
Improving Protein Density in Food Legumes Through Agronomic Interventions

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Abstract

Agronomic biofortification is the easiest and fastest way for biofortification of cereals and pulses with Fe, Zn or other micromineral nutrients especially in developing Asian and African countries, where these are considered as the staple food. Agronomic biofortification is the only way to reach the poorest of the poor rural masses, those who will never have money to buy mineral supplements nor can afford to improve the components of their diet by incorporating animal products. It is suggested by scientific evidences that biofortification is feasible technically and even it can be achievable without negotiating for agronomic productivity. Cost-benefit analyses that are predicted also do support biofortification as being imperative in the armamentarium for monitoring micronutrient deficiencies and its control. However, the pertinent challenges facing all of us are about the accessibility of biofortified crop in large scale with proven purity and quality standards and to get producers/consumers acceptance for such biofortified crops and increase their intake. With the inception of good seed production and utilisation systems, the development of markets and market-oriented products and creation of demand for these, the commercial exploitation of crops with nutrient fortification can be possible and achievable. Amongst different types of malnutrition, protein malnutrition is the foremost one as it plays the pivotal role in health and nutrition of both human and animals. Besides protein malnutrition, deficiencies due to vitamin A, iron and zinc do affect over half of the population of the world. Although ample progress has been achieved to control these deficiencies through food fortification and supplementation, yet renewed and vigorous approaches are needed, especially to reach a sizeable mass of rural poor. It is true especially for reaching the

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Millennium Development Goals (now, Sustainable Development Goals) that aims at reducing by at least two-thirds the under-five child mortality ratio and three-quarters the maternal mortality ratio by 2020. Therefore, biofortification of staple food crops including food legumes is a new public health approach to contain protein (and of course deficiencies of vitamin A, iron and zinc) in poor countries. This chapter gives a brief insight of the technology for improving protein density in food or seed legumes through agronomic interventions.

Keywords

Agronomic interventions • Food legumes • Grain legumes • Protein density • Sustainability

15.1 Introduction

India is recognised as the principal producer, consumer, processor and importer of pulses in the world. Paradoxically, the country's pulse production has reached at 19.5 million tonnes (Mt) from the acreage of 24.5 million hectares (M ha) during 2013–2014. Yet, the country is importing pulses to the extent of around 2–3 Mt every year for meeting its domestic demand. This is our major concern as an increase in the demand from India has shown to have surging effect on international prices, thereby draining the most sought-after precious foreign exchange. Expectedly, as the country's domestic requirements would be around 26.50 Mt by 2030, it further necessitates stepping up of production by almost 7.0 Mt of additional produce. This arduous task is to be accomplished even under more severe constraints in production environment including those related to biotic and abiotic stresses, climatic changes and accumulative secondary and micronutrient deficiencies in the soil. This necessitates a two-pronged proactive strategy that involves reducing cost of production and improving per unit productivity (Prahara and Dhingra 2001).

Iron and zinc deficiencies in human nutrition are widespread and rampant in developing Asian and African countries where cereal grains are the staple food. Effects are therefore underway to

develop cereal genotypes with grains denser in Fe and Zn by traditional plant breeding or using genetic engineering techniques (Grusak 2002) which require a long gestation period and adequate funds. However, the products of genetic engineering are not well accepted in many countries. Also, there is a trade-off between yield and grain biofortification. Therefore, agronomic biofortification offers to achieve this without sacrificing on yield and with no problem of product acceptance. From the viewpoint of biofortification, for example, foliar application has been reported to be better than the soil application of Fe and Zn, and for this purpose, chelated Fe and Zn fertilisers are better. When soil applied, water-soluble sources of Zn are better while soil application of Fe is not recommended. Therefore, agronomic biofortification depends upon management practices (tillage, water management, nutrient interactions and so on), soil factors (amounts of Zn present, pH, mechanisms of Zn fixation other than pH and others) and plant factors (root characteristics, excretion of phytosiderophores and organic acids by roots, utilisation of zinc at the cellular level, translocation within plant and mechanisms of Zn accumulation in grain). As genetic and agronomic biofortification are complementary to each other, once the genotypes having denser grains are developed, they will have to be adequately fertilised with Fe and Zn (Prasad et al. 2014).

15.2 Good Agronomic Practices

Good agronomic practices, popularly known as GAP, alone can help increase in productivity to the extent of 25–40 % in many field crops. Besides this, efficient production technologies are required with special importance on adequacy in crop nutritional aspects. Therefore, a need is arisen for developing appropriate production technologies for nontraditional areas involving crops and cropping systems involving pulses, for example, relay cropping and pulse-based crop rotations in rice fallows, off-season cultivation in summer/spring season and introduction of compatible crops in cropping systems. Since the availability of labour for various and diverse farm operations is reducing and cost of labour is increasing, appropriate crop management technologies need to be developed for reducing the cost of production. It also suits the two-pronged strategy of crop production, viz. increase in production/productivity of crops and/or decrease in the cost of cultivation/production (or increase in the farm income). Therefore, to harness the energy sources, efforts are already in place to assess and refine various production technologies with innovative agronomic practices (more emphasis on optimum plant population and adequate crop nutrition in pulses with desired control of both biotic and abiotic stresses). Depending upon the amount and distribution of rainfall, pulses – an important component of staple food crops – could be grown under double cropping (with annual rainfall of more than 75 cm), intercropping (60–75 cm) and monocropping (less than 60 cm rainfall) systems. In addition, efficient intercrops for pulses need to be screened/identified and popularised amongst the farmers so as to compensate against a possible crop loss through pure or monoculture (Praharaj et al. 2011).

