

GENETIC IMPROVEMENT OF MUNGBEAN (*VIGNA RADIATA* L): NECESSITY TO INCREASE THE LEVELS OF THE MICRONUTRIENTS IRON AND ZINC: A REVIEW

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ABSTRACT: Mungbean [*Vigna radiata* (L.) R.Wilczek] is an important grain legume crop, grown mainly in South Asian countries, which offers many nutritional and economic benefits. Plant breeding and genetic engineering, have a great potential to increase productivity in general and also to increase nutritional values in different plant parts such as grains, roots and tubers. Mungbean is one of the crops that can be targeted for improvement of micronutrient content. It is consumed in large parts of the developing world, especially in Asia. Increasing the content of micronutrients is only useful if the bioavailability of these micronutrients is good, this depends among others on the concentration of inhibitors such as phytic acids (PA) and phenol compounds. This review advocates the necessity of genetic improvement of mungbean, emphasizing on increasing the levels of micronutrients, particularly iron and zinc through a multi-disciplinary team approach including: genetic improvement, bioavailability and social awareness. The monotonous consumption of cereals in vegetarian populations leads to malnutrition and hence, overall deterioration in the health status of many people in the developing world. Therefore, combining breeding with good processing methods and making people aware about improved varieties available on the market further helps in improving their health status.

KEYWORDS: Genetic improvement, phytic acid, iron, mungbean, malnutrition, multidiscipline, zinc.

INTRODUCTION

The global population, which reached 7 billion in 2012, is estimated to increase to 8.3 billion in 2020; the developing world contributes most to this increase (Miflin, 2000). Plant breeding effectively contributed in preventing massive starvation by increasing the production of staple food such as rice and wheat during the green revolution in the 1960's. However, this resulted in the negligence of breeding efforts in a large number of other crops which are nutritionally important. Monotonous consumption of cereals, in absence of, for instance animal tissue and pulses, leads to deterioration in the overall nutritional status (Zimmermann and Hurrell, 2002). According to the World Health Organization (WHO, 2008), more than 2 billion people worldwide, including women, children, the middle-aged, and the elderly are suffering from vitamin and mineral deficiencies, primarily iodine, iron, vitamin A and zinc (Allen et al., 2006). For instance, two billion people - over 30 percent of the world's population - are anemic, mainly due to iron deficiency and this is still rising in an alarming rate among poor women and children below the age of 5 (World Hunger Facts, 2011). Iron deficiency also leads to anemia that is already affecting over half a billion people

worldwide. Zn is also an important trace element and it is estimated that over 30% of the world population has a Zn deficiency. Zn is required for functioning of immune system, protein synthesis, cell reproduction and wound healing; furthermore it plays a major role in fertility and conception. During the 20th century, conventional plant breeding resulted in increased yields and harvest stability. Major research objectives in the past few decades have concentrated on increasing resistance to environmental stresses, pests and pathogens (Borlaug, 2000; Zimmermann and Hurrell, 2002). But simply providing more food will not completely solve the problem of incomplete diets, therefore focus should be set on the quality and diversity of crops (e.g., Munger, 1988; Quebedeaux and Bliss, 1988; Quebedeaux and Eisa, 1990). Pulses are nutritionally as well as economically very important to vegetarians and poor people worldwide and efforts are being made for the development of high yielding varieties of pulses such as mungbeans (Khattak et al., 2006). Despite, the nutritional importance of mungbean [*Vigna radiata* (L.) R.Wilczek] limited research has been conducted to further improve its nutritional properties. One solution to micronutrient deficiency in the vegetarian

diet could be higher consumption of pulses with enhanced levels of Fe and Zn.

The genus *Vigna* is pantropical and includes about 170 species, 120 from Africa, 22 from the Indian continent and Southeast Asia, and the rest from other parts of the world. Mungbean, also known as green gram, belongs to the subgenus *Ceratotropis* and is an important crop among legumes. Mungbean is diploid with $2n=22$ and it has a small genome size of 0.60 pg/1C (579 Mbp) (Somta and Srinives, 2007). In India, mungbean occupies about 3 million ha, with a

production of 1.42 million tons (Singh and Ahlawat, 2005; Table 1). The total production of dry beans (including *Phaseolus* and *Vigna* spp.) was stagnant over the past 20 years except in Nepal, Pakistan and Myanmar where production increased. In India, the consumption pattern of mungbean depends strongly on income and price. Forty percent of all households consume mungbeans and on average, households consume 110 g per month. About 15% of the money spend to buy pulses is for buying mungbeans (Vijayalakshmi *et al.*, 2003).

