Development of iron and zinc enriched mungbean (*Vigna radiata* L.) cultivars with agronomic traits in consideration

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Contents

CHAPTER 1

General Introduction

Pulse crops are a cheap and rich source of protein, carbohydrates and micronutrients and have a special position in Indian agriculture, because it is a vegetarian society. They are particularly important for the part of the population in developing countries, who can hardly afford to consume animal protein in adequate amounts. The large number of vegetarians in India depend for a major part of their protein intake on pulses. Pulses contain 20-25% protein on dry-seed basis, which is almost 2-3 times higher as in cereals.

Pulses are grown on 68.3 million hectare and contribute 57.5 million tonnes to the world's food basket. India's share is 35.2% of the area and 27.6% of the global production. The production of pulses in India has declined during the last three decades. As a result, per capita availability of pulses per day has progressively declined from 60.7 g in 1951 to 35.9 g in 2000. Among the pulse crops, mungbean (also known as green gram, moong dal, golden gram, chiroko, oregon pea, Chickasaw pea, chickasono pea; *Vigna radiata* L.) is important and widely grown as a *Kharif* crop (crops that are sown in the rainy season). The total area in the world where mungbean is grown is 5.5 million hectare with a production of 2.5 to 3.0 million metric tonnes, of the 5.5 million hectare 4 million hectare are in South Asian countries with a production of about 1.75 million tons. India grows mungbean on 3.34 million hectare with a production of about 1.06 million tonnes (average productivity of 317 kg/ha (Singh 2006)). In South Asia, the area under pulses has been decreasing from 27 to 24 million hectare from 1963-2003 although the production increased from 13.5 to 15 million tonnes (Jat *et al.* 2006; Figure 1).

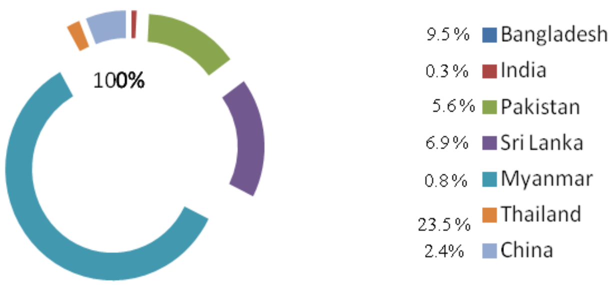

Figure 1 Average annual growth rate in production (1985 to 2000) of mungbean (*V.radiata* L.) in S. Asia

Following India, China is second in mungbean production with about 0.5 million hectare and some stable varieties (VC 1973A) of China have an average yield of 2000 kg/hectare (Jat *et al*. 2006). In Thailand and the Philippines mungbean is the most important grain legume, in Sri Lanka it is second and in India, Bangladesh, Myanmar and Indonesia mungbean ranks third. It is also grown in parts of Africa, America, Australia and Canada.

Centre of origin, domestication & dissemination

Mungbean belongs to genus *Vigna*, which includes about 150 species; 22 species are native to India and 16 to Southeast Asia. However, the largest numbers of species are from Africa. Mungbean is diploid (2n=2x=22) and has a small genome size i.e. 0.60 pg/1C (579 Mbp) (Somta and Srinives 2007). The primary centre of diversity for mungbean is thought to be the central Asia region with India as the gene centre and the probable centre of domestication and dissemination. It has been shown that mungbean has been derived from a cross between wild *V. radiata*. var. *sublobata* (Roxb.) and *V. radiata* var. *radiata* (Shanmugasundaram, 1988). A few closely related species with their common names and origin are listed in Table 1.

Table 1 Some wild and cultivated species of *Vigna* with their common names and centres of origin

Species	Common names	Origin
V. aconitifolia (Jacq.)	Moth bean	South Asia
V. angularis (Wild)	Red bean	Northeast Asia
V. glabrescens (Marechal, Mascharpa & Stainier)	Dau Xanh Vo Xam	Southeast Asia
V. mungo (L.) Hepper	Urd bean, blackgram	India
V. radiata (L.) Wilczek	Mungbean, moong	India
V. trilobata (L.) Verdc.	Jungli bean	South Asia
V. umbellate (Thunb.)	Red bean	Southeast Asia

A molecular study, using 19 SSR primers and over 415 cultivated (*V. radiata* var. *radiata*), 189 wild accessions (*V. radiata* var. *sublobata*) and 11 intermediates from different geographic locations revealed that mungbean had the highest diversity in South Asia, supporting the view that the Indian sub-continent is the centre of domestication for mungbean (Somta and Srinives 2007). The world vegetable Centre in Asia (AVRDC) is established in 1971 and works also as the major centre for genetic improvement of mungbean. It maintains about 5,900 accessions what is the largest collection of mungbean germplasm in the world (Somta *et al.* 2009; Shanmugasundaram *et al.* 2009).