In the recent past, food consumption pattern has also undergone considerable change owing to various factors like increase in income, urbanisation, change in consumer taste and preferences, awareness about safe and healthy food etc. As a result, the composition of diet

and nutrition intake has changed considerably. It is evident from the fact that the dietary plan has shifted away from cereals and pulses towards fruits, vegetables, processed food and food items of animal origin. As a result, the consumption of pulses has come down due to various possible reasons like poor availability, high prices and availability of cheaper alternatives of animal origin. Although the shift in consumption towards horticultural crops and food items of animal origin has no doubt contributed towards higher intake of calories, the intake of protein at the same time has come down mainly due to decline in the consumption of pulses which is a major source of quality protein compared to other food items. The concern is reduction in consumption of pulses by predominantly vegetarian society and poor like that of India due to high price and fluctuation in supply of pulses. Moreover, pulses could act as a low-cost substitute during high prices of vegetables and food items of animal origin. Although the production of pulses has registered an impressive growth in the recent decade yet, it is not in pace with the increase in the population. Therefore, the need of the hour is to raise both production and availability of pulses by adopting various innovative measures. This will ensure food and nutritional security by bringing sustainability in agricultural production in the country.

In order to continue with the accelerated growth in production of pulses, institutional and policy supports are also required for enhancing area under pulses, development of high-yielding varieties, supply of quality inputs (Praharaj et al. 2011, 2014; Praharaj 2013), intercropping (Sankaranarayanan et al. 2011), proper extension of production technologies (Singh and Singh 2014), development of value chain etc. The supply of pulses can also be increased by having orderly marketing of pulses. The availability of information being a vital component will make farmers respond more effectively to the various initiatives of the government. With the advent of technology, the information flow could reach to the lowest level of farming community. Popularising low-cost technology of production,

promotion of high-yielding varieties and marketing-related issues will be more effective using improved crop production techniques. The elasticity of the demand for high-value commodities is highly price sensitive, and hence, in the event of a rise in price of such commodities, pulses will act as a substitute for cheaper protein. Also, considering the fact that widespread malnutrition is prevailing amongst children and women in India, there is a need to promote consumption of pulses by linking to programmes like midday meal and rural health mission by incorporating either free distribution of pulses or by subsidising the food (Shalendra et al. 2013).

15.3 Food Legumes in Human Nutrition

Legumes have not been emphasised properly during the last five decades of research in food science and human nutrition. It is still continued as the legume species are encountered with the deficiency of sulphur amino acids and the heat lability/stability of proteinase inhibitors and phytohaemagglutinins. A survey of literatures indicates that more research papers on the negativity of legumes have been published in different food journals such as *Journal of Agriculture and Food Chemistry*, *Journal of Food Science* and *Journal of the Science of Food and Agriculture*. This trend also indicates the repeatability of certain aspects of research on the importance of food legumes so far as human nutrition is concerned. Now the time has arrived to project and depict the image of legumes in proper perspective (Deshpande 1992). The validity of our continued patronage in defining the role of legumes in human nutrition especially at a time when the research dollars worldwide are becoming increasingly harder to come by is more pertinent now than before. One such aspect is supplementation of food legumes per se in human or animal protein nutrition and biofortification of such food/seed crops for raising its protein density.

15.3.1 Protein Malnutrition

Protein malnutrition is a worldwide phenomenon occurring in every nation around the globe as deficiencies of vitamin A, iron and zinc affect over one-half of the world's population. Biofortification is the development of protein or micronutrient-dense staple crops using the best traditional breeding practices, modern biotechnology and to some extent by appropriate agrotechnologies. This approach has multiple advantages. It takes advantage of a regular daily intake of a consistent and large amount of food staples by the family members. This strategy targets low-income households as staple foods predominate in the diets of the poor. After the one-time investment for developing seeds that fortify themselves, here the recurrent costs are low, and germplasm can be shared internationally that makes it cost-effective across time and distance. Moreover, once in place, the biofortified plant system is highly sustainable. Even if government attention and international funding for such (micronutrient) issues fade, nutritionally improved varieties will continue to be grown and consumed year after year.

In addition, biofortification provides a feasible and viable means of reaching undernourished populations in relatively remote rural areas, delivering naturally fortified foods to people with limited access to these. It is also critical that improved agronomy or breeding approach for biofortification in seeds should not incur a yield penalty due to the fact that biofortification and commercial fortification are highly complementary (Graham and Welch 1996; Graham et al. 2001). They may have either important spin-off effects for enhancing farm productivity or renders yield stability in food crops in an environmentally sound way. Trace minerals embedded in seed through biofortification should be essential in enabling plants to resist pests, diseases and other environmental stresses as these plants should survive under stress with rapid initial growth and vigour (and consequently produce similar or higher yields). The details of role of pulses in nutrition especially in relation to

protein nutrition are given in subsequent sections.

15.3.2 Pulses and Protein Management

The word 'pulse' is derived from the Latin word 'puls', meaning pottage. The term 'pulse' is used for those leguminous plants containing small amount of fat. India is the leading country for high consumption of pulses with an average consumption of 35 g/person/day, but there is wide variation amongst the states. Intake per day ranged from 16 g in Tamil Nadu to 55 g in Madhya Pradesh. The highest intakes tended to be in northern states. The daily per capita availability of pulses in India has decreased, however, to a meagre of 35 g against FAO-recommended dose of 80 g/day.

Pulses are known for their cost-effectiveness and are in fact relatively a cheaper source of protein than milk, cheese, cashew, almonds, meat, fish etc. and thus valuable for developing countries. The seeds of pulses are the most commonly eaten/edible part, and most of them can be economically stored well for future use. The food value of seed of pulses is high as they have about the same calorific value per unit weight as cereals and are good sources of some vitamins and minerals. Pulses do contain about 18.0–32.0 % protein (Table 15.1 and 15.2) and about 1–5 % fat (Table 15.1). The protein density of pulses is generally higher than that of most cereals. In India, consumption of pulses is highest as compared to other pulse-growing countries due to religious restrictions on non-vegetarian diet and low purchasing power. The pulse protein in isolation has somewhat lower nutritive value than most other classes of protein like meat, fish and milk, but they contribute substantially in fulfilling the protein requirement when combined with other proteins in a mixed diet. Pulses are also a rich source of calcium than most cereals and contain about 100–200 mg of calcium/100 g of grain. They are also substantially rich in iron, thiamine, riboflavin and nicotinic acid as compared to cereals. Germinating sprouts of pulses

like, mung bean, moth bean and chickpea are popular foods in many regions.