Table 1: Area, yield and average growth rate of dry beans (including *Phaseolus* and *Vigna* spp.) in South Asian countries (2001)

| Countries | Area (1000 ha) | Yield (kg/ha) | Annual growth rates | | |
|------------|----------------|---------------|---------------------|-----------|----------------|
| | | | Area (%) | Yield (%) | Production (%) |
| Bangladesh | 84 | 680.4 | -2.4 | 0.4 | -2.0 |
| India | 7100 | 362.0 | -1.1 | 0.6 | -0.5 |
| Nepal | 39 | 693.0 | 3.1 | 0.6 | 3.7 |
| Pakistan | 219 | 476.7 | 2.4 | -0.4 | 2.0 |
| Sri Lanka | 27 | 512.2 | -1.1 | -2.0 | -3.1 |
| South Asia | 7,469 | 317.2 | -1.0 | 0.6 | -0.4 |

[FAOSTAT, 2002](#)

Growing mungbean improves the soil because it fixes atmospheric nitrogen with the help of *Rhizobium*. This partially replaces the use of inorganic fertilizers (Safdar *et al.*, 2005). The benefits of legumes to soil nitrogen fertility have been reported for various cropping systems (Ahmad *et al.*, 2001). The economic impact of mungbean as a nitrogen fixer was described by Arif and Malik (2009). They showed that the highest gross and net benefits were achieved by intercropping mungbean and groundnut.

As mungbean offers many nutritional and economic benefits and is a good source of protein, breeding efforts should concentrate on enhancing micronutrient levels (Fe and Zn). Therefore this review focuses on breeding crops

particularly mungbean, which relatively can prove helpful in solving the problem of iron and zinc deficiency.

NUTRITIONAL IMPORTANCE OF MICRONUTRIENTS (FE AND ZN) AND PROTEINS

Fe and Zn are essential nutrients for normal functioning of humans (Table 2). Their concentrations can be too low due to insufficient uptake or insufficient bioavailability. Bioavailability can be enhanced by specific promoters like ascorbate (vitamin C), β -carotene (pro-vitamin A), protein cysteine and various organic and amino acids (Table 3).

Table 2: Some of the essential functions of micronutrients (Fe & Zn) in plants, animals and humans

| Elements | Prevalence of Deficiency | Plant | Animal and Human |
|----------|--------------------------|---|---|
| Zinc | High in developing world | i. Constituent of several enzymes | i. Functions as anti-oxidant and is involved in biochemical reactions |
| | | ii. Plays essential role in DNA transcription | ii. Acts as catalyst for the enzymes involved in cell growth. It is important in metabolism of Vitamin A and collagen |
| Zinc | High in developing world | iii. Maintains the integrity of membranes and is involved in pollen formation | iii. Essential for DNA function and involved in regulation of gene transcription |
| | | iv. Regulating auxin synthesis and thus preventing diseases like "little leaf". | iv. Zn is essential in protein synthesis, cell division and growth. |
| | | v. As in animals, Zn-metalloenzymes, and Zn fingers play a role in plants | v. Reproduction and neurological function. |
| | | | vi. Zn-metalloenzymes and Zn-fingers play a role in folding of proteins |
| Iron | 2 billion | i. Cytochromes and metalloenzymes. | i. Fe is a constituent of hemoglobin and myoglobin which are essential components for storing and diffusing oxygen |
| | | ii. Necessary in photosynthesis, | ii. Important for neurological functioning and development |
| | | iii. Involved in nitrogen metabolism as it is part of enzyme nitrogenase | |
| | | iv. Iron is also part of the enzyme leg- | |

| | |
|---|--|
| hemoglobin (role in nitrogen fixation) | iii. Involved in redox reaction and thus responsible for cellular growth |
| v. Prevents plants from severe physiological disorders like necrosis and chlorosis | |
| vi. Heme is essential component of cytochrome protein and thus mediates redox reactions | |

Source: [Srivastava and Gupta \(1996\)](#)

