

Biology and Breeding of Food Legumes

Edited by

Aditya Pratap and Jitendra Kumar

*Crop Improvement Division, Indian Institute of Pulses Research,
Kanpur, INDIA*

20 Improving Protein Content and Nutrition Quality

J. Burstin, K. Gallardo, R.R. Mir, R.K. Varshney and G. Duc

20.1 Introduction

Legumes have been part of the human diet since the early ages of agriculture. Many legume species are still an irreplaceable source of dietary proteins and other nutrients for humans, especially in vegetarian diets of the developing countries (Wang *et al.*, 2003). Legume seeds contain from 16 to 50% protein and provide one third of all dietary protein nitrogen (Graham and Vance, 2003). Thus legumes, as a complement to cereals, make one of the best solutions to protein-calorie malnutrition, particularly in developing countries. Legumes constitute the main component of traditional dishes throughout the world, where maize and beans, rice and lentils, barley and peas, wheat and chickpeas are eaten together. The carbon energy supply that is needed upon germination is stored in grain legume seeds either in the form of oil (soybean, groundnut) or as starch (common bean, pea, faba bean, lentil, chickpea, cowpea, mung bean). In addition, these are also an important source of the 15 essential minerals required by man (Wang *et al.*, 2003), of complex carbohydrates, of soluble fibres and of other compounds that are alternatively considered anti-nutritional or health-promoting: trypsin inhibitors, tannins, phytate, saponins and oligosaccharides have recently been associated with various health benefits, such as protective effects against cardiovascular diseases, cancers

and diabetes (Champ, 2002; Clemente *et al.*, 2009). Since the main challenge for grain legumes in human nutrition is linked to their role as a source of protein, the genetic improvement made for protein content, bioavailability and nutritional quality in food legume crops is discussed in this chapter.

20.2 Improving the Protein Content of Grain Legume Seeds

Genetic and environmental variability and heritability

A good amount of genetic variability is required for the genetic improvement of a trait. In the recent past, studies have been conducted in estimating genetic variability for protein content among a number of accessions of various food legume crops, and it has been observed that ample amount of variability is present for this trait (Table 20.1); this varied from 26.5 to 57% in soybean, 20.9–29.2% in common bean, 15.8–32.1% in pea, 22–36% in faba bean, 19–32% in lentil, 16–28% in chickpea, 16–31% in cowpea, 21–31% in mung bean and 16–24% in pigeon pea. These results were compiled from various studies, and thus variability among these could also in part reflect the environmental variability of protein content.

Table 20.1. Principal constituents of grain legume seeds range of variation (% seed weight)