The protein density in pulses is mostly confined to the cotyledons that constitute the major proportion in the seed. Therefore, it contributes the major amount of protein to the whole seed. The seed coat of pigeon pea contains 5.6 % protein, while the cotyledon and embryonic axis have 24.3 and 48.1 %, respectively. Other pulses are also having more or less similar protein content in their seed coat, cotyledon and embryonic axis. Moreover, the pulses are subjected to various processing techniques like milling, dehulling, soaking, germination, fermentation and cooking to make it more value-added eatable products. Besides saving time, energy and fuel, these processing techniques have several nutritional advantages as these produce edible products with higher nutritional value and lower toxic compounds. In addition, the degree of elimination of toxic compound depends on the type of pulse and the processing technique. Proteins, carbohydrates, fats, minerals and vitamins are also important nutritional parameters of pulses.

Pulse proteins usually contain more than adequate levels of some of the nutritionally important amino acids such as lysine that are deficient in most cereals and other edible plant foods. Therefore, the combination of cereals and pulses provides a good balance of amino acids since cereals usually supply adequate amounts of methionine. Pulses are good source of dietary fibre also. Pulses do contain several antinutritional factors including trypsin and chymotrypsin inhibitors, lectins, polyphenols, flatulence factors, antivitamin, allergens, lathyrogens and saponins. The protease inhibitors, lectins and other antinutrients could also cause toxicity in human beings. Heat treatment is the well-established practice that destroys proteinaceous antinutrients, such as protease inhibitors and lectins, but heat treatment destroys some of the amino acids and vitamins as well. Therefore, for maintaining the nutritional value of food, it is prerequisite that heating temperature and length of processing do not exceed the optimum temperature and time required to eliminate the effect of inhibitors. Moreover, proteins in pulses are known to interact

Table 15.1 Comparative account of energy, protein and others in pulses (per 100 g edible part)^a

Pulses (dal)	Energy (KCal)	Protein (g)	Carbohydrate (g)	Fat (g)
Chickpea	360	17.1	60.9	5.3
Black gram	347	24	59.6	1.4
Cowpea	323	24.1	54.5	1.0
Beans	347	24.9	60.1	0.8
Green gram	334	24	56.7	1.3
Horse gram	330	23.6	56.5	1.1
Peas (green)	93	7.2	15.9	0.1
Peas (dry)	315	19.7	56.5	1.1
Lentil	343	25.1	59	0.7
Red gram (arhar)	335	22.3	57.6	1.7
Rajmash	346	22.9	60.6	1.3
Soybean	432	43.2	20.9	19.5

^aSource: IIPR (2009)

Table 15.2 Protein content (%) in seed of major pulses^a

Pulses	Ranges
Chickpea	18.0–30.6
Pigeonpea	18.8–28.5
Mung bean	20.8–31.8
Urd bean	21.2–31.3
Lentil	20.4–30.5
Pea	21.2–32.0
<i>Lathyrus</i>	22.7–29.6

^aAverage content is listed based on random sampling and analysis

with other compounds such as lipids, tannins, phytates, flavanone compounds and pigments. These interactions do occur when pulses are processed and converted into edible products which decrease the bioavailability of proteins in pulses. In the similar fashion, tannins and phytates do interact with minerals and vitamins resulting in a decrease in bioavailability of minerals and vitamins.

Wide variability in seed protein in pulses also exists as chickpea has protein density which varies from 18.0 % to 30.6 % with an average of 21.5 %. Protein quality of a crop depends on its amino acid composition, and the most limiting amino acids determine the nutritive value of pulses. Pulses are deficient in sulphur-containing amino acids and tryptophan, but are rich in lysine in which cereals are relatively deficient. Lentil has the lowest amount of methionine, whereas pigeon pea has the lowest amount of tryptophan (Table 15.3).

The embryo is rich in methionine and tryptophan, but it contributes only about 2.5 % of their total quantity in seed. The cotyledon, being the major component of the seed, accounted for 93 % of methionine and tryptophan of the whole seed, while the seed coat was the poorest in these amino acids. Therefore, the application of P, Mo and N has been shown to increase the level of methionine. The applications of sulphur-containing fertilisers also increase the cystine content of chickpea protein. The pulses have low biological value. The biological value of pulse protein ranges from 32 % to 78 % and a large variation is observed within varieties of the same species. Low methionine in pulse protein is one of the reasons for lower biological value of pulses. Moreover, addition of 0.3 % methionine increases the protein efficiency ratio (PER) in most of the pulses such as chickpea, lentil and pea. As tryptophan is also a limiting amino acid in pulses, addition of tryptophan also improves PER of pulses.

The protein in pulses has low digestibility. The protein digestibility and nutritive value can be enhanced by subjecting pulses to cooking or some other form of heat treatment. Low protein value and digestibility is due to the presence of protease inhibitors and other antinutritional factors. Protein digestibility and availability of amino acids can be further improved by certain processing techniques like soaking, cooking, roasting, germination and fermentation. The

Table 15.3 Major essential amino acid content (g/100 g seed) of some important pulses^a

Pulses	Lysine	Methionine	Tryptophan	Threonine
Chickpea	1.47	0.28	0.17	0.76
Pigeon pea	1.54	0.24	0.10	0.82
Mung bean	1.69	0.28	0.20	0.78
Lentil	1.57	0.20	0.20	0.91
Pea	1.75	0.29	0.26	0.91

^aSource: IIPR (2009)

Table 15.4 Effect of processing on protein and starch digestibility of mung bean

Processing method	Protein digestibility (%)	Starch digestibility (g maltose released per 100 g meal)
Soaking (12 h)	64.2	2.8
Cooking (unsoaked seed)	63.0	12.5
Cooking (soaked seed)	70.2	13.8
Pressure cooking (unsoaked seed)	71.2	17.4
Pressure cooking (soaked seed)	83.0	20.8
Germination	78.8	8.7

Source: IIPR (2009)

effect of various processing techniques on protein and starch digestibility of pulses (mung bean) is also quite evident (Table 15.4).