Table 3: Inhibitors and enhancers of iron and zinc bioavailability

| Element | RDA | RNI | UL | SUL | Inhibitors | Enhancer |
|---------|----------|------|------|------|--|--|
| Fe (mg) | 8.0-18.0 | 11.4 | 45.0 | 17.0 | Phytate, tannins, oxalate, fiber, hemagglutinins | Phytoferritin, riboflavin, ascorbate, b-carotene, cysteine, histidine, lysine, fumarate, malate, citrate |
| Zn (mg) | 8.0-11.0 | 9.5 | 40.0 | 25.0 | Phytate, tannins, fiber, hemagglutinins | Phytoferritin, riboflavin, ascorbate, b-carotene, cysteine, histidine, lysine, fumarate, malate, citrate |

Source: [White and Broadley \(2005\)](#). The US recommended daily allowances (RDA, or adequate intakes), the UK guidance daily reference nutrient intakes (RNI), the US tolerable upper intake levels (UL), and the UK guidance safe upper levels (SUL) for adults

Ironically, the spread of micronutrient deficiency is related to the spread of high-yielding rice, wheat and maize varieties during the first phase of green revolution. These varieties are generally low in micronutrients, but also have displaced a variety of crops grown previously, such as

pulses, vegetables and fruits which used to prevent a lack of micronutrients ([Rooszendaal, 1996](#)). Micronutrient malnutrition affects primarily the underprivileged population (Table 4; [Buyckx, 1993](#); [Ramalingaswami, 1995](#)).

Table 4: Effect of micronutrient deficiency on human health at different stages of life

| Age Group/ Stage | Effect over health |
|------------------|---|
| <5 | i. High mortality rate ii. Low birth weight iii. Impaired mental development |
| 5-11 | i. Growth is stunted ii. Reduced mental growth iii. Less active & susceptible to diseases iv. Delayed sexual development |
| 12-17 | i. Physically and mentally less active ii. Delayed puberty in adolescents iii. May become anemic iv. Poor immune system |
| 18-50 | i. Anemic, sometimes reach to pernicious anemia stage ii. Give birth to low weight babies iii. Depression |
| >50 | i. Retinal detachment ii. Susceptible to many diseases iii. Diminished wound healing |

Source: Seres, ACC/SCN, 2000

2.1. Iron (Fe)

In humans the uptake and absorption of iron is complex and depends on many factors. There are two forms of Fe in food: non-heme Fe and heme Fe. The heme Fe is mainly present in animal tissue, has a high bioavailability and is weakly influenced by other factors present in diets while the non-heme Fe comes from vegetables and legumes and its absorption depends on various dietary components ([Lopez and Martos, 2004](#)). Many people in poor regions of the world consume low amounts of animal tissue and rely almost entirely on non-heme Fe.

Breeding can play a vital role in lowering iron deficiency in the world by increasing the concentration of these micronutrients in edible tissues. For example, a rice variety has been developed with four times higher iron content than any normal variety ([Haas et al., 2005](#)). A similar effort should be made in developing high-level micronutrient legumes and seeds ([Pennington and Young, 1990](#)). The combination with high protein content is essential in fighting against protein energy malnutrition (PEM) and micronutrient malnutrition.

2.2. Zinc (Zn)

Zinc is the second most abundant element in organisms. It stabilizes the structure of the membranes and cellular components (<http://www.ctds.info/zinc1.html>) and it is an essential component of a large number of Zn-dependent enzymes. It also plays a major role in gene expression ([Sandstrom, 1997](#)). Deficiency of Zn in human reduces growth, sexual maturity and weakens the immune defense system ([Prasad, 1996](#)). About 70% of Zn in the US diet is provided by animal products ([Sanstead, 1995](#)). However, in many parts of the developing world this is not the case, here most Zn is provided by cereals and legume seeds.

However, these plants also have high concentrations of phytic acid, which is a potent inhibitor of Zn absorption ([Navert *et al.*, 1985](#)). Marginal Zn deficiency (10-12 mg/ day) in humans may be wide spread, but remains unnoticed because there is no established clinical method for determining marginal Zn deficiency in humans ([Endre *et al.*, 1990](#); [Larsen, 1997](#); [Shrimpton, 1993](#); [Welch and Graham, 2002](#)).