Classification, description and the importance of mungbean

The taxonomic status of mungbean by Lambrides and Godwin 2007 is as follows:

Kingdom	Plantae	**Family**	*Fabaceae*
Sub kingdom	Tracheobionta (Vascular plants)	**Tribe**	*Phaseoleae*
Division	*Magnoliophyta* (flowering plants)	**Genus**	*Vigna*
Class	*Magnoliopsida* (Dicotyledons)	**Subgenus**	*Ceratotropis*
Subclass	*Rosidae*	**Species**	*Radiata*
Order	*Fabales*	**Subspecies**	*Radiata*

Plant characteristics

Mungbeans are annuals, medium to long in size (ranging from 30 to over 100 cm), erect to sub-erect and highly branched. Leaves are trifoliate with long petioles. The inflorescence occurs in clusters from five to twenty flowers, flowers are usually yellow and have typical legume 'butterfly' floral morphology with a large standard petal, two wing petals and two fused petals that form the keel, ten anthers and a single style (Figure 2).

Figure 2 Mungbean leaf (left) and flower (right)

The plant starts flowering 30 to 45 days after sowing and continue flowering for many weeks, resulting in non-uniformity in pod maturity. Pod length varies from 4 to 11 cm and contains more but smaller seeds (< 8.0 g/100) than black gram (Figure 3).

Figure 3 Mungbean pods (left) and seeds (right)

The pods are green in color and turn darker as they mature (Figure 4). Seeds are greenish-yellow to blackish. The crop is self-pollinating and of short duration (55 to 90 days). Mungbean is fairly well adapted to sandy loam soils and dry conditions, and can be grown in the warm season in tropical and sub-tropical regions where it can tolerate even drought. Mungbean is in a symbiotic relationship with *Rhizobium* which enables it to fix atmospheric nitrogen (Table 2)

Table 2 Estimates of the amount of nitrogen fixed by different legumes

Legume Species	Nitrogen fixed* (kg/ha/yr)	Source
Faba bean	82-174	Peoples & Griffiths, 2009
Lentil	60-110	Peoples & Griffiths, 2009
Field pea	85-166	Peoples & Griffiths, 2009
Urd bean	30-74	IIPR (http://www.iipr.res.in/mullarp.htm)
Mungbean	58-109	Singh & Singh, 2011

*locations have impact on the amount of nitrogen to be fix

Importance

Mungbean has its own importance in the rural economy (Figure 5). It is nutritious and very popular because of its high digestibility and with hardly a flatulence effect commonly associated with many grain legumes. Whole seeds can be eaten after boiling, or split and made into dhal (thick soup). It is also used in various fried and spiced dishes such as noodles and balls. For household consumption and selling the seeds are the most important, mungbean fetches a very good market price and thus is good for farmers.

Figure 4 Mungbean plant in field with green (left) and fully mature (right) pods.

Dried seeds are sometimes used for animal feeding, particularly poultry. Toasting or boiling is recommended to improve its nutritional value. The green immature seed pods are occasionally eaten as a vegetable. Mungbean, being a short duration crop, is used as a rotational crop or cover crop by farmers. The leaves and stalks can be utilized for animal feeding.

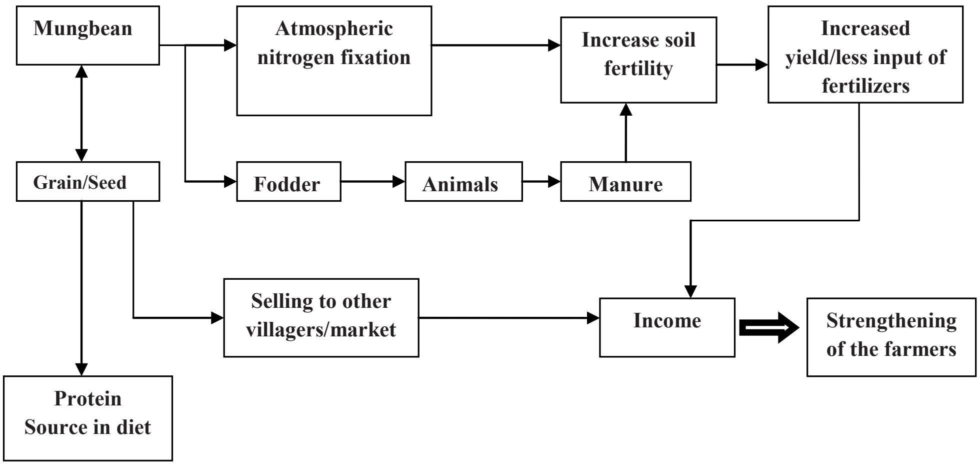

Figure 5 Socio-economic importance of Mungbean

Nutritional value

Mungbean has always been considered as a nutritional and healthy food. It can be used in many forms from salads to soup or just as a vegetable. Sprouted seeds of mungbean contain pure vitamins A, B, C, and E, and minerals such as iron, calcium and phosphorous. On a dry-weight basis mungbean contains 22 to 28% protein, 1.0 to 1.5% fat, 3.5 to 4.5% fibre, 4.5 to 5.5% ash and 60 to 65% carbohydrate (annex I). Mungbean is a good source of amino acids like aspartic acid, glutamic acid and it's a fairly good source of some essential amino acids like isoleucine, leucine, lysine, phenylalanine (Lambrides and Godwin 2007). The sprouts are free of cholesterol. One cup of mungbean sprouts contains approximately 80 kcal, 3 g of protein, 6 g carbohydrates, 2 mg of iron and only 0.2 g of fat. Mungbean sprouts are also a rich source of fibre, easily digestible and containing a high concentration of enzymes facilitating the digestive process. Mungbean can be complemented with cereals. Washing and chilling the raw mungbeans will reduce the risk of infection by harmful bacteria while cooking will destroy bacteria.