Protein	Content (%)				Reference(s)
	Oil	Starch	Fibre	Sucrose	
<i>Soybean</i>					
35.1–42.0	17.7–21.0	1.5	20.0	6.2	Hedley (2001)
34.7–55.2	6.5–28.7	–	–	–	NGRP (2001)
40.0–45.0	19.0–21.5	–	–	–	Hyten <i>et al</i> (2004)
41.8–49.4	15.2–20.7	–	–	–	Chung <i>et al</i> (2003)
40.4–50.6	13.4–21.2	–	–	–	Brummer <i>et al</i> (1997)
31.7–57.4	–	–	–	–	Jun <i>et al</i> (2008)
26.5–47.6	–	–	–	–	Vollman <i>et al</i> (2000)
<i>Groundnut</i>					
–	44.0–50.0	–	–	–	Lord and Wakelam (1950)
20.7–28.1	–	–	–	–	Dwivedi <i>et al</i> (1990)
16.0–34.0	–	–	–	–	Jambunathan <i>et al</i> (1985)
<i>Common bean</i>					
20.9–27.8	0.9–2.4	41.5	10.0	5.0	Hedley (2001)
23.0–29.2	–	–	–	–	Coelho <i>et al</i> (2009)
<i>Pea</i>					
18.3–31.0	0.6–5.5	45.0	12.0	2.1	Hedley (2001)
24.0–32.4	–	45.5–54.2	8.9–11.9	–	Gabriel <i>et al</i> (2008b)
21.9–34.4	1.4–4.7	18.6–54.5	5.9–12.7	1.3	Bastianelli <i>et al</i> (1998)
20.6–27.3	–	–	–	–	Burstin <i>et al</i> (2007)
15.8–32.1	–	–	–	–	Blixt (1978)
<i>Faba bean</i>					
26.1–38.0	1.1–2.5	37.0–45.6	7.5–13.1	0.4–2.3	Duc <i>et al</i> (1999)
22.4–36.0	1.2–4.0	41.0	12.0	3.3	Hedley (2001)
26.0–29.3	–	42.2–51.5	–	–	Avola <i>et al</i> (2009)
<i>Lentil</i>					
23.0–32.0	0.8–2.0	46.0	12.0	2.9	Hedley (2001)
25.1–29.2	–	46.0–49.7	13.1–14.7	2.1–3.2	Wang <i>et al</i> (2009)
18.6–30.2	–	–	–	–	Hamdi <i>et al</i> (1991)
<i>Chickpea</i>					
15.5–28.2	3.1–7.0	44.4	9.0	2.0	Hedley (2001)
18.7–21.1	–	42–45.1	–	–	Frimpong <i>et al</i> (2009)
17.1–19.8	–	48.0–54.9	–	–	Frimpong <i>et al</i> (2009)
–	–	–	2.7–11.7	–	Cho <i>et al</i> (2002)
12.4–31.5	–	–	–	–	Hulse (1975)
<i>Cowpea</i>					
23.5	1.3	–	–	–	Hedley (2001)
24.8	1.9	–	6.3	–	Kabas <i>et al</i> (2006)
20.9–36.0	2.6–4.2	–	–	–	Oluwatosin (1997)
16.0–31.0	2.4–4.3	–	–	–	Adekol and Oluleye (2007)
23.1–27.3	–	–	–	–	Bliss <i>et al</i> (1973)
<i>Mung bean</i>					
22.9–23.6	1.2	45.0	7.0	1.1	Hedley (2001)
21.0–31.3	1.2–1.6	–	8.9–12.9	–	Anwar <i>et al</i> (2007)
23.7–31.4	–	–	–	–	Lawn and Rebetzke (2006)
<i>Pigeon pea</i>					
19.5–22.9	1.3–3.8	44.3	10.0	2.5	Hedley (2001)
15.9–24.1	–	–	–	–	Upadhyaya <i>et al</i> (2007)

The heritability of a trait is an important factor for efficient selection. It has been shown that environmental conditions significantly influence the seed protein content in most legumes (Dwivedi *et al.*, 1990; Hamdi *et al.*, 1991; Oluwatosin, 1997; Saxena *et al.*, 2002; Frimpong *et al.*, 2009). However, environmental effects in seven different environments had a similar magnitude of effects on protein content to genetic effects when studied in 255 genotypes of pea (Matthews and Arthur, 1985). Gueguen and Barbot (1988) found protein content varying from 18.1 to 27.8% for the cultivar 'Amino', depending on the environment. Karjalainen and Kortet (1987) showed that protein content was positively associated with the sum of temperature from sowing to maturity, and temperature during flowering and beginning of seed filling, while it was negatively associated with July precipitation. Larmure *et al.* (2005) further specified the effect of temperature during seed filling on seed protein content through its effect on the nitrogen:carbon (N:C) ratio. All environmental factors affecting nitrogen nutrition, such as drought stress, soil compactness, root diseases and pests may also influence seed protein content through their impact on nitrogen availability (Biarnès *et al.*, 2000). Foroud *et al.* (1993) described a variable effect of the level and timing of water stress on the protein content of soybean. Aerial disease could also have negative effects by increasing the N:C ratio of assimilates reaching the seeds (Garry *et al.*, 1996). Several authors also reported intraplant variability resulting from fluctuating environmental factors and N:C availability during seed filling of different fruiting nodes (Escalante and Wilcox, 1993; Atta *et al.*, 2004).

Genotypic/environmental effects are also usually significant, even though often of lower magnitude (Matthews and Arthur, 1985; Oluwatosin, 1997; Lawn and Rebetzke, 2006; Burstin *et al.*, 2007; Frimpong *et al.*, 2009). As a result, seed protein content heritability values are variable across studies, depending on the extent of genetic variability analysed, unpredictable environmental variation and experimental design. None the less, seed protein content heritability is generally moderate to high among accessions (20–80%), suggesting that selection for protein can be successful.