Zn deficiency in plants can be caused by Zn deficiency in soils ([Cakmak, 2002](#); [Nube and Voortman, 2006](#)) and about 50% of the agricultural soil in India is Zn deficient ([Gupta, 2005](#)). Zn deficiency became more prevalent during the green revolution which involved heavy use of soil for the cultivation of crops such as rice. The lowest Zn concentrations in India are in the soils of Haryana and Madhya Pradesh ([Gupta, 2005](#)) and a correlation was observed between low soil Zn content and the occurrence of human Zn deficiency ([Pathak *et al.*, 2003](#)). Low Zn level can be overcome by adding Zn to the soil. It has been shown that Zn increase in the soil leads to Zn increase in grains ([Rengel *et al.*, 1999](#)). However, whether adding Zn in soils can lead to enough increase in levels of Zn in the plants to prevent human Zn deficiency is still questionable ([Cakmak, 2002](#); [Welch, 2002](#); [Slaton, 2005](#)).

MICRONUTRIENTS ASSIMILATION MECHANISMS IN PLANTS

Plants get their minerals from the soil. The process of micronutrient uptake, accumulation and their regulation is a dynamic process that should avoid deficiency or toxicity in the plant. This process is dependent on various factors like transporters within the plant, genotype of the plant and the environment (soil). To start a successful breeding program there is the necessity to understand physiological mechanisms of micronutrient absorption, translocation, remobilization in leaves and re-translocation into seeds.

About 80% of the Fe is stored in chloroplasts and this accumulation is developmentally controlled. In roots some essential proteins and enzymes like leg-hemoglobin and nitrogenase are required for iron accumulation ([Kaiser *et al.*, 2003](#)). Plants can also uptake elements in gaseous or ions forms through their stomata and cuticles. Cations like Fe^{2+} can be absorbed by the plants in gaseous forms with the help of ectodesmeta i.e. non-plasmic channels in the leaves ([Prasad, 2007](#)).

3.1. Zinc accumulation in plants

Two mechanisms are functionally active in heavy metal uptake (i) energy independent non-metabolic uptake and (ii) energy dependent metabolic uptake. In the first mechanism Zn is taken across the plasma membrane of root cells as Zn^{2+} or as a Zn-phytosiderophore complex while in the second mechanism Zn uptake takes place through calcium (Ca^{2+}) channels using energy (ATP).

Along with these two mechanisms several transporter gene families play a role in Zn uptake and accumulation. One of the most important is the ZIP family ([Palmgren *et al.*, 2008](#)). Other transporter families involved in Zn accumulation and transport include P-type ([Monchy *et al.*, 2007](#)), ATPase-HMA (ATP dependent High metal accumulator), MATE (multi drug and toxic compound extrusion) ([Durrett *et al.*, 2007](#)), OPT (oligo-peptide transporter). Besides these gene families, cation diffusion facilitators (CDFs) or MTPs (metal transporter proteins) are involved in transport of Zn^{2+} from cytoplasm to the vacuoles and the endoplasmic reticulum. MTP1 is highly expressed in both roots and shoots ([Verbruggen *et al.*, 2009](#)). The CaCA (Ca^{2+} /cation antiporter) super-family is thought to play a role in Zn^{2+} vacuolar storage via Zn^{2+}/H^{+} exchange ([Shaul *et al.*, 1999](#)). ZIPs, MTPs, HMAs, CaCA, APCS had high expression levels in those plants which hyper accumulate Zn^{2+} ([White *et al.*, 2009](#)) and can be targets for breeding.

3.2. Iron accumulation in plants

Iron, which is widely distributed in the lithosphere, is taken up by plants in two different ways: mechanism I (non-graminaceous species) and mechanism II (cereals and grasses). In mechanism I, the Fe^{3+} present in the soil is chelated by phenolic compounds secreted by the roots; this reduces Fe^{3+} to Fe^{2+} with the enzyme, ferric reductase. Further, IRTs (iron regulated transporters) help in Fe^{2+} uptake and IRT1 is the major root plasma membrane transporter. Iron uptake is regulated by signals from the shoot

when there is an iron deficiency. The nature of these signals is still unknown (Vert *et al.*, 2003). Once iron is taken up by roots using active roots transporters, it is translocated via the xylem sap to aerial parts (Elizabeth and Jean, 2004). The flow of iron from source to acceptor tissues via phloem sap and the sub-cellular distribution is poorly understood and documented.

Seed is a store-house of food and nutrients and for obtaining high micronutrient levels it is important to understand the overall signalling networks involved in accumulation of these

metals in the various organs and at different stages of development (Curie and Brieat, 2003).