15.3.3 Legumes Contain Antinutritional Proteins

Legume seed protein contains a number of components that can be classified as antinutritional or undesirable. Many of these belong to the albumin fraction which is often considered with a more favourable amino acid composition than the globulins although it is little understood to what extent these proteins are replaceable either to the seed or to the germinated plant. It is in fact due to some known cases in which appropriate mutants have been identified. So far as mutants are considered, for example, lipooxygenases in pea (*Pisum sativum*) and soybean (Forster et al. 1999), a concomitant loss of seedling vigour or seed has not been reported (Hedemann et al. 1999). Much attention is received by enzyme inhibitors as legume albumin proteins with negative attributes. It could have been inferred from

many studies of near-isogenic pea lines with different quantity of trypsin inhibitor (TI) proteins that these benefit animal performance as a derivative of null mutants (Hedemann et al. 1999). Mutants and/or transgenic 'knockouts' also could provide answers to the pertinent question of whether or not these TI proteins are essential for viability of plant or seed.

Many compounds present in pulses have been found to have antinutritional effect. These include protease inhibitors, lectins, polyphenols, saponins, phytates and lathyrogen toxins. These antinutrients either reduce the digestibility of pulses or cause toxic effect on their consumption. It is therefore necessary to eliminate these substances by processing or remove them by genetic manipulation (Domoney et al. 2002). Processing plays an important role in the nutritive value of pulses. Cooking is commonly employed for heat treatment of pulses. Moist heat is effective in destroying trypsin inhibitor activity of pulses as application of dry heat to the meal or seed is not effective in inactivating the chymotrypsin inhibitory activity (CIA) and trypsin inhibitory activity (TIA). However, water

soaking for 24 h followed by cooking for 20 min is effective in destroying both CIA and TIA. That is why germination (soaking in water and consequent physiological activities) also reduces the trypsin inhibitor activity in most of the pulses. In related processes where autoclaving is done for 15–20 min at 120 °C or extrusion cooking is undertaken at 150 °C or even microwave radiation is given at 107 °C for 30 min, these help in destroying most of the trypsin inhibitor activity of pulses and are equally effective as that of soaking cum cooking.

15.3.4 Some Other Nutritional Drawbacks

Some of the important drawbacks of dal – the finished product – include many important constituents that contribute to the human/animal nutrition. Due to the presence of oxalic acid in high proportion, black gram is not usually consumed by persons with rheumatic diseases. Therefore, those persons with uric acid in urine or rheumatism should not consume urad dal to avoid gout and arthritis. Moreover, pregnant women suffering from constipation should strictly avoid food dishes made from black gram so as to avoid stomach problems. While preparing it for medicinal purposes, since black gram may cause flatulence, other compatible products such as asafetida, pepper and ginger may be added to it so as to make its digestion better. Similarly, eating of mung beans can be avoided if one has diarrhoea or cold hands and feet. Green peas are an extremely low-fat food (with one-third gram of total fat per cup), but it supplies fat-soluble nutrients from this legume, including vitamin E and beta-carotene. In addition, lentils do have some antinutritional factors characterised by trypsin inhibitors and relatively high phytate content. Trypsin is an enzyme responsible for digestion, while phytates help reduce the bioavailability of many dietary minerals. Therefore, the phytates can also be reduced by soaking the lentils overnight in warm water (Creveieu et al. 1997).

15.3.5 Agronomic Feasibility in Increasing Protein Density

The potential to increase the micronutrient density and protein of staple foods by conventional breeding does exist (Graham and Welch 1996; Graham et al. 1999, 2001) as there exists an adequate genetic variation amongst cultivars in respect of concentrations of β -carotene, other functional carotenoids, iron, zinc and other minerals. Therefore, selection of nutritionally appropriate breeding materials is feasible. Moreover, micronutrient-density traits are stable across environments. Therefore, it is possible to combine the high-micronutrient-density trait with high yield in almost all the field crops including pulses. However, increasing protein density agronomically and any other means with increase in seed yield are rather difficult to realise as these are mostly negatively correlated. Therefore, although it is possible to improve the content of several limiting micronutrients together thereby pushing populations towards nutritional balance, yet enriching protein density with increase in seed yield in food crops is difficult.

Micronutrient supply to plants as per its need is more often limited and controlled. A lab incubation study undertaken for 140 days so as to measure potential release of Cu, Mn and Zn from city waste compost (CWC) and rock mineral flour (RMF) as compared to inorganic micronutrient fertilisers revealed that release of the micronutrients from CWC and RMF had different trends (Shivay et al. 2010a). It is because of the fact that restricted supply of micronutrients for plant growth is a common constraint worldwide. It is more so in organic farming systems where nutrient supply to crops is mostly dependent upon decomposition of applied manures and crop residues and mineralisation of native soil organic matter. The above study suggested that irrespective of the quantity of the RMF applied, about 4.6 % of Cu added as RMF was released. However, as the amount of compost added was increased, Cu release from CWC increased from 0.7 % to

3.5 %. The maximum recovery of copper (98 %) was from copper sulphate and that of zinc from zinc sulphate was 98 %. As the RMF level was increased, manganese release from RMF decreased from 114 % to 103 %, whereas the corresponding decrease in Mn release from CWC was from 14 % to -3 %. In the case of manganese sulphate, manganese recovery was maximum (100 %). Similarly, with an increase in the amount of RMF applied, zinc release from RMF increased from 5.8 % to 15.5 % with no Zn release from CWC. These results concluded that both CWC and RMF could be used for organically grown cereals to meet their Cu, Mn and Zn requirements. The results of this study have, however, general applicability in organic farming (Shivay et al. 2010a) with some limitations.

