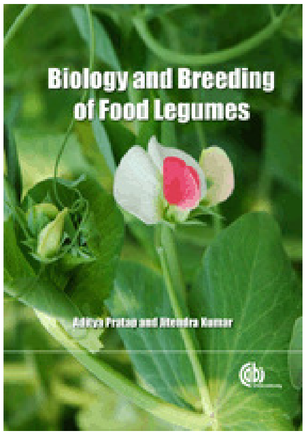

Published in **Biology and Breeding of Food Legumes**

ISBN : 9781845937669

http://bookshop.cabi.org/default.aspx?site=191&page=2633&pid=2338

Chapter 20: Improving protein content and nutrition quality

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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 for humans (Table 1), especially in the mainly vegetarian diets of developing countries (Wang et al. 2003).

Table 1, Worldwide production and cultivation data for the main food legume species (FAOSTAT 2008)

Species	Cultivated area (x10^6 Ha)	World Production (10^6 t)
Soybean (G. max (L.) Merrill)	97	230
Groundnut (A. hypogaea)	24.6	38.2c
Common bean (P. vulgaris L.)	28a	20.4a
Pea (P. sativum L.)	5.9a	9.8a
Fababean (V. faba L.)	2.5b	3.7b
Lentil (L. culinaris Medic L.)	3.8	3.5
Chickpea (C. arietinum L.)	11.6	8.8
Cowpea (V. unguiculata (L.) Walp)	11.8	5.4
Pigeonpea (C. cajan (L.) Millsp.)	4.9	4.1

a: Figures for dry peas and dry beans; b: Broad beans, horse beans, dry in FAOSTAT database; c: Including shells.

In most centres of crop domestication, legumes and cereals have been domesticated together (Gepts, 2004). Associated with cereals, 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. Legumes are consumed in many forms: seedling and young leaves are eaten in salads, fresh immature pods and seeds provide a green vegetable, and dry seeds are cooked in various dishes. However, researches have been mainly devoted to the dry seeds. Legume seeds provide an exceptionally varied nutrient profile, including proteins, fibres, vitamins and minerals (Mitchell et al. 2009). Nitrogen that is used by the young seedling during germination is stored in the seed in the form of storage proteins. Seeds contain from 16% to 50% of protein and provide one third of all dietary protein nitrogen (Graham and Vance, 2003). Anticipating the increasing demand for protein food sources, the Protein Advisory Group of the United Nations has identified the improvement of legumes as a critically important area of research. The protein-rich legumes as a complement to cereals make one of the best solutions to protein-calorie malnutrition, particularly in developing countries. The carbon energy supply that is needed upon germination is stored in grain legume seeds either mainly in the form of oil (soybean, groundnut), or as starch (common bean, pea, fababean, lentil, chickpea, cowpea, mungbean). Grain legume seeds 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, oligosaccharides have recently been associated with various health benefits, such as protective effect against cardiovascular diseases, cancers and diabetes (Champ et al. 2002, Clemente et al. 2009).

Because we think that the main challenge for grain legumes in human nutrition is linked to their role as a source of protein, the main topic of this chapter will be the improvement of protein content, bio-availability, and nutritional quality.

1. Improving the protein content of grain legume seeds: results and prospects

1.1 Genetic variability, environmental variability and heritability

In order to devise the best strategy to improve protein content in legumes, we surveyed the genetic variability of seed protein content in major food legumes, its relationship with other important traits such as yield components, its heritability and interactions with environment, and its genetic determinants.

To improve seed protein content, there should be enough genetic variability for this trait. In Table 2, a survey of the literature illustrates the presence of a large variability in protein content in grain legume germplasm collections, segregating populations, mutant populations, and cultivated varieties. In soybean, seed protein content data vary from 26.5 and 57%; in common bean, it varies from 20.9 and 29.2%; in pea from 15.8 to 32.1%; in fababean from 22 to 36%; in lentil from 19 to 32%, in chickpea from 16 to 28%; in cowpea from 16 to 31%; in mungbean from 21 to 31% and in pigeonpea from 16 to 24%. These results were compiled from various experiments, and thus variability among experiments could also in part reflect the environmental variability of protein content.

Table 2: Principal constituents of grain legume seeds: range of variation (% of seed weight)