Current status of molecular marker research in mungbean

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 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 *et al*. 2004 and Betal *et al*. 2004. High levels of polymorphisms were found by using ISSRs, SSRs and RAPDs (Chattopadhyay *et al*. 2005; Gwag *et al*. 2006; Dieu and Le, 2005; Lavanya *et al.* 2008). SSRs developed in other pulse crops like common beans and cowpea can sometimes 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 (RIL) 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 (Lambridges *et al.* 1999) and seed related characters (Humphry *et al*. 2005) have been identified. Mungbean yellow mosaic virus (MYMV) causes yellowing of leaves in legumes including mungbean. It causes heavy losses. Lambrides *et al.* 1999 used the BSA strategy to identify markers for MYMV in mungbean. One RAPD marker (primer OPAJ 20) was found to be distantly linked to the resistance gene. Further markers (ISSR & SCAR) linked to disease resistance in black gram have potential for locating genes in mungbean (Somata *et al.* 2007). Maiti *et al.* 2011 developed QTL for MYMV from consensus motifs resistance (R) genes from other crops. QTL for Phytic acid (PAP), total P (TP) and inorganic P (IP) in mungbean F2 population were identified (Sompong *et al.* 2012). In grain legumes seed weight is one of the primary components for yield. In an F2 population of the cross between VC3890 and TC1966, four QTLs were identified by Fatokun *et al.* 1992, these QTLs accounted for 49% of the trait variation. Phytic acid inhibits the absorption of certain mineral micronutrients like Fe and Zn in cereals and legumes. Seed derived dietary PA contributes the mineral micronutrients deficiency in humans. In seeds of mungbean seven QTL were identified for P; two for PAP; four for IP and one for TP. 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.

Scope and outline of thesis

In this thesis, efforts are made to analyze mungbean production and consumption in India, to find agronomic and molecular diversity in the mungbean germplasm and to develop recombinant inbred lines based on two crosses between one high and one low micronutrient genotype. The following and selected crosses were used in the experiment: BG39 X 2KM138 and SMH 99-1 X BDYR1.

Chapter 2 focuses on the mungbean quality traits with respect to production, consumption, processing and nutritional which can enhance the food sovereignty concept. For this objective combined effort was carried out by the TELFUN (Indian) team. A survey of 100 farmers, 150 consumers, 100 processors and 116 rural women were carried out individually by each team member. The results were interpreted against the background of food sovereignty.

Chapter 3 gives a review about potential genetic improvement of mungbean in regard to micronutrient levels. Micronutrient deficiency especially iron and zinc is rising at an alarming rate throughout the world especially in women and children. Therefore focus should be set on providing healthy food like legumes.

Chapter 4 presents an agronomic study carried over the mungbean germplasm lines. An effort was made to see the variation in the germplasm lines in context to maturity, yield, yellow mosaic virus resistance, micronutrients (iron and zinc) and protein.

Chapter 5 introduces the requirement and limitations in the mungbean production in the Indian society. Further genetic diversity analysis using AFLP's and ISSR was carried out in cultivars selected by farmers in varietal selection programme and few other cultivars. For this objective, a combination of the conventional survey strategy, participatory varietal selection, molecular markers and chemical analysis was used.

Chapter 6 reports about the genotype and environment interaction studies with a number of genotypes from the previous molecular experiment. In this chapter, efforts were made to select the best lines with main emphasis on micronutrients. Different doses of fertilizers and micronutrients were given and the allocation in the plant was determined.

Chapter 7 describes genetic studies with iron and zinc content in the individuals of the RIL population derived from two crosses i.e. BG39 X 2KM138 and SMH99-1 X BDYR1. All individual lines were analyzed through chemical analysis for iron and zinc content. A start was made with introducing molecular markers in these populations.