In the case of soybean, it is interesting to note that the selection of varieties with significantly increased protein content was achieved quite rapidly using backcrossing or recurrent selection (Brim and Burton, 1978; Wilcox and Cavins, 1995; Cober and Voldeng, 2000). This was achieved due to: (i) the large variability present for protein content in germplasm; (ii) the sufficiently high heritability values; and (iii) the mostly additive inheritance (Chung *et al.*, 2003).

To study the genetics of protein content in soybean quantitative trait loci (QTL) analysis was conducted, which resulted in the identification of two major QTL controlling seed protein content in a population derived from a cross between a *Glycine soja* accession from China and a *Glycine max* breeding line (Diers *et al.*, 1992). The *G. soja* parent possessed positive alleles of the QTL-LGI and QTL-LGE. Sebolt *et al.* (2002) investigated the stability of these QTL alleles in the *G. max* genetic background by developing the near-isogenic lines having homozygous alleles of *G. soja* QTL responsible for high protein content. Among the two QTL, only the QTL-LGI allele showed a significant effect in the *G. max* background, although it increased plant height and reduced yield and oil, seed size and maturity. Subsequently, a genetic association was shown between protein content QTL-LGI and other QTL controlling oil content, maturity and yield in different lines recombining within the target regions under introgress (Nichols *et al.*, 2006). These results have confirmed that the linkage between the protein content QTL and yield QTL can be broken. The effect of this QTL-LGI was further validated by marker-assisted selection (MAS) involving improvement in protein content of soybean lines carrying homozygous alleles from the high-protein parent (Yates *et al.*, 2004). Many other soybean seed protein content QTL have been identified in a range of environments and in several genetic backgrounds, which are presented elsewhere (Vuong *et al.*, 2007). QTL controlling seed protein content were investigated on 17 soybean mapping populations and found to be located on all the linkage groups of soybean genome, except for LG B1, D1b, D2, J and O. The identified QTL may be efficiently utilized

for developing future soybean varieties with desirable seed components through MAS (<http://www.SoyBase.org>).

In pea, the selection for yield has led to a rapid and undesirable decrease in protein content. Burstin *et al.* (2007) analysed the variation of protein content in five environments using a recombinant inbred line (RIL) mapping population segregating for *afila* controlling leaf tendril formation, *le* controlling internode length and plant height, and *rms6* controlling plant branching. In this population, eight QTL controlling seed protein content variation were observed. Among these, five were stable across at least two environments, of which two were located in the same genomic region, where the above three genes were located suggesting their pleiotropic effects on several traits. Tar'an *et al.* (2004) also reported two (of three) seed protein QTL in pea showing consistency in many environments. Irzykowska and Wolko (2004) reported five QTL in a cross segregating for the *r* gene controlling starch synthesis and wrinkled seed phenotype. Genetic variability for seed protein content was studied in pigeon pea using wild relatives and improved varieties (Saxena *et al.*, 2002). The results indicated the possibility of developing genotypes possessing high protein content similar to their wild relatives and seed characters similar to cultivated types.

Seed protein content, yield and related traits

Highly negative correlations between protein and oil are well documented in soybean and other food legume crops (Dwivedi *et al.*, 1990; Oluwatosin, 1997; Hyten *et al.*, 2004). Correlation between starch and protein content has also been reported as negative when the gene pool was considered in pea (Bastianelli *et al.*, 1998) and chickpea (Frimpong *et al.*, 2009). There are often negative correlations between protein content and yield, but it has also been reported variously as either non-significant or positive (Bliss *et al.*, 1973; Hamdi *et al.*, 1991; Oluwatosin, 1997; Cober and Voldeng, 2000; Lawn and

Rebetzke, 2006; Burstin *et al.*, 2007; Frimpong *et al.*, 2009). Similarly negative correlations have been reported between seed size and protein content in pigeon pea (Saxena *et al.*, 2002), but some promising lines with high protein content and large seed size have been obtained at ICRISAT, suggesting the possibility of simultaneous improvement in protein content and yield-contributing traits.

