin fraction of seed protein (Casey et al. 1998; Hughes et al. 2001). *Glycine max* 2S-1 (post-translationally processed 2S-albumin) was reported as a candidate gene for overexpression approach to enhance protein quality in legumes including soybean (Galvez et al. 2008). Modification of nucleotide

sequence in an endogenous gene has also found to be promising approach to enhance SAA; for instance, a part of gene (45 bp sequence) that encodes Met-rich region was isolated from maize and incorporated into a β-*phaseolin* gene from *Phaseolus vulgaris* for overexpression (Hoffman et al. 1988). Several Met-rich proteins were detected in soybean which helps in utilizing these proteins in enhancing protein quality (George and De Lumen 1991). Transgenic soybean and canola plants were developed by overexpressing bacterial DHPS (dihydrodipicolinate synthase) gene and observed elevated levels of free lysine in mature seeds (Falco et al. 1995). Protein quality of rice was enhanced by overexpression of synthetically designed fusion proteins and observed higher lysine and threonine content (Jiang et al. 2016). Gibbon and Larkins (2005) developed "quality protein maize" and showed two fold increase in lysine content in maize seeds, and it was achieved by using high lysine maize mutant *opaque2* (Mertz et al. 1964) as a parent line. Higher lysine content (14%) in rice plants was achieved through regeneration from calli (Schaeffer and Sharpe 1987). Through modification of biosynthetic and catabolic fluxes, increased essential amino acid content, especially free lysine and methionine, was reported in tobacco (Shaul and Galili 1992), canola (Falco et al. 1995), and Arabidopsis (Ben-Tzvi Tzchori et al. 1996). Slight increase in free lysine was observed in rice and barley through overexpression of bacterial *DHPS* (Lee et al. 2001; Brinch-Pedersen et al. 1996). Improved amino acid contents like Lys and Thr in rice were reported through silencing of *LKR/SDH* by RNA interference (RNAi) (Frizzi et al. 2008; Houmard et al. 2007). Increased free lysine (~60-fold) in rice seeds was reported by overexpression of *AK* and *DHPS* as well as silencing *LKR/SDH* genes by RNAi (Long et al. 2013). Transgenic maize and rice seeds with 10–65% and 20.6% lysine, respectively, were achieved through overexpression of 3 lysine-rich genes in maize (Yue et al. 2014) and RLRH1 and RLRH2 in rice (Wong et al. 2015). The salient achievements in the field of protein quality improvement in pulses and other crops are summarized in Table 6.2.

6.5 Concluding Remarks

As the world population, especially developing countries, is suffering from protein malnutrition (hidden hunger), attaining nutritional security has become a major challenge for the countries. The protein digestibility is determined by an amount of protein absorbed by an organism relative to the total protein consumed; it depends on the structure of proteins, processing methods, and the prevalence of antinutritional compounds in the food that limit the digestion. Thus, the application of suitable processing method to inactivate antinutritional factors and to modulate the protein structure in favor of its digestibility can bring practical application of plant protein as a animal protein substitution.

Table 6.2 The list of genes and proteins exploited for enhancing protein quality in pulses and other crop plants using genetic engineering approach

Genetic tools	Crop	Protein quality	Genes involved	References
Transgenic	Soybean	Enhanced methionine content	Synthetic protein (MB16)	Zhang et al. (2014)
Transgenic	Soybean	Methionine enhanced by 6.8%	Gene encoding methionine-rich protein	Yamada et al. (2008)
Transgenic	Narbon bean	Enhanced SAA	2S Albumin storage protein and bacterial aspartate kinase gene	Demidov et al. (2003)
Transgenic	Pea	Enhanced protein	Gene encoding albumin fraction of seed protein	Casey et al. (1998) and Hughes et al. (2001)
Transgenic	Soybean	Enhanced methionine-rich protein	Gm2S-1 (posttranslationally processed 2S-albumin)	Galvez et al. (2008)
Transgenic	Common bean	Enhanced SAA	Fused protein of 45 bp sequence encoding Met-rich region from maize and gene encoding β-phaseolin	Hoffman et al. (1988)
Transgenic	Soybean	Elevated levels of lysine	Bacterial *DHPS*	Falco et al. (1995)
Transgenic	Rice	Higher lysine and methionine	Synthetic fusion proteins	Jiang et al. (2016)
Plant breeding	Maize	Higher lysine	–	Gibbon and Larkins (2005)
Transgenic	Tobacco	High lysine and methionine	Bacterial *DHPS*	Shaul and Galili (1992)
Transgenic	Canola	High lysine and methionine	Bacterial *DHPS*	Falco et al. (1995)
Transgenic	*Arabidopsis*	High lysine and methionine	Bacterial *DHPS*	Ben-Tzvi Tzchori et al. (1996)
Transgenic	Rice	Slight increase in lysine	Bacterial *DHPS*	Lee et al. (2001)
Transgenic	Barley	Slight increase in lysine	Bacterial *DHPS*	Brinch-Pedersen et al. (1996)
Transgenic	Rice	High lysine and threonine	Silencing of *LKR/SDH*	Frizzi et al. (2008) and Houmard et al. (2007)
Transgenic	Rice	Higher lysine (60-fold)	*AK* and *DHPS*	Long et al. (2013)

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