Species	Protein	Oil	Starch	Fiber	Sucrose	Reference
Soybean	35.1 - 42	17.7-21	1.5	20	6.2	Hedley, 2001
	34.7-55.2	6.5-28.7	-	-	-	NGRP, 2001, USDA germplasm collection
	40-45	19-21.5	-	-	-	Hyten et al. 2004, RIL population
	41.8-49.4	15.2-20.7	-	-	-	Chung et al. 2003, RIL population
	40.4-50.6	13.4-21.2	-	-	-	Brummer et al. 1997, parents of RIL populations
	31.7-57.4	-	-	-	-	Jun et al. 2008, Association mapping population
	26.5-47.6	-	-	-	-	Vollman et al. 2000, 60 lines, 6 environments
Groundnut	25.8	49.2	-	8.5	-	Anonymous
	-	44-50	-	-	-	Lord and Wakelam 1950
	20.7-28.1	-	-	-	-	Dwivedi et al. 1990, 64 accessions
	16 - 34	-	-	-	-	Jambunathan et al. 1985 ICRISAT collection
Common bean	20.9 - 27.8	0.9 – 2.4	41.5	10	5	Hedley, 2001
	23-29.2	-	-	-	-	Coelho et al. 2009, 20 accessions
Pea	18.3 - 31	0.6 – 5.5	45	12	2.1	Hedley, 2001
	24-32.4	-	45.5-54.2	8.9-11.9	-	Gabriel et al. 2008, dehulled seeds, 8 varieties
	21.9-34.4	1.4-4.7	18.6-54.5	5,9 -12,7a	1.3-11.1[1]	Bastianelli et al. 1998, 213 or 54 (1) accessions
	20.6-27.3	-	-	-	-	Burstin et al. 2007, RIL population
	15.8-32.1	-	-	-	-	Blixt 1978, 2200 accessions
Fababean	26.1 - 38	1.1 – 2.5	37-45.6	7.5-13.1a	0.4 -2.3[z]	Duc et al. 1999 , 37 or 12 (z)spring varieties
	22.4 - 36	1.2 – 4	41	12	3.3	Hedley, 2001
	29.4-32	1.3-2	41.2-44.3	8.7-9.9	-	Duc et al. 2010, 8 varieties
	26-29.3	-	42.2-51.5	-	-	Avola et al. 2009, 15 accessions
Lentil	23 - 32	0.8 – 2	46	12	2.9	Hedley, 2001
	25.1-29.2	-	46-49.7	13.1-14.7	2.1-3.2	Wang et al. 2009, 8 varieties
	18.6-30.2	-	-	-	-	Hamdi et al. 1991, 987 germplasm accessions
Chickpea	15.5-28.2	3.1 – 7	44.4	9	2	Hedley, 2001
	18.7-21.1	-	42-45.1	-	-	Frimpong et al. 2009, 7 Desi chickpea varieties
	17.1-19.8	-	48-54.9	-	-	Frimpong et al. 2009, 9 Kabuli chickpea varieties
	-	-	-	2.7-11.7	-	Cho et al. 2002, RIL population
	12.4-31.5	-	-	-	-	Hulse 1975
Cowpea	23.5	1.3	-	-	-	Hedley, 2001
	24.8	1.9	-	6.3	-	Kabas et al. 2006, mean of 8 varieties
	20.9-36	2.6-4.2	-	-	-	Oluwatosin 1997, 15 accessions
	16-31	2.4-4.3	-	-	-	Adekola and Oluleye 2007, 15 mutants
	23.1-27.3	-	-	-	-	Bliss et al. 1973, 11 varieties
Mungbean	22.9-23.6	1.2	45	7	1.1	Hedley, 2001
	21-31.3	1.2-1.6	-	8.9-12.9	-	Anwar et al. 2007, dehulled seed, 4 varieties
	23.7-31.4	-	-	-	-	Lawn and Rebetzke 2006, 121 accessions
Pigeonpea	19.5-22.9	1.3 – 3.8	44.3	10	2.5	Hedley, 2001
	15.9-24.1	-	-	-	-	Upadhyaya et al. 2007, 310 accessions

a: Acid-detergent fiber

A second important factor for efficient selection is the heritability of the trait. Seed protein content in grain legumes is strongly influenced by the environment. In pea, Mathews and Arthur (1985) underlined that environmental effects in seven environments had similar magnitude effects on protein content than genetic effects in 255 genotypes. Gueguen and Barbot (1988) found protein content varying from 18.1 to 27.8% for cultivar Amino depending on the environment. Significant environmental effects are reported for most grain legumes (cowpea: Oluwatosin 1997, Bliss et al. 1973, chickpea: Frimpong et al. 2009, lentil: Hamdi et al. 1991, pigeonpea: Saxena et al. 2002, groundnut: Dwivedi et al. 1990). Environmental variability is probably caused by several factors. Karjalainen and Kortet (1987) showed that protein content was positively associated with the sum of temperature from sowing to maturity, and with the temperature during flowering and beginning of seed filling, while it was negatively associated with July precipitations. Larmure et al. (2005) further specified the effect of temperature during seed-filling on seed protein content through its effect on N/C ratio. All environmental factors that impact nitrogen nutrition, such as drought stress, soil compacting, 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 have opposite effects by increasing N/C ratio of assimilate reaching the seeds (Garry et al. 1996). Several authors also reported intra-plant variability resulting from fluctuating environment and N/C availability during seed filling of different fruiting nodes (Atta et al. 2004, Crochemore et al. 1994, Escalante and Wilcox 1993). Genotype-by-environment effects are also usually significant even though often of lower magnitude (Burstin et al. 2007, Biarnès et al. 2000, Matthews and Arthur 1985, Oluwatosin 1997, Bliss et al. 1973, Lawn and Rebetzke 2006, Frimpong et al. 2009, Hamdi et al. 1991, Dwivedi et al. 1990). As a result, seed protein content heritability values are very variable across experiments, depending on the extent of genetic variability analysed, unpredictable environment variation and experimental design. However, despite significant influence of environment and the presence of frequent genotype-by-environment interactions, seed protein content heritability is generally moderate to high among accessions (20 to 80%), suggesting that selection for protein can be successful.