15.3.6 Mobilisation of Minerals to Seeds

Studies made on pulses indicated that there existed a differential, dynamic and selective mobilisation of minerals to seeds. A typical study was carried out for examination of the mineral nutrition of fruiting plants of *Pisum sativum* L., *Lupinus albus* L. and *Lupinus angustifolius* L. in sand cultures supplied with adequate and balanced quantity of essential nutrients. Changes in content of specific minerals in leaves, pods, seed coat and embryo were described while studying mobilisation of minerals to developing seeds of legumes (Hocking and Pate 1977). It was observed that about 60–90 % of the N, P and K were lost from the leaf, pod and seed coat during senescence, whereas 20–60 % of the Mg, Zn, Mn, Fe and Cu and less than 20% of the Na and Ca were lost during the stage. Relative to dry matter accumulation, P, N and Zn tend to increase in an organ. Other elements showed the trend more or less parallel with K, Mn, Cu, Mg and Fe or significantly behind Ca and Na as dry weight increased. Endosperm minerals were of only minor significance in embryo nutrition. Compared with 4–27 % for testa transfer to the embryo, mobilisation returns from pods were

estimated to supply 4–39 % of the seeds' accumulations of specific minerals. The study was also made for comparisons of the mineral balance of plant parts of *Lupinus* spp. with that of stem xylem sap and fruit tip phloem sap. It was supporting the view that leaves and pods were principal recipients of xylem-borne minerals and that exported from these organs via phloem was the major source of minerals to the seeds. Since embryo and endosperm differ substantially in mineral composition from phloem sap, the study suggested that during seed development selective uptake occurred from the translocation stream. Substantial differences were also noticed between species in the effectiveness of transfer of specific minerals to the seeds and in mineral composition of plant organs as the said differences between species mostly related to Ca, Na and certain trace elements (Hocking and Pate 1977).

15.4 Agronomic Approaches

The approaches involved with improved agronomy are primarily concerned with enhancing both seed yield and protein density in pulses.

15.4.1 Agronomic Approaches for Biofortification

Most of the agronomic approaches for biofortification revolve around exogenous application of nutrient at critical stage of crop growth for absorption and subsequent mobilisation to seed from aerial parts. For example, application of sulphur and other micronutrients, viz. Zn, Mn, Cu and Mo, has been found to improve crop protein production and its quality significantly in respect of micronutrients. However, low solubility of micronutrients in soils (not the low total amount) is the major reason for the widespread occurrence of their deficiency in crop plants. Therefore, agronomic fortification through application of fertiliser nutrient alone may not be effective in many situations constrained with alkalinity or calcareousness, reduced levels of soil organic matter and soil moisture. On the

other hand, large variation has been found in many crops with respect to underground structure/root characteristics and nutrient acquisition from relatively non-available pool. Therefore, genetic and agronomic fortification approaches when combined together may provide benefits both under long- and short-term strategy. Moreover, superiority of new technologies over the local practices is also shown by large-scale on-farm demonstrations (OFD) in India. Therefore, increase in pulse production to the tune of at least 13–42 % in the country (Table 15.5) is possible with adoption of these technologies. Alternatively, increase in yield also results in increase in protein substantially with relation to both quality and quantity.

In a large number of frontline demonstrations conducted across the country, it revealed that package technology (comprising improved varieties and integrated management of nutrients and pests) holds promise to increase productivity at least by 25–42 % in comparison to farmers' practice. However, improved varieties of different pulse crops caused at least 20–25 % yield advantage (Table 15.5) over the farmers' practices (Masood and Gupta 2012). Farmer-participatory testing or evaluation will also help in refining technologies, pinpoint and eliminate adoption constraints. Therefore, large-scale demonstrations on farmer's fields are considered important and crucial for bridging these yield gaps (Prahara et al. 2013) and protein density with the involvement of extension agencies.

Similarly, improved production practices or agrotechnologies like ridge furrow planting, furrow irrigated raised bed (FIRB) planting or simply raised bed planting (50–120 cm raised beds), pre-emergence application of pendimethalin at 1–1.25 kg/ha followed by early post-emergence application of recommended herbicides, foliar spray of 2 % urea or DAP, seed treatment with *Rhizobium* and P solubiliser, application of sulphur at 20 kg/ha (for legumes and oilseeds) and bio-intensive IPM modules have been recommended or advocated for pulses. These were carefully made or recommended for farmers so as to increase in yield of both seed and protein after a lot of experimentation and large-scale frontline demonstrations. Similarly,

the farmer participatory research (FPR) under complex rainfed areas needs to be developed and involve farmers more closely in on-farm research for better in situ understanding and execution of crop- and region-specific recommendation (Prahara et al. 2013; Singh et al. 2011b). This is otherwise called site-specific recommendations. These efforts may further push average productivity from around 650–750 kg/ha, which means expected production of 17.7 Mt by 2020 (Masood and Gupta 2012) with ample increases in protein density in seed (percent basis and total). Fortunately, the country has produced 19.5 Mt pulses in 2013–2014 with collective application of these farmers' friendly technologies.

Other targets for legume seed protein improvement include removal of potential allergens, removal of antinutritional factors and activities that generate undesirable flavours, improved digestibility and improved functional behaviour for processing although the molecular basis of many of these has not been sufficiently well defined to enable directed improvement, either by genetic manipulation or by breeding using genetic variation. There have also been several attempts to alter the amino acid composition of the globulins through genetic manipulation and through the use of natural variation either to directly modify globulin amino acid sequence or to express exogenous sulphur-rich proteins (Krishnan 2000). There exists a great potential of these approaches as these are still largely unrealised and underutilised but should yield seed protein with enhanced quality in the future. Therefore, studies of model legumes can play an important role in biofortification per se through improved understanding of the effects of environment and genetic background on protein quality and of the regulation of the amounts of the individual seed proteins (Casey et al. 1986, 1993).