Potential limitations in protein content improvement

The rate of protein accumulation depends on both the sink strength capacity of seeds and the source strength of vegetative parts for nitrogenous assimilates resulting from nitrogen acquisition, assimilation, transport and mobilization (Salon *et al.*, 2001; Munier-Jolain *et al.*, 2008). The rate of dry matter accumulation depends on the accumulation of all constituents, including carbohydrates and oil, and relates mostly to carbon supply through efficient photosynthesis and effective biosynthetic pathways. De-podding and defoliation studies were conducted in several legume species in order to analyse the effect of source:sink ratio variation on seed constituents' accumulation. Burstin *et al.* (2007) analysed the genetic variability of the effect of de-podding on the seed protein content of eight pea genotypes. The effects of genotype, de-podding and genotype \times de-podding on seed protein content were all significant. For all genotypes, seed protein increased dramatically when the source:sink ratio increased. However, there was still a significant variation for seed protein content among the eight genotypes once de-podded. This suggests that N source capacity is the major limiting factor for seed protein content in pea, but also that the maximal rate of protein accumulation in the seed is significant. Similar results were obtained for soybean (Proulx and Naeve, 2009; Rotundo *et al.*, 2009). Three types of genes/QTL could be identified for seed protein content (Burstin *et al.*, 2007; Gallardo *et al.*, 2008): (i) major genes controlling developmental processes and having pleiotropic effect on the whole plant phenotype and on

the source–sink structure; (ii) genes of plant metabolism controlling source–sink relationships at the plant metabolism level; and (iii) genes solely controlling the capacity of seeds to accumulate storage compounds. The impact of these different types of effectors on yield will probably be different.

Improving nitrogen supply to the seed

The establishment and optimal functioning of symbiosis in food legumes, together with efficient mobilization of assimilates from vegetative parts to the seeds, controls the availability of nitrogen to the growing seeds. Many genes involved in the control of nodulation have recently been identified (Ferguson *et al.*, 2010). Pea mutants with absence of N₂ fixation activity produce lower seed yield and protein content, which can be alleviated by adequate mineral fertilization, whereas an autoregulation mutant of pea displaying a supernodulating phenotype has a reduced shoot biomass and seed yield, associated with higher seed protein content (Sagan *et al.*, 1993). A reduced root development was detected in supernodulating mutants of soybean or pea (Olsson *et al.*, 1989; Bourion *et al.*, 2007), which may be explained by a competition effect of nodules for C, with a secondary effect of lower access to soil resources. The importance of fine tuning between root and nodule establishment and functioning for final C:N equilibrium in seeds is illustrated by these extreme mutant phenotypes. The efficiency of N-fixing symbiosis relies on the carbon supply from aerial parts to the root parts. In a recent study, Bourion *et al.* (2010) located QTL for root development in the region of QTL for seed protein content. However, QTL should be refined and further work is needed in order to define the ideotype of root/nodule/shoot development.

Improving seed sink strength

Functional interactions exist among different seed constituents: for example, the disruption of the *r* gene abolishes starch synthesis in pea seeds, leading to a wrinkled seed phenotype having a profound impact on seed metabolism, where elevated sucrose content impacts

the accumulation of storage protein families (Wang and Hedley, 1993). By knocking down the accumulation of one of the constituents, the percentage of the others will increase. However, this may have a detrimental effect on seed yield. This strategy is viable if it allows production of specific seed products for market purposes. Strategies to increase seed sink strength have been tested through the manipulation of amino acids and sucrose flux to the developing embryo (Weber *et al.*, 2005). It has been shown that seed-specific overexpression of an amino acid permease in pea increases amino acid supply to, and the level of protein in, the seed (Weigelt *et al.*, 2008). This indicates a stimulation of storage protein synthesis by increased amino acid availability. Seed-specific overexpression of a bacterial phosphoenol pyruvate carboxylase in *Vicia narbonensis* increased seed protein content with a compensatory effect on seed number and seed weight (Rolletschek *et al.*, 2004). In general, the genetics of seed protein content largely remain a mystery. However, with the advent of high-throughput genotyping and phenotyping tools, we think that two directions should be pursued in order to gain on efficiency in breeding: whole genome selection and plant modelling of interacting processes.