Chapter 8 gives a general discussion about the results found in the different experimental chapters and the importance of these findings for the mungbean improvement programme are discussed. The path and stability analysis shows positive genotypic and phenotypic studies in various characters. Further characters were studied to see the direct and indirect effects (Gill *et al.* 1995; Maht and Mahto 1997; Hassan *et al.* 1995). The correlation studies between micronutrients (fe & zn) shows a positive corelation (r = 0.47). The results were supported by various studies in other legumes and crops like Tryphone and Masolla 2010 in common bean, Thavarajah *et* *al.* 2010 in lentils, Anandan *et al.* 2011 in rice, Velu *et al.* 2011 in wheat etc.. Further molecular studies revealed moderate genetic variability. This is supported by Bhat *et al.* 2005 study in mungbean genotypes. In GxE experiment, it was noticed that there was variable pattern of response for different characters in different environments was observed. Similar results were observed by Singh *et al.* 1990; Singh *et al.* 1991 and Popalghat *et al.* 1999. An effort was made to initiate the molecular studies in RIL's population. The chemical analyses of RILs show a positive correlation between micronutrients which was supported by Beebe *et al.* 2000; Guzman-Maldonado *et al.* 2003. In the last part, an overall impact of plant breeding, food sovereignty and TELFUN in the Indian society was discussed along with the positive and negative aspects of such interdisciplinary projects.

References

Afzal MA, Haque MM, Shamugasudaram S (2004) Random amplified polymorphic DNA (RAPD) analysis of selected mungbean (*Vigna radiata* L. Wilczek) cultivars. Asian Journal of Plant Science 3 (1): 20-24.

Anandan A, Rajiv G, Eswaran R, Prakash M (2011) Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. Journal of Food Science 76: H122-H130.

Beebe S, Gonzalez A, Rengifo J (2000) Research on trace minerals in the common bean. Food and Nutrition Bulletin 21:387–391.

Betal IS, Chaowdhury PR, Kundu S, Raychaudhuri SS (2004) Estimation of genetic variability of Vigna radiata cultivars by RAPD analysis. Biologia Plantarum 48 (2) 205-209.

Chattopadhyay K, Ali MN, Sarkar HK, Mandal N, Battacharya S (2005) Diversity analysis by RAPD and ISSR markers among the selected mungbean (*Vigna radiata* L. Wilczek) genotypes. Indian Journal of Genetics and Plant Breeding 65 (3): 173-175.

Cheng XZ, Yang CV (2001) study on relationships among species in mungbean group using RAPD markers. Scientia Agricultura Sinica 34 (2) 216-218.

Dieu TMH, Le TB (2005) Genetic diversity of 57 mungbean cultivars (*Vigna radiata* L.) by RAPD. Cong nghe sinh hoc. 3 (1): 57-66.

Fatokun CA, Menancio-Hautea DI, Danesh D, Young ND (1992) Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping.Genetics 132: 841–846.

Fatokun CA, Danesh D, Young ND, Stewart EL (1993) Molecular taxonomic relationship in the genus *Vigna* based on RFLP analysis. Theoretical Applied Genetics 86: 97-104.

Gill JS, Verma MM, Gumber RK, Balwant S (1995) Character association in mungbean lines derived from three interval crosses in mungbean. Crop Improvement 22 (2): 255-260.

Gwag JG, Chung HK, Lee JH, Ma KH, Dixit A, Park YK, Cho EG, Kim TS, Lee SH (2006) Characterization of new microsatellite markers in mungbean, *Vigna radiata* L. Molecular Ecology Notes 6 (4): 1132-1134.

Hassan MS, Siddique A, Malek MA (1995) Correlation studies on mungbean. Bangladesh Journal of Agriculture Research 20: 12-13.

Humphry M, Lambrides C, Chapman S, Aitken E, Imrie B, Lawn R, McIntyre C, Liu C (2005) Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. Plant Breeding 124: 292-298.

Indian Institute of Pulse Report (IIPR). AICRP MULLARP Mission (http://www.iipr.res.in/mullarp.htm)

Jat ML, Raj K, Gupta OE, Ortiz R (2006) Diversifying the intensive cereal cropping systems of the Indo-Ganges through horticulture. Chronica Horticultrae 46 (3): 16–20.

Lakhanpaul S, Chadha S, Bhat KV (2000) Radom amplified polymorphic DNA (RAPD) analysis in Indian mungbean (*Vigna radiata* (L.) Wilczek) cultivars. Genetica 109 227-234.

Lambrides CJ, Diatloff AL, Liu CJ, Imrie BC (1999) Proceedings of the 11th Australasian Plant Breeding Conference, Adelaide, Australia.

Lambrides CJ, Godwin ID (2007) School of Land and Food Sciences, Plant Improvement Group, The University of Queensland, St Lucia, QLD 4072, Australia (chapter mungbeans).

Lavanya GR, Srivastava J, Ranade SA (2008) Molecular assessment of genetic diversity in mung bean germplasm. Journal of genetics 87: 65–74.

Maht RN, Mahto JL (1997) Correlation and regression study in black gram for yield attributes under rainfed conditions. Environment and Ecology 15 (3): 556-558.

Maiti S, Basak J, Kundagrami S, Kundu A, Pal A (2011) Molecular marker-assisted genotyping of mungbean yellow mosaic India virus resistant germplasm of mungbean and urdbean. Molecular Biotechnology 47: 95-104.

Maldonado SHG, Martínez O, Acosta-Gallegos JA, Lara FG, Octavio Paredes-López (2003) Putative Quantitative Trait Loci for Physical and Chemical Components of Common Bean. *Crop Science* 43: 1029-1035

Menancio-Hautea D, Fatokun CA, Kumar L, Danesh D, Young ND (1993) Comparative genome analysis of mungbean (*Vigna radiata* (L.) Wilczek) and cowpea (*V.unguiculata* L.) using RFLP mapping data. Theoretical Applied Genetics 86: 797-810.