Fortunately, breeding for protein content can benefit from a very efficient analysis method, Near-Infrared Spectrometry (Williams et al. 1978, Biston et al. 1992). Protein content measurements can be done very effectively for large number of seed samples in a non-destructive way after the development of a calibration curve based on reference crude protein content measures (generally, Kjehldal %N × 6.25). This method is largely used by breeders.

This is for soybean that most of the information on seed protein content genetics is available. Improving seed protein content has been a major breeding target in soybean over the last 40 years and it is interesting to note that the selection of varieties with significantly increased protein content was achieved quite rapidly, through back-cross or recurrent selection (Brim and Burton 1978, Wilcox and Cavins 1995, Wilcox 1998, 2001, Helms and Orf 1998, Cober and Voldeng 2000). This was achieved thanks 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). Protein content variation in initial crosses was probably under the control of major genes. For example, the donor parent from the back-crossing scheme and from the recurrent breeding scheme of Wilcox (1998) and Wilcox and Cavins (1995) was Pando. The variety likely possesses the high protein content allele of *G. soja* at QTL LG-I identified in one of its derived line. Indeed, Diers et al. (1992) identified two major QTL controlling seed protein content in a population derived from a cross between a *G. soja* accession from China and a *G. max* breeding line. The *G. soja* parent possessed positive alleles of the QTL-LGI and QTL-LG E. Sebolt et al. (2002) checked the stability of these QTL alleles in the *G.max* genetic background by crossing *G. Soja* line (homozygous for the two *G. Soja* QTL alleles responsible for high protein content) with *G. max* breeding lines as recurrent parent. Among the two QTL alleles, only QTL-LGI allele showed significant effect in *G. max* background. The *G. soja* allele was associated with taller plant, reduced yield and oil, smaller seed and earlier maturity. Nichols et al. (2006) specified the location of the protein content QTL-

LGI. The genetic association of the protein and oil QTL at this locus was confirmed in lines recombining in the region. The association with the maturity QTL was confirmed in three recombining lines out of 4 whereas the association with the yield QTL was confirmed only in one line out of 4. This result has to be confirmed in order to be sure 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 of 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 (Mansur et al. 1993, Brummer et al. 1997, Csanádi et al. 2001, Hyten et al. 2004, Jun et al. 2008). A summary of all the major QTL (explaining more than 10% of phenotypic variation) has been 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 utilised for developing future soybean varieties with desirable component in the seed through MAS (http://www.SoyBase.org).

In pea, the selection for protein content is relatively recent. In France, it followed a decision taken in 1994 by the French official service for variety registration (CTPS) to fix a minimum seed protein content threshold to accept varieties for registration. Indeed, the selection for yield had lead to a rapid and undesirable decrease in protein content. Burstin et al. (2007) analysed in five environments the variation of protein content using a recombinant inbred line (RIL) mapping population segregating for three major developmental genes: *afila* controlling leaf tendril formation, *le* controlling internode length and plant height, and *rms6* controlling plant branching. Eight QTL controlled seed protein content variation in this population, among which 5 were stable across at least two environments. Two of these QTL were located in the same genomic region which harbours developmental genes '*afila*' and '*le*', suggesting pleiotropic effects of these genes on several traits. Tar'an (2004) reported 3 seed protein QTL in pea, two of them being consistent in many environments. Irzykowska and Wolko (2004) reported 5 QTL in a cross segregating for the *r* gene controlling starch synthesis and the wrinkled seed phenotype.

In pigeonpea, genetic variability for seed protein content was studied 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.

1.2 Seed protein content, yield and related traits: carbon costs and the possible role of C/N interplay

Highly negative correlations between protein and oil are well documented in soybean and varieties improved for protein content had lower level of oil (Wilcox 1998, Cober and Voldeng 2000, Wilcox and Guodong 1997, Hyten et al. 2004). Negative correlation between seed protein and oil content were also reported in cowpea (Oluwatosin 1997) and groundnut (Dwivedi et al. 1990). Similarly, negative correlations were reported between starch and protein content, whatever the genepool considered, in pea (Bastianelli et al. 1998) and chickpea (Frimpong et al. 2009). *De facto*, there is an intrinsic relationship between protein content and other major constituents content in the seeds: modifying one of them necessarily has an impact on the percentage of the others, as a result of the distribution of a finite amount of assimilates in different seed constituents. Similarly, correlations between protein content and yield have been reported to be often negative, but also sometimes non-significant, and sometimes positive (Cober and Voldeng 2000, Burstin et al. 2007, Bliss et al. 1973, Oluwatosin 1997, Leleji et al. 1972, Lawn and Rebetzke 2006, Frimpong et al. 2009, Hamdi et al. 1991) and selection for high protein content has often but not always led to reduced yield (Leleji et al. 1972, Brim and Burton 1978, Wilcox and Cavins 1995). Similarly negative correlations have been reported between seed size and protein content in pigeonpea (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 improvement of protein content and yield contributing traits simultaneously.