15.4.2 Agronomic Interventions

Biofortification is a novel approach that can lead to the development of micronutrient-dense staple crops. A study involving one bacterial

Table 15.5 Seed yield advantages (%) from improved techs of pulse crops under FLDs (2006–09)

Technology	Chickpea	Pigeon pea	Lentil	Mung bean	Urd bean	Field pea
Number of demonstrations	3480	3773	1454	1640	1098	686
Full package	24.9	34.6	41.9	33.9	27.8	40.1
Improved varieties	22.4	24.7	23.6	3.3	21.9	20.0
S application	15.4	17.4	20.3	19.0	19.9	24.1
<i>Rhizobium</i> inoculation	13.4	13.5	21.0	11.1	14.2	13.2
Weed management	40.0	30.0	24.7	29.6	18.3	26.4
Integrated pests management	19.9	28.1	13.1	20.4	17.6	20.2

Source: Masood and Gupta 2012

(*Providencia* sp. PW5) and three cyanobacterial strains CW1, CW2 and CW3 (*Anabaena* sp., *Calothrix* sp. and *Anabaena* sp., respectively) for enhancing the nutritional quality of wheat grains (protein, Fe, Cu, Zn and Mn) revealed that an enhancement to the tune of 18.6 % in protein density was measured with PW5 þ N₆₀P₆₀K₆₀ as compared to fertiliser control (N₆₀P₆₀K₆₀). Inoculation of *Providencia* sp. PW5 þ N₆₀P₆₀K₆₀ also resulted in an increase of 105.3 %, 36.7 % and 150.0 % in Fe, Mn and Cu, respectively. For reducing malnutrition in developing countries, the study clearly underlines the need for the inclusion of PGPR to complement the existing biofortification strategies (Rana et al. 2012).

As cereals are staple foods in most developing countries in Asia including India, Zn fortification of cereal grains benefits all including the poorest of the poor. Moreover, zinc deficiency has emerged as the fourth important micronutrient deficiency in humans (Singh et al. 2011a; Cakmak et al. 1990). Moreover, zinc fertilisation has great relevance to India, because about half of its soils are deficient in available Zn. Therefore, fertilisation of cereals with Zn is a faster, quicker and certain way to increase zinc content in cereals. This practice needs to be put in place without further delay. In a study involving an aromatic rice production, 1.0 % Zn-enriched urea (ZnSO₄) was most effective in realising higher grain yield and economic return. This study also suggests that ZnSO₄ is the better source so as to enrich prilled urea than ZnO (zinc oxide) (Yadav et al. 2010).

There was a paucity of information on biofortification in pulses. However, both cereal

and pulses come under the gamut of food grains, and the agronomic approaches applicable for cereals are equally applicable to pulses. Moreover, many of these trials were made on rice in a rice–wheat cropping system as it covered about 24 million hectares in SE Asian countries including China, India, Pakistan, Nepal and Bangladesh, and zinc deficiency is widespread in rice–wheat belts of all these five countries. Keeping in view of low quality of nutrients in ZnSO₄ 7H₂O (zinc sulphate heptahydrate), the study suggested higher efficiency in Zn availability following coating with 2 % urea. Zinc sulphate was also a better coating material than ZnO. It was also revealed that various efficiency factors such as agronomic efficiency, apparent recovery, partial factor productivity and physiological efficiency of applied Zn decreased as the level of Zn coating was increased (Shivay et al. 2008).

Similarly, a confirmatory 2-year study showed that an increase in grain/straw yields along with zinc fortification of oat grains is feasible by Zn fertilisation. For higher grain and straw yields as well as Zn fortification of oat grains, coating oat seeds with Zn sulphate or Zn oxide before sowing was found to be the best method practised. This was followed by the next best method where deep placement of Zn fertilisers at sowing was made to garner higher nutrient use efficiency (Shivay et al. 2013). While studying protein, hardness score index, sedimentation value and Zn and Fe concentration in organically and conventionally grown wheat (both *T. aestivum* and *T. durum* cultivars), it revealed that protein density was more in conventionally grown cultivar than in organically grown one although grain hardness was not influenced by nutrient

management, while sedimentation value was higher when wheat was conventionally grown (Shivay et al. 2010b). On the other hand, Zn concentration in both the cultivars was more in organically grown (with farmyard manure) than in conventionally grown wheat. Bread wheat cultivar 'HD 2733' had significantly more Fe in grain when organically grown compared to conventionally grown variety, while the reverse was true in the case of *Triticum durum* cultivar 'PDW 215' (Shivay et al. 2010a).

Another study on zinc levels (0, 2.5, 5.0 and 7.5 kg/ha) and chickpea variety (desi 'Pusa 372' and 'Pusa 5028' and Kabuli 'Pusa 2024') revealed that 'Pusa 372' had the highest grain yield (2.13 t/ha), Zn concentrations in both grain and straw (42.9 mg/kg grain and 36.8 mg/kg straw resp.), total Zn uptake (229.0 g/ha), protein yield (480.6 kg/ha) and protein density (22.7 %) in chickpea grains. Kabuli-type 'Pusa 2024' chickpea had the lowest protein density (19.9 %). In addition, application of Zn significantly increased grain and straw yields, Zn concentrations in grain and straw and its total uptake and protein yield, protein density in grain, N uptake and net returns (Shivay et al. 2014c). It also increased the efficiency of applied fertiliser (26.6 kg grain/kg applied NPK with 7.5 kg Zn/ha and 22.3 kg grain/kg applied NPK with no Zn) (Shivay et al. 2014c).