Popalghat GR, Patil JV, Deshmukh RB, Mhase LB (1999) Stability for yield and yield components in chickpea (*Cicer arietinum* L.). Legume Research 22:254-258.

People M, Griffiths J (2009) The legume story- how much nitrogen do legumes fix? *Farming ahead*, Khondinin group. June: 50-52.

Santalla M, Power JB, Dave MR (1998) Genetic diversity in mungbean germplasm revealed by RAPD markers. Plant Breeding 117 (5): 473-478.

Shanmugasundaram S (1988) A catalog of mungbean cultivars released around the World. AVRDC, Shanhua, Taiwan. 20.

Shanmugasundaram S, Keatinge JDH, Hughes JA (2009) The Mungbean Transformation, Diversifying Crops, Defeating Malnutrition. IFPRI Discussion Paper 00922, Supported by CGIAR.

Singh DP, Singh BB (2011) Breeding for tolerance to abiotic stresses in mungbean. Journal of Food Legumes 24(2): 83-90.

Singh J (2006) Country report for India (1). In Processing and utilization of legumes, ed. S. Shanmugasundaram. Tokyo, Japan: Asian Productivity Organization.

Singh G, Brar HS, Verma MM (1991) Genotype environment interaction and phenotypic stability in chickpea. Crop Improvement 18:135-140.

Singh KB, Williams PC, Nakkoul H (1990) Influence of growing season, location and planting time on some quality parameters of kabuli chickpea. Journal of the Science of Food and Agriculture 53: 429-441.

Sompong Utumporn, Somta Prakit, Raboy Victor, Srinives Peerasak (2012) Mapping of quantitative trait loci for phytic acid and phosphorus contents in seed and seedling of mungbean (*Vigna radiata* (L.) Wilczek). Breeding Science 62: 87–92.

Somta Prakit, Srinives Peerasak (2007) Genome Research in Mungbean [V*igna radiata* (L.) Wilczek] and Blackgram [*V. mungo* (L.) Hepper] ScienceAsia 33 69-74 Supplement 1.

Somta P, Sommana W, Srinives P (2009) Molecular diverstity assessment of AVRDC- The World Vegetable Centre elite- parental mungbeans. Breeding Science 59: 149-157.

Thavarajah D, Thavarajah P, See Chai-Thiam, Vandenberg A (2010) Phytic acid and Fe and Zn concentration in lentil (*Lens culinaris* L.) seeds is influenced by temperature during seed filling period. Food Chemistry 122: 254-259.

Tryphone GM, Msolla SN (2010) Diversity of common bean (*Phaseolus vulgaris* L.) genotypes in iron and zinc contents under screen house conditions. African Journal of Agriculture Research 5 (8): 738-747.

Velu G, Ortiz-Monasterio I, Singh RP, Payne T (2011) Variation for grain micronutrients concentration in wheat core-collection accessions of diverse origin. Asian Journal of Crop Science 3: 43-48.

Vijayalakshmi P, Amirthaveni S, Devadas RP, Weinberger K, Tsou SCS, Shanmugasundaram S (2003) Enhanced bioavailability of iron from mungbeans and its effects on health of school hildren. AVRDC Technical Bulletin No. 30 Shanhua, Taiwan.

CHAPTER 2

Genetic improvement of mungbean (*Vigna radiata* L): Necessity to increase the levels of the micronutrients iron and zinc.

A review

Renu Singh, Adriaan W. van Heusden, Ram Kumar and Richard G.F. Visser

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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 ii. Plays essential role in DNA transcription iii. Maintains the integrity of membranes and is involved in pollen formation iv. Regulating auxin synthesis and thus preventing diseases like "little leaf". v. As in animals, Zn-metalloenzymes, and Zn fingers play a role in plants	i. Functions as anti-oxidant and is involved in biochemical reactions ii. Acts as catalyst for the enzymes involved in cell growth. It is important in metabolism of Vitamin A and collagen iii. Essential for DNA function and involved in regulation of gene transcription iv. Zn is essential in protein synthesis, cell division and growth. 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. ii. Necessary in photosynthesis, iii. Involved in nitrogen metabolism as it is part of enzyme	i. Fe is a constituent of hemoglobin and myoglobin which are essential components for storing and diffusing oxygen ii. Important for neurological functioning and

	nitrogenase iv. Iron is also part of the enzyme leg-heamoglobin (role in nitrogen fixation) v. Prevents plants from severe physiological disorders like necrosis and chlorosis vi. Heme is essential component of cytochrome protein and thus mediates redox reactions	development iii. Involved in redox reaction and thus responsible for cellular growth

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, hemaglutinins	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, hemaglutinins	Phytoferritin, riboflavin, ascorbate, b-carotene, cysteine, histidine, lysine, fumarate, malate, citrate

Source: White & 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 (Roozendaal 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

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.