1.3 Potential levers for protein content improvement

Seed protein content can be viewed as the result of the relative accumulation of proteins and dry matter in the seeds (Lhuiller-Soundele et al. 1999). The rate of protein accumulation depends on the sink strength capacity of seeds (i.e. their ability to synthesize and accumulate proteins), and on the 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 on carbon supply through efficient photosynthesis and effective biosynthetic pathways. Depodding or defoliation experiments 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 depodding on the seed protein content of eight pea genotypes. The effects of genotype, depodding and genotype × depodding 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 8 genotypes, once depodded. This suggests that N source capacity is the major limiting factor of seed protein content in pea, but that the maximal rate of protein accumulation in the seed is also significant. Similar results were obtained for soybean (Proulx and Naeve 2009, Rotundo et al. 2009). We hypothesize that three types of genes/QTL could be identified for seed protein content (Burstin et al. 2007, Gallardo et al. 2008): primarily, major genes controlling developmental processes and having pleiotropic effect on the whole plant phenotype and on the source-sink structure, secondly genes of the plant metabolism controlling source-sink relationships at the plant metabolism level, and ultimately genes controlling solely the capacity of seeds to accumulate storage compounds. The impact of these different types of effectors on yield will probably be different.

1.3.1. Improving nitrogen supply to the seed

Among all plant species, legumes have the unique ability to fix nitrogen through their symbiotic interaction with nitrogen-fixing bacteria. The establishment and optimal functioning of this symbiosis together with efficient mobilization of assimilates from vegetative parts to the seeds control the availability of nitrogen to the growing seeds. Many genes involved in the control of nodulation have been identified recently (Ferguson et al., 2010). Pea mutants with absence of N_2 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 a secondary effect of lower access to soil resources. The importance of a 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 the N-fixing symbiosis relies on the carbon supply from aerial parts to the root parts. In a recent study, Bourion et al. (2010) have 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.

1.3.2. Improving seed sink strength

Functional interactions exist among the different seed constituents: for example, the disruption of the *r* gene abolishes starch synthesis in pea seeds, leading to a wrinkled seed phenotype. This defect in starch accumulation had a profound impact on seed metabolism, where elevated sucrose content impacted the accumulation of storage protein families (Wang and Hedley 1991). 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 possible if it allows producing specific seed products dedicated to specialty markets. However, in the perspective of increasing protein production in the world, strategies allowing maintained yield should be preferred. 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). Weigelt et al. (2008) showed that seed-specific over-expression of an amino-acid permease in pea increases amino acid supply to the seed and the level of protein in the seed. This indicated a stimulation of storage protein synthesis by increased amino acid availability. However, compensatory changes lead to decreased seed weight, as discussed in paragraph 1.2. Seed-specific over-expression of a bacterial phosphoenol pyruvate carboxylase in *Vicia* narbonensis similarly increased seed protein content with compensatory effect on seed number and seed weight (Rolletschek et al. 2004). Few studies analysed the transcriptional control of common bean seed storage protein gene expression (Li et al. 1999, Ng et al. 2008).

In conclusion, the genetics of seed protein content largely remain a black-box. 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.

2. 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). Legumins are compact hexamers of 350 to 400 kDa associating acidic alpha-polypeptides and basic beta-polypeptides. Vicilins and convicilins are trimers of 150 to 280 kDa composed of very heterogeneous and differently processed polypeptides. Proteome reference maps have been developed for soybean (Hadjuch 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, glutelins) complete the protein fraction of the seed and the composition in the different protein fractions depends on the species (Boulter and Croy 1997, Montoya et al. 2010, Gallardo et al. 2008).

2.1 Protein composition and digestibility

The different storage proteins have different *in vitro* and *in vivo* digestibility depending on their structural characteristics (Crévieu et al. 1997, Le Gall et al. 2007, Gabriel et al. 2008a, b, Montoya et al. 2010, Clemente et al. 2000). Studies on grain legume seed protein digestibility in human are scarce (an example of digestibility values on pea protein fractions is provided by Mariotti et al 2001). But 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 over-expression of a particular storage protein family (for example Burow et al. 1993) but did not generally yield the expected outcome. Another strategy in order to improve protein digestibility could be to search for protein composition patterns favourable to digestibility in the germplasm variability (Montoya et al. 2010). Indeed, several authors reported intra-specific seed protein composition variability (in pea, Bastianelli et al. 1998, Burstin and Duc 2005, Tzitzikas et al. 2006; in lentil, Scippa et al. 2010; in soybean, Natarajan et al. 2006). Other minor protein resistant to hydrolysis may also have a role in protein digestibility and their variability could be exploited (for example, the albumin PA2 in pea, Vigeolas et al. 2008).