IRON AND ZINC BIOAVAILABILITY AND BIOFORTIFICATION

Bioavailability of Fe & Zn in a vegetarian diet

Generally the vegetarian diet contains equal amounts of iron as a non-vegetarian diet but in the vegetarian diet the micronutrients have a lower bio-availability (Hunt, 2003). The chemical form (heme and non-heme) of iron is an important factor affecting the iron availability of vegetarian diets (Table 5).

Table 5: Bioavailability of iron from different food sources

| Diet | Iron forms | Bioavailability | Reference |
|-----------------|---|-----------------|--|
| Red meat supply | 10-12% of total iron is of heme form | 15-40% | Hunt and Roughead, 1999 |
| Fish & Poultry | Heme concentration lower than non-vegetarian diet | 1-15% | Monsen <i>et al.</i>, 1978 |
| Vegetarian diet | Non-heme | - | Roughead and Hunt, 2000 |

As legumes have good concentrations of Fe and Zn, their inclusion in diets is desirable (Table 6). But some diets alter or enhance the bioavailability of micronutrients because of anti-nutrients and promoters. Plant diets are high in phytates (6-phosphoinositol) and polyphenols, such as tannins which inhibit absorption of iron and zinc (Holm, 2002). Phytic acid binds essential micronutrients and also forms complexes with micronutrients of other foods during intestinal digestion. These complexes are not absorbed and result in low bioavailability. The concentration of these anti-nutrients varies greatly between varieties and is usually high in seeds and grains. Low phytate mutants (*lpa*) are known in major crops and legumes like rice (*O. sativa* L.), wheat (*T. aestivum* L.), common bean (*P. vulgaris* L.) and soybean (*Glycine max* L.) (Thavarajah *et al.*, 2010; Campion *et al.*, 2009; Guttieri *et al.*, 2006; White and Broadley, 2005). Lower levels of anti-nutrients indirectly results in a higher bioavailability.

Zinc bioavailability in a vegetarian diet is lower. Food, rich in zinc and protein, like legumes,

whole grains etc. (Sandstrom *et al.*, 1980) are needed despite their high phytate content. Overall there is a positive zinc balance (Johnson and Walker, 1992; Hunt, 2003).

4.1. Biofortification using plant breeding and biotechnology

In order to increase the concentration of micronutrients in edible tissue like seed, two strategies can be employed i.e., application of mineral fertilization and improvement in mobilization of these minerals in the soil. Micronutrients can be added to the soil or sprayed on the leaves. For example, although there is a fair amount of Fe in soils little is available and Zn, Fe and Mg compete for uptake (Neue *et al.*, 1998; Lind *et al.*, 2003; Berger *et al.*, 2006). Therefore it is good to use Fe-chelates and Zn-chelates as soil fertilizers. Especially in the case of high concentrations of phosphate in soils because they strongly reduce Zn availability (Marschner, 1995).

Table 6: Variation in concentration of micronutrients

| Legume | Fe (mg kg ⁻¹) (max-min)* | Zn (mg kg ⁻¹) (max-min)* | References |
|-----------------------------------|---|---|--|
| Bean (<i>P. vulgaris</i>) | 35-92 | 21-59 | Islam <i>et al.</i>, 2002 |
| Pea (<i>P. sativum</i>) | 23-105 | 16-107 | Grusak and Cakmak, 2005 |
| Soybean (<i>G. max</i>) | - | 59-83 | Raboy <i>et al.</i>, 1984 |
| Chickpea (<i>C. arietinum</i>) | 24-41 | 35-60 | Haq <i>et al.</i>, 2007 |
| Mungbean (<i>V. radiata</i>) | 15-92 | 15-38 | This thesis |
| Lentils (<i>L. culinaris</i> L.) | 114 | 65 | Thavarajah <i>et al.</i>, 2010 |

*range of concentration from minimum to maximum

CURRENT STATUS OF MUNGBEAN RESEARCH

The cereal-cereal based cropping system pushed mungbean production to more marginal environments. Despite mungbean's productivity and nutritional benefits, its production was either stagnant or decreasing. Disadvantages of

growing mungbeans are lack of good quality seed, unfamiliarity with good management practices and susceptibility to various diseases especially mungbean yellow mosaic virus (MYMV). Furthermore growing mungbeans is labour-intensive and low-yielding. But

nowadays the potential of mungbean to supply protein and to provide farmers with an income-generating opportunity are recognized (Shanmugasundaram, 2006). The efforts are now aimed at solving the major constraints limiting mungbean production and also to improve its nutritional composition.