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 (Prased 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.* 2003a). 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 2005a).

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).

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^{+} 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.

Iron accumulation in plants

Iron, which is widely distributed in the lithosphere, is taken up by plants in two different ways: mechanism I (non-graminacious 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 *et al.* 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).

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 (*P. vulgaris*)	35-92	21-59	Islam *et al.* 2002
Pea (*P. sativum*)	23-105	16-107	Grusak and Cakmak 2005
Soybean (*G. max*)	-	59-83	Raboy *et al.* 1984
Chickpea (*C. arientinum*)	24-41	35-60	Haq *et al.* 2007
Mungbean *(V. radiata)*	15-92	15-38	This thesis
Lentils (*L. culinaris* L.)	114	65	Thavarajah *et al.* 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 F_2 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 (Lambridges *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 (Tryphone 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).

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.

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, processers, 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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References

ACC/SCN (2000) Fourth Report on the World Nutrition Situation, Geneva: ACC/SCN in collaboration with the International Food Policy Research Institute.

Afzal MA, Shamugasudaram S (2004) Random amplified polymorphic DNA (RAPD) analysis of selected mungbean (*Vigna radiata* L. Wilczek) cultivars. Asian Journal of Plant Science 3 (1): 20-24.

Ahmad T, Mahmood FY, Malik KA (2001) Residual effect of nitrogen fixed by mungbean and blackgram on subsequent rice and wheat crops. Australian Journal of Experimental Agriculture 41: 245–248

Allen L, Benoist B, Dary O, Hurrell R (2006) Guidelines on food fortification with micronutrients. World Health Organization and Food and Agricultural Organization of the United Nations. Geneva: World Health Organization.

Arif M, Malik MA (2009) Economic feasibility of proposed cropping patterns under different soil moisture regimes of Pothwar plateau. International Journal of Agricultural Biology 11 (1): 27–32.

Beebe S, Gonzalez AV, Rengifo J (1999) Research on trace minerals in common bean. Food and Nutrition Bulletin 21 (4): 387-391.

Berger J, Ninh NX, Khan NC, Nhien NV, Lien DK, Trung NQ, Khoi HH (2006) Efficacy of combined iron and Zn supplementation on micronutrient status and growth in Vietnamese infants. European Journal of Clinical Nutrition. 60: 443-454.

Betal IS, Chaowdhury PR, Kundu S, Raychaudhuri SS (2004) Estimation of genetic variability of Vigna radiata cultivars by RAPD analysis. Biologia Plantarum 48 (2): 205-209.

Borlaug NE (2000) Ending world hunger. The promise of biotechnology and the threat of antiscience zealotry. Plant Physiology 124: 487-490.

Buyckx M (1993) The international community's commitment to combating micronutrient deficiencies. Food Nutrition and Agriculture 7: 2–7.

Cakmak I (2002) Plant nutrition research: Priorities to meet human needs for food in sustainable ways. Plant and Soil 247: 3-24.

Campion BS, Doria F, Tagliabue E, Galasso G, Fileppi IM (2009) Isolation and characterisation of an LPA (low phytic acid) mutant in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics 118 (6): 1211–1221.

Chattopadhyay K, Ali MN, Sarkar HK, Mandal N, Battacharya S (2005) Diversity analysis by RAPD and ISSR markers among the selected mungbean (*Vigna radiata* L. Wilczek) genotypes. Indian Journal of Genetics & Plant Breeding 65 (3): 173-175.

Cheng XZ, Yang CV (2001) Study on relationships among species in mungbean group using RAPD markers. Scientia Agricultura Sinica 34 (2): 216-218.

Cichy KA, Forster S, Grafton KF, Hasfield GL (2005) Inheritance of seed Zn Accumulation in navy bean. Crop Science 45: 864-870.

Curie C, Brieat JF (**2003)** Iron transport and signaling in plants. Annual Review of Plant Biology 183–206.

Dieu TMH, Le TB (2005) Genetic diversity of 57 mungbean cultivars (*Vigna radiata* L.) by RAPD. Cong nghe sinh hoc. 3 (1): 57-66

Durrett TP, Gassmann W, Rogers EE (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiology 144: 197–205.

Elizabeth CT, Jean FC (2004) Plant ferritin and non-heme iron nutrition in humans, HarvestPlus Technical Monograph 1, Washington DC and Cali: International food policy research institute and CIAT.

Endre L, Beck FWJ, Prasad AS (1990) The role of Zn in human health. Journal of Trace Elements Experimental Medicine 3: 337–375.

FAOSTAT (2002) FAOSTAT on-line. Rome: United Nations Food and Agriculture Organization.

Fatokun CA, Danesh D, Young ND, Stewart EL (1993) Molecular taxonomic relationship in the genus *Vigna* based on RFLP analysis. Theoretical and Applied Genetics 86: 97-104.