2.2. Improving amino acid balance in legume seeds: progress and prospects

Essential amino acids are important in the nutrition of humans, who are unable to synthesize them and are dependent on dietary sources of these amino acids. Among them, tryptophan and the sulphur-containing amino acid methionine are the most limiting in legume seeds (Table 3). To the contrary, legume seed proteins are rich in lysine while cereal seed proteins are low in this amino-acid (Wang et al. 2003).

Table 3: Four amino acids of grain legume seeds: range of variation (g/100g protein)

Species	Lys	Met	Cys	Trp	Reference
Soybean	22.4-24.1	4.4-8.8	5.1-7.3	4.4-5.1	Panthee et al. 2006, RIL population
Pea	15.5-19.7	2-2.4	2.9-3.6	2-2.7	Gabriel et al. 2008, 8 varieties [a]
	14.8-23	2.1-3.3	2.9-4.2	1.6-3.2	Bastianelli et al. 1998, 54 acc. incl. *rug* mutants
Fababean	17.3-21.6	2.3-2.9	2.9-4.3	2.0-3.2	Duc et al. 1999, 12 spring varieties
	19.2-20.3	2.1-2.7	3.6-3.9	2.4-2.7	Duc et al. 2010, 8 varieties
Lentil	4.5-12.6	1.2-1.7	0.4-0.5	-	Rozan et al. 2001, 5 lens species
Cowpea	-	1.9-2.8	1.6-2.1	3-3.7	Bliss et al. 1973, 11 varieties
	4.9-9	0.52-2.05	0.84-2.24	0.72-1.91	Oluwatosin 1997, 15 accessions

[a] dehulled seeds

Genetic manipulations have been used in attempts to improve amino acid balance in legume seeds, particularly towards increasing methionine level. The main strategy employed was to modify storage protein composition in favor of accumulation of sulphur-rich proteins. For example, the sulphur-rich 2S albumin genes from Brazil nut and sunflower were expressed in seeds of soybean and lupins (*Lupinus angustifolius* L.), respectively (Altenbach et al. 1989, Molvig et al. 1997). Although these transgenic plants had increased seed methionine level, the introduced sulphur-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 sulphur compounds, such as free sulphur amino acids and glutathione (Tabe and Droux, 2002), thus indicating that the rate of synthesis of sulphur amino acids during legume seed development is limiting. Activating the synthesis of essential amino acids might therefore be a possible route for improvement of amino acid balance in legume seeds.

An aspect to be considered in view of stimulating the synthesis of essential amino acids is the crosstalk between the amino acid biosynthetic pathways, balancing the flux of carbon and nitrogen backbone atoms between them. This has been well documented for the aspartate family biosynthetic pathways leading to the synthesis of methionine and of two other essential amino acids (threonine and lysine) from a common precursor, aspartate (for a review, see Jander and Joshi, 2010). Molecular studies using *Arabidopsis* and tobacco as model plants have demonstrated that enzymes of these pathways are feedback-inhibited by several products along the pathways. Noteworthy, lysine negatively regulates the activity of the first enzyme of its own pathway, "dihydropicolinate synthase" (Galili, 2002), but also the amount of *S*-adenosylmethionine that is a negative regulator of a key enzyme for methionine synthesis, cystathionine gamma synthase (Hacham et al. 2007). Thus, lysine regulates the flux of carbon and nitrogen towards methionine synthesis. A similar feedback inhibition was observed for the tryptophane biosynthetic pathway, tryptophane inhibiting its own biosynthetic pathway by regulating negatively anthranilate synthase, which catalyses the conversion of chorismate to anthranilate (Ufaz and Galili, 2008). Interestingly, the modulation of feedback inhibition in these pathways allowed increasing the synthesis of some amino acids. As for example, the introduction of genes encoding anthranilate synthase forms insensitive to feedback inhibition enhanced the accumulation of tryptophane in seeds, including soybean seeds (Ufaz and Galili, 2008 ; Ishimoto et al. 2010). These findings open perspectives towards modifying 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. It should be noted that the up-accumulation of amino acids under free forms in seeds could have negative effects on agronomic traits. As for example, the germination ability of transgenic seeds containing very high levels of free lysine or tryptophane was reduced (Zhu and Galili, 2003; Wakasa et al. 2006). This could be inferred to the toxicity of free amino acids and/or to modifications in the synthesis of compounds derived from these amino acids. To avoid such deleterious effects on agronomic traits, a challenge would be to increase the synthesis of essential amino acids while working to increase their incorporation into storage proteins.