On Fe nutrition, study also suggests that growing of aerobic rice with *Sesbania* mulch and Fe fertilisation produced higher grain and straw yield of aerobic rice. Moreover, application of three foliar sprays of 2.0 % FeSO_4 (at maximum tillering, pre-flowering and flowering) was found to increase higher Fe concentration in plant parts. This caused significant improvement in uptake in grain as well as in straw of aerobic rice (Yadav et al. 2013). Moreover, Fe application increased Fe concentration to the extent of 5.4–19.9 % and 5.8–13.3 % in grain and straw of aerobic rice, respectively. Besides that, methods of rice production also increased Fe concentration from 5 % to 11 % and from 4.1 % to 6.3 % over bare soil (no mulch) in grain and straw, respectively (Yadav et al. 2013).

From the viewpoint of biofortification, foliar application is better and requires lesser amount of Fe and Zn fertilisers than their soil application. When cultivars or GM crops with grains denser in Fe and Zn are developed, adequate Fe and Zn fertilisation will be necessary. *The genetic and agronomic approaches are therefore complementary to each other and should progress in tandem.* However, a better understanding of the various reactions that micromineral nutrients undergo in soil and the mechanisms involved in their absorption and translocation in plants, especially to grains is required. This calls for adequate funding of agronomy, soil science and plant physiology departments in agricultural institutes in developing as well as in developed countries (Prasad et al. 2014).

Ferti-fortification offers a rapid solution for increasing Zn concentration in grain and straw. For example, green manuring and Zn-coated fertilisers increased nutrient concentration and their uptake in grain and straw. Foliar fertilisation of 0.2 % zinc sulphate led to higher Zn concentration in rice, while Zn-coated urea (ZCU) as monohydrated ZnSO_4 measured highest total Zn uptake (Pooniya and Shivay 2015). Similarly, residue recycling of summer green manuring crops and Zn-coated urea significantly enhanced soil microbial activities resulting in better nutrient turnover in addition to improved long-term productivity of soil and plant system (Pooniya et al. 2012; Singh et al. 2011a).

Studies were also carried out on nutrient accumulation through residues and its effect on nutrient fortification. While studying the effect of summer legumes grown as a sandwich crop in rice–wheat cropping system, it showed that cowpea residues accumulated significantly higher amount of N (97.7 and 4.56 kg/ha in shoot and root, respectively) over that in mung bean. Nutrients like, P, K, Mn and Zn accumulation in cowpea residues were significantly higher than those in mung bean although system productivity of aromatic hybrid rice–wheat–summer legume was highest with mung bean compared to cowpea and summer fallow (Jat et al. 2012). A field experiment to study the effect of genotypic

variation and K nutrition on K accumulation and utilisation efficiency in barley (*Hordeum vulgare* L.) under rainfed conditions with low available soil potassium also revealed that successive increases in K nutrition had a significant effect on the dry matter and K accumulation either in the total or in various plant parts of barley at the tillering, stem elongation, heading and maturity growth stages (Shivay et al. 2003). K nutrition also led to significantly higher grain yield with each unit K application than without K application. Amongst 11 genotypes of barley tested, genotype 98–6 had the highest grain yield, and the K use efficiency of this genotype was 10.4 kg grain per kg K applied (Shivay et al. 2003). This shows the potential of existing genotypes for further use in breeding programmes.

Besides this, type of fertiliser materials also affect the nutrient uptake and its use efficiency. In an experiment carried out to study the effect of coating prilled urea with eco-friendly neem (*Azadirachta indica* A. Juss.) formulations for improving the efficiency of nitrogen use in hybrid rice (NDHR-3), it revealed that increasing levels of nitrogen significantly increased grain and straw yields and N uptake with a significant decline in agronomic nitrogen use efficiency (NUE). However, *Pusa* neem golden urea proved to be significantly superior to other nitrogen sources with regard to panicle length, grain yield, N uptake, NUE, apparent N recovery and (lesser) environmental hazards with reduced N loss (Singh and Shivay 2003).

In another study on the residual effect of N sources, S and B levels applied to sunflower on productivity, nutrient concentrations and their uptake by the succeeding crop of mung bean in sunflower–mung bean cropping system, it revealed that remarkable higher mung bean seed yield (961 kg/ha) was realised with 50 kg/ha S applied to preceding sunflower crop. Due to the residual effects of nutrients applied to the preceding sunflower crop, the concentrations and uptake of N, S and B were also greater in the succeeding mung bean crop (Shekhawat and Shivay 2012).

Similarly, a study on sulphur nutrition indicated that 40 kg/ha bentonite sulphur with 90 % pellets (or 20 kg/ha bentonite sulphur with 90 % pellets applied at 15 DAT + 20 kg/

ha bentonite sulphur with 90 % pellets at 45 days after transplanting or DAT) significantly increased growth, yield attributes, productivity, sulphur and nitrogen concentrations in grain and straw and also their uptake in an aromatic rice in north-western plain zone of India (Shivay et al. 2014a). In a separate study, it was also observed that Zn application not only increased grain yield, net profit and B: C ratio but also protein concentration/density in grain and nitrogen uptake by chickpea. Three sprays of zinc sulphate heptahydrate (ZSHH) or Zn-EDTA (one each at maximum vegetative growth, flowering and grain filling stage) also yielded the best results (Shivay et al. 2014b).

Addition of organic matter maintains optimum soil structure, enhances water holding capacity and exchangeable ions and reduces leaching of nutrients and toxicities (Choudhary et al. 2008). Crop residue, green manures, composts, animal manures, growing cover crops, reducing tillage and avoiding burning of crop residues can significantly lead to improved plant growth and acquisition of micronutrients in final output (Fageria et al. 2002; Paul et al. 2014; Pooniya and Shivay 2015). Although accumulation of micronutrients varies amongst and within plant species yet, it is more or less attributed to genetics, environment, physiological or biochemical mechanism, agronomic management practices and pest and diseases.