20.3 Improving Seed Protein Composition for Better Digestibility and Nutrient Balance

Seed storage proteins are synthesized during seed development and confined in membrane-bound organelles until they are hydrolysed upon germination to provide carbon and nitrogen skeletons for the developing seedlings. Grain legume storage proteins include two major classes of salt-soluble globulins: the 7/8S vicilins and 11/12S legumins, each of which consists of a family of closely related molecules (Boulter and Croy, 1997). Proteome reference maps have been developed for soybean (Hadjdouch *et al.*, 2005), pea (Bourgeois *et al.*, 2009) and lentil (Scippa *et al.*, 2010) revealing a complex composition of grain legume globulins. Other proteins (albumins,

Table 20.2. Range of variation (g/100g protein) in four amino acids of grain legume seeds.

Species	Lysine	Methionine	Cysteine	Tryptophan	Reference
Soybean	22.4–24.1	4.4–8.8	5.1–7.3	4.4–5.1	Panthee <i>et al.</i> (2006)
Pea	15.5–19.7	2.0–2.4	2.9–3.6	2.0–2.7	Gabriel <i>et al.</i> (2008b)
	14.8–23.0	2.1–3.3	2.9–4.2	1.6–3.2	Bastianelli <i>et al.</i> (1998)
Faba bean	17.3–21.6	2.3–2.9	2.9–4.3	2.0–3.2	Duc <i>et al.</i> (1999)
Lentil	4.5–12.6	1.2–1.7	0.4–0.5	–	Rozañ <i>et al.</i> (2001)
Cowpea	–	1.9–2.8	1.6–2.1	3.0–3.7	Bliss <i>et al.</i> (1973)
	4.9–9.0	0.52–2.05	0.84–2.24	0.72–1.91	Oluwatosin (1997)

glutelins) complete the protein fraction of the seed, and the composition in the different protein fractions depends on the species (Boulter and Croy, 1997; Gallardo *et al.*, 2008; Montoya *et al.*, 2010).

The various storage proteins have different *in vitro* and *in vivo* digestibility depending on their structural characteristics (Créviu *et al.*, 1997; Gabriel *et al.*, 2008a, b; Montoya *et al.*, 2010). Although studies on grain legume seed protein digestibility in the human are scarce (Mariotti *et al.*, 2001), some relevant information can be found in digestibility surveys on monogastric animals. Generally, β -sheet structures are less digestible than α -helix structures and glycosylated proteins are less susceptible to hydrolysis. Several attempts have been made in order to improve protein digestibility through the suppression or overexpression of a particular storage protein family (Burow *et al.*, 1993), but these did not generally yield the expected outcome. Therefore, searching variability among germplasms for protein composition patterns favourable to digestibility has been suggested as an alternate strategy for improvement (Montoya *et al.*, 2010). Several authors reported intra-specific variability for seed protein composition in food legume crops such as pea (Tzitzikas *et al.*, 2006), lentil (Scippa *et al.*, 2010) and soybean (Natarajan *et al.*, 2006). This variability was also observed for other minor proteins, which may also have a role in protein digestibility (Vigeolas *et al.*, 2008).

Essential amino acids are important in human nutrition, as they cannot be synthesized and there is therefore dependency on dietary sources of these amino acids. Among these, tryptophan and the sulfur-containing amino acid methionine are the most limiting

in legume seeds (Table 20.2). By contrast, legume seed proteins are rich in lysine while cereal seed proteins are low in this amino acid (Wang *et al.*, 2003).

Genetic manipulations have been used in attempts to improve seed quality, in particular towards increasing methionine levels. The strategy employed in improving amino acid balance in legumes is to modify storage protein composition in favour of the accumulation of sulfur-rich proteins. To this end, transgenic plants expressing the sulfur-rich 2S albumin genes from Brazil nut and sunflower in the seeds of soybean and lupin (*Lupinus angustifolius* L.), respectively, were developed (Altenbach *et al.*, 1989; Molvig *et al.*, 1997). Although these transgenic plants increased seed methionine levels, the introduced sulfur-rich sink proteins generally had allergenic properties (Pastorello *et al.*, 2001). Importantly, the accumulation of such foreign proteins in seeds occurred at the expense of other sulfur compounds, such as free sulfur amino acids and glutathione (Tabe and Droux, 2002), which thus limited the rate of synthesis of sulfur amino acids during seed development. Activating the synthesis of essential amino acids might therefore be a possible route for improvement of amino acid balance in legume seeds.