The accumulation of the different classes of storage proteins during seed filling, which largely determines the seed amino acid balance in mature seeds, varies according to the availability of sulphur and nitrogen in the environment. Legume seeds produced in limiting sulphur conditions, but with adequate nitrogen, generally contained reduced levels of sulphur-rich storage proteins and accumulate more sulphur-poor proteins (Higgins et al. 1986, Tabe and Droux, 2002). In soybean, Paek et al. (1997) reported an increase in the proportion of sulphur-poor protein as protein concentration increased. Wilcox and Shibles (2001) to the contrary found a constant sulphur/nitrogen ratio in a population segregating for seed protein content; but seed yield was not high in this population, and thus sulphur was probably not limiting in this context. In cowpea, Bliss et al. (1973) found a positive correlation between seed protein content and the methionine content of proteins. In chickpea, the application of nitrogen, phosphorus and sulphur fertilizers improves the levels of protein and essential amino acids (Gupta and Singh, 1982; Williams and Singh, 1987). In pea seeds, the reduced levels of sulphur-rich proteins in conditions of limited sulphur 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 signaling molecules controlling expression of genes for sulphur-rich storage proteins in legume seeds (Tabe et al. 2010, and references therein). These finding indicate that the capacity of legume plants to regulate the flux of sulphur and nitrogen compounds to the seeds should be considered if the accumulation of sulphur-rich storage proteins has to be increased. Sulphate is one of the dominant forms of sulphur found in the phloem supplying pods during legume seed development (Tabe and Droux, 2001). In plants, sulphate can be reduced to sulfide, leading to the synthesis of cysteine, the precursor for methionine synthesis, or it can be stored in the vacuoles. A recent study in *Arabidopsis* reports that sulphate stored in the vacuoles contributes only a little to the establishment of seed protein composition, but is likely to be important in maintaining redox homeostasis in seeds (Zuber et al. 2010). Hence, considering the importance of sulphate for the synthesis of sulphur compounds, one limiting step for accumulation of sulphur-rich proteins could be the uptake of sulphate by the root and its distribution within the plant by membrane-localized sulphate transporters. Several sulphate transporters of high affinity have been identified in several species that were strongly regulated by sulphur deficiency to facilitate the uptake of sulphate by the root (SULTR1;1 and SULTR1;2) or its translocation from source-to-sink (SULTR1;3) (for a review, see Hawkesford and De Kok, 2006). Other transport forms of sulphur in the phloem are glutathione and *S*-methylmethionine that can be respectively reconverted into cysteine and methionine (Bourgis et al. 1999). Interestingly, a characterization of knockdown *Arabidopsis* mutants for the isozyme 2 of homocysteine methyltransferase, which converts *S*-methylmethionine into methionine, suggests that increasing the transport of *S*-methylmethionine from vegetative tissues to seeds could increase seed methionine level (Lee et al. 2008).

Furthermore, optimization of sulphur assimilation requires coordination with carbon and nitrogen pathways, and multiple processes have been proposed to contribute to this balance (see Hawkesford and De Kok, 2006). For example, cysteine synthesis from sulfide and *O*-acetyl-L-serine is a reaction interconnecting sulfate, nitrogen, and carbon assimilation. Indeed, *O*-acetyl-L-serine is dependent upon adequate nitrogen and carbon availability and regulates positively gene expression and activity of sulphate transporters, and of several enzymes of sulphate reduction and assimilation (see Hesse et al. 2004, and references therein). Moreover, *O*-acetyl-L-serine has been shown to be induced by sulphur deficiency (Hirai et al. 2003). In contrast, other metabolites acting as signals in response to the nutrient status are negative regulators of sulphate uptake and metabolism, such as glutathione and cysteine (Hawkesford, 2003). The combination of these feedback loops regulates the flux of sulphur and nitrogen atoms in the different metabolic pathways, thus controlling their distribution in plant parts, including seeds.

3. Seed protein bio-availability for human: breeding for minor compounds effective on the protein value or acceptability

Grain legume seeds bring in the diet carbohydrates (lipids, starches, fibres) and minor seed compounds which will influence positively or negatively protein bio-availability by impacting digestibility or acceptability (health benefits or taste and colour requirements). Numerous studies have documented the possibility of improving the nutritional values of grain legumes as animal feeds (mainly for monogastric animals) but considering minor constituents, results cannot be easily extrapolated to humans: (i) even if monogastric, human beings have their own physiology varying with age, (ii) human diet is composed of a diversity of ingredients generating high dilutions and complex interactions. This situation is completely different from the simple, repetitive diets given to homogeneous genetic populations of young animals. This is why seed compounds called antinutritionals in feeds have been removed by breeding, when on the contrary some of them may have positive role on human health like on chronic diseases prevention, i.e. cancer, cardiovascular disease, diabetes, and obesity prevention. In this part, we will review the genetic variability available for minor legume seed compounds which may help breeders to significantly improve their protein contribution in human diets.