Gelin JR, Forster S, Grafton KF, McClean PE, Rojas-Cifuentes GA (2007) Analysis of Seed Zn and Other Minerals in a Recombinant Inbred Population of Navy Bean (*Phaseolus vulgaris* L.). Crop Science 47: 1361-1366.

Ghandilyan A, Vreugdenhill D, Mark G, Aarts M (2006) Progress in the genetic understanding of plant iron and Zn nutrition. Physiologia Plantarum 126: 407–417.

Gregorio GB (**2001)** Symposium "Plant Breeding: A New Tool for Fighting Micronutrient Malnutrition" given at the Experimental Biology 2001 meeting, Orlando, Florida, on April 1, 2001. International Food Policy Research Institute, Washington, DC.

Grusak MA, Cakmak I (2005) Methods to improve the crop-delivery of minerals to humans and livestock. In Plant nutritional genomics. Edited by M.R. Broadley and P.J. White. Blackwell Publishing, Oxford. 265-286.

Gupta AP (2005) Micronutrient status and fertilizer use scenario in India. Journal of Trace Elements in Medicine and Biology 18: 325-331.

Guttieri MJ, Peterson KM, Souza EJ (2006) Agronomic performance of low phytic acid wheat. Crop Science 46 (6): 2623–2629.

Gwag JG, Chung HK, Lee JH, Ma KH, Dixit A, Park YK, Cho EG, Kim TS, Lee SH (2006) Characterization of new microsatellite markers in mungbean, *Vigna radiata* L. Molecular Ecology Notes 6 (4): 1132-1134.

Haas JD, Beard JL, Murray-Kolb AM, Felix A, Gregorio G (2005) Iron bio fortified rice improves the iron stores of non-anaemics Filipino women. Journal of Nutrition 135: 2823-2830.

Haq MZ, Shaid I, Ahmed S, Imran M, Niaz A, Bhanger MI (2007) Nutritional and compositional study of Desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. Food Chemistry 105: 1357-63.

Holm PB (2002) Transgenic approaches in commonly consumed cereals to improve iron and zinc content and bioavailability. Journal of Nutrition 132: 514–516.

Humphry M, Lambrides C, Chapman S, Aitken E, Imrie B, Lawn R, McIntyre C, Liu C (2005) Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. Plant Breeding 124: 292-298.

Hunt JR (2003) Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. American Journal of Clinical Nutrition 78(Suppl): 633S-639S.

Hunt JR, Roughead ZK (1999) Non-heme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lacto-ovovegetarian diets for 8 wk. American Journal of Clinical Nutrition 69: 944–52.

Islam FMA, Basford KE, Jara C, Redden RJ, Beebe SE (2002) Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genetic Resources and Crop Evolution 49: 285–293.

Johnson JM, Walker PM (1992) Zinc and iron utilization in young women consuming a beef-based diet. Journal of the American Dietetic Association. 92:1474–1478.

Kaiser BN, Moreau S, Castelli J, Thomson R, Lambert A, Bogliolo S, Puppo A, Day DA (2003) The soybean NRAMP homologue, GmDMT1, is a symbiotic divalent metal transporter capable of ferrous iron transport. Plant Journal 35: 295–304.

Karunaratne AM, Amerasinghe PH, Sadagopa R, Sandstead VM, Perera HH (2008) Zn, Iron and Phytic Acid levels of some popular foods consumed by rural children in Sri Lanka. Journal of Food Composition and Analysis 21: 481-488.

Khattak GSS, Ashraf M, Saeed I, Alam B (2006) A new yielding mungbean (*Vigna radiate* (L.) Wilczek) variety "*Ramzan*" for the agro climatic conditions of NWFP. Pakistan Journal of Botany 38 (2): 301-310.

Lakhanpaul S, Chadha S, Bhat KV (2000) Radom amplified polymorphic DNA (RAPD) analysis in Indian mungbean (*Vigna radiata* (L.) Wilczek) cultivars. Genetica 109: 227-234.

Lambrides CJ, Diatloff AL, Liu CJ, Imrie BC (1999) Proceedings of the 11th Australasian Plant Breeding Conference, Adelaide, Australia.

Larsen T (1997) Erythrocyte membrane enzymes as indicators of zinc status, *In* Trace Elements in Man and Animals – 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals. Eds. PWF. National Research Council of Canada, Ottawa 105–106.

Lind T, Lonnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekstrom EC, Persson LA (2003) A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. American Journal of Clinical Nutrition 77: 883-890.

Lopez MA, Martos FC (2004) Iron availability: an updated review. International Journal of Food Sciences and Nutrition 55: 597–606.

Maldonado Guzman SH, Acosta-Gallegos J, Paredes-López O (2000) Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L). Journal of the Science of Food and Agriculture 80: 1874–1881.

Maldonado SHG, Martínez O, Acosta-Gallegos JA, Lara FG, Octavio PL (2003) Putative Quantitative Trait Loci for Physical and Chemical Components of Common Bean. Crop Science 43: 1029-1035

Marschner H (1995) Mineral Nutrition of Higher Plants, Academic Press, London.