In several South Asian countries like India, Sri Lanka, Bangladesh and Pakistan research on mungbean is being conducted (Vijayalakshmi *et al.*, 2003). Recently, attention has been focused on developing nutritionally enriched varieties. In order to identify beneficial alleles of relevant genes in mungbean, genetic diversity within the available germplasm needs to be studied. Genetic diversity studies in mungbean have been carried out by Santalla *et al.*, 1998; Lakhanpaul *et al.*, 2000; Cheng and Yang, 2001; Afzal and Shamugasudaram, 2004 and Betal *et al.*, 2004. High levels of polymorphisms were found by Chattopadhyay *et al.*, 2005 using ISSRs and by Dieu and Le, 2005 using RAPDs. Microsatellites (Gwag *et al.*, 2006) gave similar results. SSRs developed in other pulse crops like common beans and cowpea can be used in mungbean. Similarly, RFLP probes from common beans, cowpea and soybeans have been used in mungbean research (Somta and Srinives, 2007). Comparative genomics between *V. radiata* with *V. unguiculata* and *P. vulgaris* showed that there were conserved blocks of considerable size with some genes for important traits (Fatokun *et al.*, 1993; Menacio-Hautea *et al.*, 1993). Six molecular linkage maps of mungbean using F₂ or recombinant inbred lines (RILs) were published. These maps differ in length (737.9-1570 cM), number of markers (102-255 markers) and number of linkage groups (Somta and Srinives, 2007). In mungbean QTLs for major traits such as insect and disease resistance (Lambrides *et al.*, 1999) and seed-related characters (Humphry *et al.*, 2005) have been identified.

CURRENT STATUS OF BREEDING EFFORTS FOR INCREASING MICRONUTRIENT CONTENT

Breeding for improved mineral content is quite complicated because the effects of individual loci are small and difficult to identify (Maldonado *et al.*, 2003). It was shown in common beans that wild varieties can have a higher ability to accumulate iron (71-280 mg kg⁻¹ compared to a mean Fe content of 100 mg kg⁻¹ in cultivated varieties) and Zn (24-38mg kg⁻¹ compared to 17 mg kg⁻¹) (Maldonado *et al.*, 2000). Maldonado *et al.*, (2003) did a QTL mapping study and identified QTLs for seed mass, Fe, Zn, and Ca concentration. They identified two unlinked QTLs for iron content and one for Zn content. The two QTLs associated with Fe content

explained ~ 25% of variance whereas Zn QTL explained 15% of the variance. Gelin *et al.*, (2007) found a QTL in a RIL population for Zn concentration and a marker assisted breeding program resulted in an increase in Zn content by 11.7% resp. 15.3%. Zn content was not associated with iron content and higher levels of Zn didn't result in lower levels of Fe. Recent studies show that higher Zn concentrations in the seed can be caused by only of a single dominant gene (Singh and Westermann, 2002; Cichy *et al.*, 2005). Improving seed Zn accumulation through plant breeding efforts should be possible. Gelin *et al.*, (2007) described a single QTL for seed Zn concentration which explained 17.8% of the variability. They developed a recombinant inbred population and found the QTL responsible for improved Zn accumulation in bean to be located on linkage group 9. In common bean the genetic variability can result in an 80% increase of the iron content and 50% of the Zn content. No QTLs for iron and zinc content are described yet in mungbean. Identification of any molecular marker for high iron and zinc will allow marker assisted selection (MAS) for the improvement of these important micronutrients in mungbean.

Beebe *et al.*, (1999) found seven QTLs for iron content and QTLs for Zn content on almost all chromosomes in common beans. Researchers at CIAT, found a highly significant positive correlation of 0.52 between Fe and Zn concentration in 1000 accessions (Welch and Graham, 2004). This positive correlation was confirmed in ninety other genotypes (Tryphon and Masolla, 2010). Thus, genetic factors for increasing Fe and Zn might be pleiotropic or co-segregating. To understand the mode of action of genes involved in the mineral uptake and cellular import and export and intracellular sequestration these genes have to be identified and to be studied thoroughly. With enough knowledge about the involved genes genetic modification might also play a role in the future in increasing micronutrient content in edible parts of the mungbean crop (Ghandilyan *et al.*, 2006) for instance by over-expressing of some of the key genes.

For both Fe and Zn seed concentrations in beans; there were significant location and location x genotype effects, demonstrating that environments influence the concentrations of Fe and Zn (Gregorio, 2001; Beebe *et al.*, 1999).