3.1 Minor compounds effective on digestibility and /or health

3.1.1 Trypsin inhibitors:

Trypsin inhibitors are present in most grain legume seeds (Table 4). High inhibiting activities are found in soybean seeds which are usually reduced by processing, But null mutants for both Bowman–Birk and Kunitz trypsin inhibitors have been identified in soybean, allowing low trypsin inhibitor cultivars to be produced (Clarke and Wiseman, 2000). In pea, large genetic variability is available for the activity of Bowman-Birk trypsin/chymotrypsin inhibitor proteins (TIA) (Bastianelli et al. 1998). The polymorphism in coding and promoter sequences of genes at *Tri* locus accounts for most of the variation in TIA and this allowed to initiate marker-assisted selection (Page et al. 2002). However, if low TIA activity is a benefit in pig or poultry feed digestibility, recent data suggest that high contents of trypsin inhibitors in foods should be positive, since a reduction of HT29 colon cancer cells has been shown when grown *in vitro* in the presence of pea TI (Domoney et al. 2009). If validated *in vivo*, this would encourage breeding for high content of TI for food or nutraceutical applications.

Table 4: Minor constituents of grain legume seeds: range of variation

Species	TIA (TIU/mg)	Tannins (g/kg)	Saponin (g/kg)	Total alpha-galactosides (%DM)	Phytic acid (g/kg)	Reference
Soybean	-	-	6.5	-	-	Kadlec et al. 2001
	-	-	-	-	6.2-20.5	Saghai Maroof et al. 2009, 31 lines
	-	-	-	-	32.4-41.3	Chitra et al. 1995, 6 lines, dehulled seeds
	43-83	-	-	-	-	Guillamon et al. 2008
Common bean	-	-	2.3 - 3.5	0.4-8	-	Kadlec et al., Koslovska et al. 2001
	-	0-38.5	-	-	-	Caldas and Blair 2009, 3 RIL populations
	-	-	-	-	2.9-17.8	Blair et al. 2009, RIL population
	17-51	-	-	-	-	Guillamon et al. 2008
Pea	-	-	1.1	2.3-9.6	-	Kadlec et al., Koslovska et al. 2001
	1-14.6	0.04-7.4	0.3-1	3.6 - 10b	1.3 - 10.2	Bastianelli et al. 1998, 54 pea accessions
	1.9-6.8	-	-	-	-	Gabriel et al. 2008, dehulled seeds, 8 varieties
	6-15	-	-	-	-	Guillamon et al. 2008
Fababean	0.3 -5.3	0.1 - 10.4	-	1.4 - 6.2b	3.8 - 13.4	Duc et al. 1999, 12 spring varieties
	-	-	0.1	1-4.5	-	Kadlec et al., Koslovska et al. 2001
	-	2.1-3.2	-	-	-	Avola et al. 2009, 15 accessions
	0.8-3.6	-	-	-	-	Filipetti et al. 1999, 6 lines
	5-10	-	-	-	-	Guillamon et al. 2008
Lentil	-	-	1.1	1.8-7.5	-	Kadlec et al., Koslovska et al. 2001
	1.9-2.8	3.4-6.1	-	-	6.2-8.8	Wang et al. 2009, 8 varieties
	3-8	-	-	-	-	Guillamon et al. 2008
Chickpea	-	-	2.3	2-7.6	-	Kadlec et al., Koslovska et al. 2001
	-	-	-	-	7.7-12.3	Chitra et al. 1995, 13 desi lines, dehulled seed
	-	-	-	-	5.4-11.7	Chitra et al. 1995, 3 kabuli lines, dehulled seed
	12.7					Singh and Jambunatham, 1981, desi lines
	10.3					Singh and Jambunatham, 1981, kabuli lines
	15-19	-	-	-	-	Guillamon et al. 2008
Cowpea	12-16.6a	-	-	-	-	Vasconcelos et al. 2010, 3 varieties
	-	0.3-6.9	-	-	-	Plahar et al. 1997
Mungbean	-	-	-	-	10.2-14.8	Chitra et al. 1995, 3 lines, dehulled seeds
Pigeonpea	-	-	-	-	6.8-14.9	Chitra et al. 1995, 16 lines, dehulled seeds
	-	-	-	-	9.9-16.4	Singh 1999, dehulled seeds

a: g inhibited /kg seed flour, b: Raffinose + stachyose + verbascose

3.1.2 Lectins:

Most grain legumes cotyledons contain lectins (haemagglutinins), polysaccharide-binding proteins that bind to glycoprotein in epithelial surface of the small intestine, interfering with nutrient absorption and increasing production of mucins and a loss of plasma proteins in the intestinal lumen (Pusztai, 1989). Duranti (2006) listed numerous bioactive effects of lectins in humans including small intestine hyperplasia, change in intestinal flora, immuno-modulating activity, hormone secretion, access to the systemic circulation, and this complex role has hampered their medical use for limiting tumour growth which was suggested by experiments on animal models. In plants, lectins are very diverse and are involved in plant defense (Etzler, 1985) or symbiosis with *Rhizobia* (van Rhijn et al. 2001). Some natural variability exists for lectin hemagluglutinin activity in germplasm (Valdebouze et al. 1980). However, the low content and toxicity of lectins together with the complexity of lectin roles did not allow for the definition of a breeding target for this trait.