Therefore, the application of nutrients such as N, P, K, S, Zn, Mo and Fe is known to enhance protein density in seeds of legumes. However, the efficiency of such enhancement depends upon many underlying conditions that have a bearing on increasing the level of different amino acid component of protein. For example, the application of P, Mo and N has been shown to increase the level of methionine and that of S-containing fertilisers increase the cystine content of chickpea protein. It is in fact low methionine in pulse protein that is one of the reasons for lower biological value of pulses. It is in this context, studies shows that addition of 0.3 % methionine increases the protein efficiency ratio (PER) in most of the pulses including chickpea, lentil and pea. Similarly, addition of tryptophan – another limiting amino acid in pulses – also improves PER of pulses.

15.4.3 Cost-Effectiveness of the Biofortification Strategy

The major cost components for biofortification relate to research and development needed to develop biofortified genotypes and its implementation. Since an internationally recognised agricultural research system is in place to develop modern varieties of staple food crops, the costs towards research are essentially the incremental costs for increasing or enhancing micronutrient density in food crops. These research costs are likely to be the single largest cost component of biofortification and are of course a one-time investment which is incurred at the start of this. It is also estimated that costs associated with plant breeding fall around an average of about \$ 0.4 million/year/crop over a period of a decade globally. Therefore, once biofortified genotypes or varieties are developed with recurring costs on in-house trials and local adaptation research, thereafter the costs are minimal as it involves routine maintenance breeding costs to keep the trait stable. Moreover, where adequate and proper systems for dissemination of modern genotypes are in place (such as in South Asia), implementation costs are nil or negligible. Where such systems are underdeveloped or developing (such as in parts of sub-Saharan Africa), additional costs are incurred in establishing seed multiplication and delivery systems and creating both markets and consumer demand. In addition, the success of biofortification products available to consumers should be viable and continue in a sustainable manner. Therefore, biofortified crops should necessarily be incorporated into existing marketing chains and new market opportunities that are developed in due course of time.

Therefore, centres should facilitate in the dissemination of biofortified varieties to achieve this and create the demand for these varieties by linking producers and consumers with the strategy for product and market development. The strategy focuses on engaging and developing the capacity of users (processors/retailers, consumers and producers) and diffusers (institutions and people in organisations that

interact directly with enablers to move a technology to implementation) to adopt the new technology. At the same time, they should transfer knowledge and create awareness of the new technology amongst enablers. Moreover, the people working in organisations and institutions who can create a favourable environment for the adoption, dissemination and increased consumption of biofortified crop varieties are important and crucial in developing certain leadership to spread the requisite message to people all around. Therefore, these considerations play vital for rapid spread of biofortification products in the masses which involve all the stakeholders in food chain.

15.4.4 HarvestPlus Biofortification Program: A Case study

Biofortification approach requires that agricultural research make direct linkages with the human health and nutrition sectors (Bouis 2003) that require an inter- or multidisciplinary research approach, an innovative funding strategy to support the research, adequate willingness amongst scientists and, of course, ultimate dissemination of the biofortified products or seeds. For example, in the *HarvestPlus Biofortification Program*, the major functional activity includes plant breeding at the NARES (National Agricultural Research and Extension Services) and CGIAR centres for maize, rice, sweet potato, common bean, cassava and wheat to develop varieties with the best nutritional and agronomic traits in these crops. The other activities include application of novel advances in biotechnology, genomics, genetics and molecular biology to identify and understand plant biosynthetic genes and pathways of nutritional importance, including those for nutrient absorption enhancers and inhibitors, screening of promising lines for micronutrient bioavailability, efficacy studies involving human subjects to evaluate nutritional impact of the most promising lines intended for release and, above all, food science and human nutrition research to measure the retention of

nutrients in processing and cooking. In addition, although breeding may be a viable option, applying the above knowledge in marker-assisted selection for conventional breeding of crops and in the initial development (but not release) of transgenic lines is also very important. Moreover, the programme impact and policy research intends to target the following areas of the subject (biofortification):

1. Understand social and economic factors that determine the dietary quality of the poor and their micronutrient status, as well as policy advocacy based on that research
2. Target regions or states where biofortification will have the greatest social benefit and proven impact
3. Improving seed dissemination systems, demand creation, market and product development by reaching and, of course, fully engaging the end users
4. To provide support to internal project collaborators and external audiences (including donors, the academic and development communities, public officials and the general media) through coordinated communication activities

15.4.5 Biofortification for the Future

In addition to the traditional objectives of disease resistance, yield, drought tolerance etc. and based on micronutrient deficiency rates, there is compelling evidence that biofortification can be a key objective for plant breeders and agronomists in the future. Scientific evidences also suggest that biofortification is inevitable as it is technically feasible. Predictive cost–benefit analyses have also shown that biofortification is useful for controlling micronutrient deficiencies. The challenge is always there to get the desired consumer acceptance for biofortified crops, thereby increasing the intake of the target nutrients. Improved agronomy for seed protein enrichment could have biological impact. However, it is without compromising agronomic

traits/yield as it has been amply demonstrated for food crops including pulses. On a positive note, this can become a reality with the advent of good seed supply systems, the development of markets and products and future demand creation. Therefore, from a given food crop(s), every effort is to be targeted at to yield more with proven quality and nutritional considerations.

15.5 Conclusion

The micronutrient deficiency is probably the main cause for hidden hunger. Biofortification or enriching the nutrition contribution of staple food crops through agronomic or plant breeding or any other appropriate approach is in fact the option for solving food-related malnutrition. Agronomic interventions for biofortification of food crops, food fortification/ supplementation through micronutrients and dietary diversification are very much required on priority basis. Improved agronomy has a significant role in productivity changes. It has a minor role in enhancing protein density as both are negatively correlated and protein is genetically controlled. However, total seed protein density is increased following productivity increases. Improved agronomy has a major role in micronutrient (Zn, S and Fe) biofortification which could possibly reduce protein and micronutrient malnutrition.

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