Lysine regulates the flux of carbon and nitrogen towards methionine synthesis (see Jander and Joshi, 2010). A similar feedback inhibition was also observed for the tryptophan biosynthetic pathway, where tryptophan itself inhibits its own biosynthetic pathway by negatively regulating anthranilate synthase, which catalyses the conversion of chorismate to anthranilate (Ufaz and Galili, 2008). Interestingly, the modulation of feedback

inhibition in these pathways allowed an increase in the synthesis of some amino acids. For example, the introduction of genes encoding anthranilate synthase forms insensitive to feedback inhibition enhanced the accumulation of tryptophan in seeds (Ufaz and Galili, 2008; Ishimoto *et al.*, 2010).

These findings open perspectives towards modification of the synthesis of essential amino acids in legume seeds through the identification of feedback-insensitive natural allelic variants in genes of amino acid biosynthetic pathways. However, the up-accumulation of amino acids under free forms in seeds could have negative effects on agronomic traits. For example, the germination ability of transgenic seeds containing very high levels of free lysine or tryptophan was reduced (Zhu and Galili, 2003; Wakasa *et al.*, 2006).

Availability of sulfur and nitrogen in the environment determines the amino acid balance in mature seeds. Legume seeds produced in sulfur-limiting conditions, but with adequate nitrogen, generally contained reduced levels of sulfur-rich storage proteins and accumulated more sulfur-poor proteins (Tabe and Droux, 2002). In soybean, Paek *et al.* (1997) reported an increase in the proportion of sulfur-poor protein as protein concentration increased. Wilcox and Shibles (2001), by contrast, found a constant sulfur:nitrogen ratio in a population segregating for seed protein content, but seed yield was not high in this population and thus sulfur was probably not limiting in this context. In cowpea, Bliss *et al.* (1973) found a positive correlation between seed protein content and protein methionine content. In chickpea, the application of nitrogen, phosphorus and sulfur fertilizers improves the levels of protein and essential amino acids (Gupta and Singh, 1982; Williams and Singh, 1987). In pea seeds, reduced levels of sulfur-rich proteins in conditions of limited sulfur availability were shown to be primarily a consequence of reduced levels of their mRNA (Higgins *et al.*, 1986). *O*-acetylserine and free methionine, but not free cysteine, were implicated as signalling molecules controlling the expression of genes for sulfur-rich storage proteins in legume seeds (Tabe *et al.*, 2010 and references therein).

These findings indicate that the capacity of legume plants to regulate the flux of sulfur and nitrogen compounds to the seeds should be considered if the accumulation of sulfur-rich storage proteins is to be increased. Sulfate is one of the dominant forms of sulfur found in the phloem supplying pods during legume seed development (Tabe and Droux, 2001). In plants, sulfate can either be reduced to sulfide leading to the synthesis of cysteine, the precursor for methionine synthesis, or it can be stored in the vacuoles. Considering the importance of sulfate for the synthesis of sulfur compounds, one limiting step for accumulation of sulfur-rich proteins could be the uptake of sulfate by the root and its distribution within the plant by membrane-localized sulfate transporters. In several species strongly regulated by sulfur deficiency, sulfate transporters of high affinity have been identified that facilitate the uptake of sulfate by the root (SULTR1-1 and SULTR1-2) or its translocation from source to sink (SULTR1-3) (see Hawkesford and De Kok, 2006). Other transport forms of sulfur in the phloem include glutathione and *S*-methylmethionine, which can be reconverted to cysteine and methionine (Bourgis *et al.*, 1999). Interestingly, a characterization of knockdown *Arabidopsis* mutants for isozyme 2 of homocysteine methyltransferase, which converts *S*-methylmethionine to methionine, suggests that increasing the transport of *S*-methylmethionine from vegetative tissues to seeds could increase seed methionine levels (Lee *et al.*, 2008).