3.1.3 Alpha-galactosides:

Major alpha-galactosides in grain legume seeds are raffinose, stachyose and verbascose. They are not degraded in upper gastrointestinal tract and thus pass into large intestine where bacterial enzymes decompose them in short chain fatty acids and gases responsible of digestive discomfort; they have however highly probable prebiotic properties which may be of interest against colorectal cancer (Guillon and Champ 2002). Even if some genetic variation exists (Table 4), genetic tool to monitor these contents have never been worked out, due to competition with easy cooking or technological treatments such as soaking in water added with bicarbonate, germination or adding commercial exogenous alpha-galactosidase in the diet (Guillon and Champ 2002).

3.1.4. Vicine and convicine:

The glycosides vicine and convicine (VC) are concentrated in cotyledons of faba bean seeds. Conventional cultivars contain from 6 to 14 g/kg DM of VC in mature seed. A mutant allele *vc-* has been discovered which reduces 10 to 20 fold VC contents (Duc et al. 1989). This allele has a positive effects on egg production by laying hen and energy value of feeds for chicken (Crepon et al. 2010). VC are causing favism, an acute hemolysis caused by the ingestion of faba beans occurring only in G6PD-deficient human individuals (Arese and De Flora, 1990). Because of high cost of chemical determinations, molecular markers were proposed to assist the selection for low VC content genotypes (Gutierrez et al. 2006). Low VC cultivars are presently in test in humans in order to evaluate their nutritional safety on individuals susceptible to favism.

3.1.5 Tannins and flavonoid compounds:

Flavonoids are major phenolic compounds involved in the determination of seed coat colours and in the tanning power on proteins (Nozzozilo et al. 1989, Plahar et al. 1997, Caldas and Blair 2009). Tannins or proanthocyanidins are synthesized through the flavonoid pathway (Dixon et al. 2005). These polyphenolic compounds bind to proteins and reduce their digestibility. In pea and faba bean, a single gene mutation has a pleiotropic effect eliminating tannins from seed coat and determining a white flower trait. They increase protein digestibility in pigs or poultry by ca 10 % when compared to tannin-containing lines (Grosjean et al. 1999, Crepon et al. 2010). This quality trait was economically valuable for feed efficiency and zero tannin varieties were bred for 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). However, limited data is available for individual phenolic compounds. Removal of tannins for humans may have positive nutritional effect but is certainly impacting the level of astringent taste with positive or negative consumer reactions according to habits. Moreover, the health benefit of proanthocyanidins may deserve some attention. The diverse colours of common beans were suggested to be important sources of dietary antioxidants (Beninger and Hosfield, 2003).

3.1.6 Phytic acid:

It is commonly found in cereal and legume seeds and its anti-nutritional effect is associated with mineral-complexing (especially Zn, Ca and Fe) and inactivation of digestive enzymes. In feeds the small magnitude in anti-nutritional effects did not justify a breeding action. In foods, the reduction of bioavailability of minerals and proteins induced by phytic acid may be a nutritional concern (Frossard et al. 2000), but on the other hand phytic acid may have protective effects such as a decrease of the risk of iron-mediated colon cancer and lowering serum cholesterol and triglycerides (Champ, 2002). In common bean, 5 QTL were identified that controlled total and net seed phytate content (Blair et al. 2009). Any breeding activity to improve the contents of phytic acid should also be evaluated in comparison with technological processes able to eliminate them and to health benefits.

3.2. Acceptability of legume seed proteins in food: Compounds effective on taste and colour

Lipoxygenase activity can cause unpleasant tastes and aromas when reacting with seed lipids. In soybean and pea, null mutants were found for 3 and 2 LOX genes respectively. Their molecular characterization (Forster et al. 1999, Lenis et al. 2010) has well progressed and offers possibilities of breeding for lipoxygenase-free varieties.

Saponins are triterpenoid glycosides with detergent like effects resulting in haemolytic capabilities when incubated with erythrocytes. However, their anti-nutritional role on animal performances has not been clearly established. A large number of different saponins exists in legumes (Heng et al 2006) and they contribute to the bitterness of peas as well as that of soybean. Saponins have been studied for their positive hypo-cholesterolaemic effects and also because may also have anti-carcinogenic effects (Champ, 2002) Some genetic diversity has already been described for quantity and quality of seed saponins (Table 4, Heng et al. 2006) but its genetics basis is unknown.

Finally, seed-coat as well as cotyledon colours define the appealing value for local or specific food markets. These traits are generally easy to breed because of oligogenic control and high heritabilities. They are of primary importance for specific food ingredient market niches.

In conclusion, there is an urgent need to acquire new references on health and nutritional values of grain legumes. Determining the value of particular fractions in nutraceutic applications may provide new markets with higher added value. We did not develop here the effects and cost of the technological treatments on bioactive components, but this may help to choose between genetic strategies or technological processes (Champ 2002). Finally, several studies have demonstrated effectiveness of proteins in protection against parasitic insects (Rahbé et al. 2003) or fungi attacks. Attempts to modify contents in minor bioactive compounds should be associated with an appraisal of their consequences on plant behaviour against biotic or abiotic stresses.

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