6.1. Status of Mungbeans: In developed world

In many parts of the developed world, mungbean is used in sprouted form as a salad vegetable or for cooking purposes. The area of mungbean production in the developed world is

increasing day by day. Presently in USA it is 50,000 ha and in Australia about 40,000 ha (Weinberger, 2003). Extensive research is required on quality traits such as sprouting quality and protein quantity. Mungbean research was initiated in USA in early 1990s and later on more molecular and field work was started in countries like Canada, India, Thailand, Australia, Japan, Taiwan etc. In countries like Canada, there has been a constant interest in developing mungbean as a potential pulse crop. Park and Anderson, (1977) developed and evaluated mungbean cultivars under Canadian conditions.

6.2. A multi-disciplinary team approach

Most of the research is now being concentrated in the area of increasing the micronutrient content in the edible parts of plants species including grain legumes, but other barriers like bioavailability of the micronutrients, impact of these high nutrients varieties on humans, acceptability of a particular micronutrient dense crop etc. can only be tackled in an interdisciplinary way. Phytic acid to iron (PA: Fe) molar ratio is an index of iron bioavailability. Relatively high phytic acid to iron molar ratio results in a low iron bioavailability and vice-versa (Karunaratne, 2008). Thus, breeding/molecular techniques should be used to lower the level of the anti-nutrients like PA (Sandberg, 2002). This strategy has already been used successfully in improving the nutritional status of maize grown for animal feed. To increase the acceptability of micronutrient enriched legumes attention should be given to its sensory aspect. In India, a high yielding variety was rejected just because its taste was not acceptable by the consumers (Shobha *et al.*, 2006). So the taste should be improved or people should get acquainted with other household processing methods (resulting in a different taste). Improved techniques to prepare local dishes can also improve iron and other nutrients bioavailability. Finally, the whole chain should be studied to make the people aware about the benefits of mungbean.

In order to make farmers aware about the benefits of growing pulses, the public sector should initiate extension and development programs which involve stake-holders such as producers, processors, nutritionists. Figure 1 shows some pictures of the effort that was made to aware farmers about the benefits of mungbean. Farmers were also involved in development of a mungbean variety in the ongoing project Telfun (www.telfun.info).



Figure 1: Involvement of farmers in mung bean development programme under project TELFUN (Source: www.telfun.info)

Maximum efficacy will occur when alliances are formed between breeders improving the micronutrient content), food scientists and nutritionists, who can alter the ratio of enhancers to inhibitors of bioavailability in recipes consumed, and the social scientists will help in bringing awareness to the people about new techniques and products available in the market. In this way the whole chain can be strengthened, from on-farm research and development leading to empowering end-users, and enhance the technology adaptation and utilization. It is important to recognize the perspective of indigenous people which plays a significant role in acceptance of varieties.

CONCLUSIONS AND FUTURE PERSPECTIVES

Although, very low amounts of micronutrients (Fe, Zn etc) are required in a diet, they all play a very important role in human physiology. Plant breeding in general focuses more on increasing yield and disease resistance. Now the time has come to improve micronutrient concentrations in legumes. Developing cultivars with higher capacity to accumulate Fe and Zn will contribute significantly to the improvement of the micronutrient status of people. In order to achieve this objective scientists have to first understand the genetics of high micronutrient traits and formulate a breeding strategy for improving micronutrient density in the edible parts of the crops. Application of modern techniques in the breeding process can fasten the process and thus helps in achieving the objective. Secondly, investigations are required to check the bioavailability of these micronutrients by modernizing the indigenous techniques and/or developing new techniques. Thirdly how much these fortified legumes can elevate Fe and Zn deficiency especially among women and children in the developing world. Fourthly, socioeconomic studies with farmers, consumers and processors are required to check the acceptance of the resulting products. And finally, the farmers should be involved in the

study so that, they can be updated from time to time about new varieties and crop.

Conclusion:

- The nutritional importance of legumes has to be recognized.
- Adequate genetic variation is present in the legume germplasm. High micronutrient content is positively correlated with yield.
- Anti-nutrient factors should be minimized to maximize the micronutrients bioavailability.
- Nutritional genomics and biotechnology research can complement conventional breeding to improve breeding efficiency.
- A combined effort involving a multidisciplinary approach and preferably in different countries should lead to more nutritional balance of the people.

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