20.4 Breeding for Minor Compounds (Seed Protein Bioavailability for Humans)

Minor seed compounds in grain legumes exert either positive or negative influence on protein bioavailability by impacting digestibility or acceptability. Studies have documented the possibility of improving the nutritional values of grain legumes as animal feeds, mainly for monogastric animals. However, these findings cannot be easily extrapolated to humans because, even if monogastric, human beings have their own physiology varying with age,

and the human diet is composed of a diversity of ingredients generating high dilutions and complex interactions. This is why seed compounds termed anti-nutritionals in feeds have been removed by breeding, even though some of them may have a positive role in human chronic disease prevention, i.e. cancer, cardiovascular disease, diabetes and obesity. The genetic variability available for these compounds, including trypsin inhibitors, lectins, α -galactosides and phytic acids, may thus help breeders significantly improve the protein component of human diets (Table 20.3).

Trypsin inhibitors are present in most grain legume seeds, but these inhibitory activities in soybean seeds are usually reduced by processing. However, in soybean, null alleles have been identified for both Bowman–Birk and Kunitz trypsin inhibitors, facilitating the development of low-trypsin-inhibitor cultivars (Clarke and Wiseman, 2000). In pea, large genetic variability is available for the activity of Bowman–Birk trypsin/chymotrypsin inhibitor proteins (TIAs) (Bastianelli *et al.*, 1998). The polymorphism in coding and promoter sequences of genes at the *Tri* locus accounts for most of the variation in TIAs, and this allows the initiation of MAS (Page *et al.*, 2002). Even if low TIA activity is of benefit in pig or poultry feed digestibility, its high content in foods should be of positive value because the presence of trypsin inhibitors in pea reduced levels of HT29 colonic cancer cells *in vitro* (Domoney *et al.*, 2009). If validated *in vivo*, this would encourage breeding for high trypsin inhibitor content in food or nutraceutical applications.

Most grain legume cotyledons contain lectins (haemagglutinins), polysaccharide-binding proteins that bind to glycoprotein on the epithelial surface of the small intestine, interfering with nutrient absorption and increasing the production of mucins and a loss of plasma proteins in the intestinal lumen (Pusztai, 1989). In plants, lectins are very diverse and are involved in plant defences (Etzler, 1985) or symbiosis with *Rhizobia* (van Rhijn *et al.*, 2001). Although some natural variability exists for lectin haemagglutinin activity in germplasm (Valdebouze *et al.*, 1980), its low content and toxicity do not allow for the definition of a breeding target for this trait.

The glycosides vicine and convicine (VC) are in abundance in the cotyledons of faba bean seeds; in general, their concentration varies from 6 to 14 g/kg DM in mature seeds of released cultivars. The presence of VC causes favism, which is an acute haemolytic problem caused by the ingestion of faba beans in G6PD-deficient human individuals (Arese and De Flora, 1990). A mutant allele, *vc-* has been identified that reduces VC contents by ten- to 20-fold (Duc *et al.*, 1989). Since the determination of VC content by chemical analysis in seeds is costly, molecular markers have been proposed to assist in selection for genotypes of low VC content (Gutierrez *et al.*, 2006).

Flavonoids are major phenolic compounds involved in the determination of seed coat colour and tannin synthesis (Nozzolillo *et al.*, 1989, Caldas and Blair, 2009), tannins are binding to proteins and reducing their digestibility. In pea and faba bean, a single gene mutation has a pleiotropic effect in eliminating tannins from the seed coat and determining the white flower trait. The zero tannin trait increases protein digestibility in pigs or poultry by about 10% when compared with tannin-containing lines (Grosjean *et al.*, 1999; Crepon *et al.*, 2010). This quality trait is economically valuable for feed efficiency, and zero-tannin varieties have been bred in Europe. In common bean, the genetics of seed coat colour and tannin content was shown to be under the control of 12 QTL (Caldas and Blair, 2009), but limited data are available for individual phenolic compounds. The removal of tannins from the human diet may have positive nutritional effects, but it has a certain impact on the level of astringency, with positive or negative consumer reactions according to dietary habits. The health benefits of proanthocyanidins may deserve some attention. The diverse colours of common beans are suggested to be important sources of dietary antioxidants (Beninger and Hosfield, 2003).

The anti-nutritional effect of phytic acid is associated with mineral complexing (especially Zn, Ca and Fe) and the inactivation of digestive enzymes; it induces a reduction in bioavailability of minerals and proteins in foods and hence may be of nutritional concern (Frossard *et al.*, 2000). On the other hand, phytic acid may have protective effects such

Table 20.3. Levels of some minor constituents of grain legume seeds.

Species	TIA (TIU/mg)	Tannins (g/kg)	Saponin (g/kg)	Total α -galactos- ides (% DM)	Phytic acid (g/kg)	Reference
Soybean	–	–	6.5	–	–	Kadlec <i>et al.</i> (2001)
	–	–	–	–	6.2–20.5	Saghai Maroof <i>et al.</i> (2009)
	43–83	–	–	–	–	Guillamon <i>et al.</i> (2008)
Common bean	–	–	2.3–3.5	0.4–8.0	–	Kadlec <i>et al.</i> (2001)
	–	0–38.5	–	–	–	Caldas and Blair (2009)
	–	–	–	–	2.9–17.8	Blair <i>et al.</i> (2009)
	17–51	–	–	–	–	Guillamon <i>et al.</i> (2008)
Pea	–	–	1.1	2.3–9.6	–	Kadlec <i>et al.</i> (2001)
	1.0–14.6	0.04–7.4	0.3–1.0	3.6–10	1.3–10.2	Bastianelli <i>et al.</i> (1998)
	1.9–6.8	–	–	–	–	Gabriel <i>et al.</i> (2008)
	6.0–15.0	–	–	–	–	Guillamon <i>et al.</i> (2008)
Faba bean	0.3–5.3	0.1–10.4	–	1.4–6.2	3.8–13.4	Duc <i>et al.</i> (1999)
	–	–	0.1	1.0–4.5	–	Kadlec <i>et al.</i> (2001)
	–	2.1–3.2	–	–	–	Avola <i>et al.</i> (2009)
	0.8–3.6	–	–	–	–	Filipetti <i>et al.</i> (1999)
	5.0–10.0	–	–	–	–	Guillamon <i>et al.</i> (2008)
Lentil	–	–	1.1	1.8–7.5	–	Kadlec <i>et al.</i> (2001)
	1.9–2.8	3.4–6.1	–	–	6.2–8.8	Wang <i>et al.</i> (2009)
	3.0–8.0	–	–	–	–	Guillamon <i>et al.</i> (2008)
Chickpea	–	–	2.3	2.0–7.6	–	Kadlec <i>et al.</i> (2001)
	12.7	–	–	–	–	Singh and Jambunatham (1981)
	10.3	–	–	–	–	Singh and Jambunatham (1981)
	15.0–19.0	–	–	–	–	Guillamon <i>et al.</i> (2008)
Cowpea	12.0–16.6 ^a	–	–	–	–	Vasconcelos <i>et al.</i> (2010)
	–	0.3–6.9	–	–	–	Plahar <i>et al.</i> (1997)
	–	–	–	–	9.9–16.4	Singh (1999)

TIA, trypsin/chymotrypsin inhibitor activity. ^a De-hulled seeds.

as a decrease in the risk of iron-mediated colonic cancer and lowering of serum cholesterol and triglycerides (Champ, 2002). In common bean, five QTL were identified that controlled total and net seed phytate content (Blair *et al.*, 2009).

Lipoxygenase activity can cause unpleasant tastes and aromas when reacting with seed lipids. In soybean and pea, null mutants were found for three and two LOX genes, respectively. Their molecular characterization (Forster *et al.*, 1999; Lenis *et al.*, 2010) has been accomplished and offers the possibility of breeding for lipoxygenase-free varieties. On the other hand, a large number of different saponins also exist in legumes (Heng *et al.*, 2006). These compounds, although contributing to the bitterness of pea as well as of soybean, have positive hypocholesterolaemic and anti-carcinogenic effects that have also been studied (Champ, 2002). Some genetic diversity has already been described for both the quantity and quality of seed saponins

(Table 20.3; Heng *et al.* 2006), but its genetic basis is unknown.

20.5 Conclusion

There is an urgent need to develop new references on the health-promoting and nutritional values of grain legumes. Determining the value of particular fractions in nutraceutical applications may provide new markets with higher added value. Although the effects and cost of the technological treatment of bioactive components have not been calculated, this may help in choosing between genetic strategies and technological processes. Several studies have demonstrated the effectiveness of proteins in protection against parasitic insects or fungi. Attempts to modify the contents of minor bioactive compounds will involve an appraisal of their consequences on plant behaviour in regard to biotic or abiotic stresses.

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