Menancio-Hautea D, Fatokun CA, Kumar L, Danesh D, Young ND (1993) Comparative genome analysis of mungbean (*Vigna radiata* (L.) Wilczek) and cowpea (*V.unguiculata* L.) using RFLP mapping data. Theoretical and Applied Genetics 86: 797-810.

Miflin B (2000) Crop biotechnology. Where now**?** Plant Physiology 123: 17-28.

Monchy S, Benotmane MA, Janssen P, Vallaeys T, Taghavi S, van der Lelie D, Mergeay M (2007) Plasmids pMOL28 and pMOL30 of *Cupriavidus metallidurans* are specialized in the maximal viable response to heavy metals. Journal of Bacteriology 189: 7417–7425.

Monsen ER, Hallberg L, Layrisse M (1978) Estimation of available dietary iron. American Journal of Clinical Nutrition 31:134–41.

Munger HM (1988) Adaptation and breeding of vegetable crops for improved human health. In: *B. Quebedeaux and F.A. Bliss (eds.).* Horticulture and human health. Prentice Hall, Englewood, N.J. 177–184.

Navert B, Sandstrom B, Cederblad A (1985) Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. British Journal of Nutrition 53: 47-53.

Neue HU, Quijano C, Senadhira D, Setter T (1998) Strategies for dealing with micronutrient disorders and salinity in lowland rice systems, Field Crops Research 56: 139-155.

Nube M, Voortman RL (2006) Simultaneously addressing micronutrient deficiencies in soils, crops, animal and human nutrition: opportunities for higher yields and better health Stichting Onderzoek Wereldvoedselvoorziening van de Vrije Universiteit. Centre for World Food Studies. Staff Working Paper (September).

Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK, Sanders D (2008) Zinc biofortification of cereals: problems and solutions. Trends in Plant Science 13: 464–473.

Park SJ, Anderson TR (1977) AC Harosprout mungbean. Canadian Journal of Plant Science 77: 111-112.

Pathak P, Kapil U, Kapoor SK, Dwivedi SN, Singh R (2003a) Magnitude of zinc deficiency among nulliparous nonpregnant women in a rural community of Haryana State, India, Food and Nutrition Bulletin 24: 368-371.

Pennington JAT, Young B (1990) Iron, zinc, copper, manganese, selenium, and iodine in foods from the United States total diet study. Journal of Food Composition Analysis 3**:** 166-184.

Prasad MNV (2007). Plant-mineral: macro-and micro nutrients, uptake, functions, deficiency and toxicity symptoms.

Prased AS (1996) Zinc deficiency in women, infants and children American Journal of Clinical Nutrition 15: 113-120.

Quebedeaux B, Bliss FA (1988) Horticulture and human health. Contributions of fruits and vegetables. Proc. 1st Intl. Symp. Hort.and human health. Prentice Hall, Englewood, N.J.

Quebedeaux B, Eisa HM (1990) Horticulture and human health. Contributions of fruits and vegetables. Proc. 2nd Intl. Symp. Hort. and Human Health. HortScience. 25: 1473–1532.

Raboy V, Dickinson DB, Below FE (1984) Variation in Seed Total Phosphorus, Phytic Acid, Zinc, Calcium, Magnesium, and Protein among Lines of Glycine max and G. soja. Crop Science 24 (3): 431-434.

Ramalingaswami V (1995) New global perspectives on overcoming malnutrition. The American Journal of Clinical Nutrition 61: 259–263.

Rengel Z, Batten GD, Crowley DE (1999) Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Research 60: 27-40.

Roozendaal Gerda van (1996) Enhancing the Nutritional Qualities of Crops: A second Green Revolution? By in biotechnology and development monitor no-29 Dec.

Roughead ZK, Hunt JR (2000) Adaptation in iron absorption: iron supplementation reduces non-heme-iron but not heme-iron absorption from food. The American Journal of Clinical Nutrition 72:982–989.

Safdar AM, Riffat A, Sohail S (2005) Enhancing water use efficiency, Nitrogen fixation capacity of mashbean and soil profile nitrate content with phosphorous and potassium application. Pakistan Journal Agronomy 4: 340–344.

Sandberg AS (2002) Bioavailability of minerals in legumes. British Journal of Nutrition 3: S 281-285.

Sandstrom B (1997) Bioavailability of zinc. European Journal Clinical Nutrition 51: S17-S19.

Sandstrom BA, Cederblad B, Bjorn-Rasmussen AE (1980) Zinc absorption from composite meals, I: the significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. The American Journal of Clinical Nutrition 33:739–45.

Sanstead HH (1995) Is zinc deficiency a public health problem? Nutrition 11: 87-92.

Santalla M, Power JB, Dave MR (1998) Genetic diversity in mungbean germplasm revealed by RAPD markers. Plant Breeding 117 (5): 473-478.

Shanmugasundaram S (2006) Improving income and nutrition by incorporating mungbean in cereal fallows in the Indo-Gangetic Plains of South Asia, Proceedings of the final workshop and planning meeting, Punjab Agricultural University, Ludhiana, Punjab, India, May 27–31 2004. AVRDC Publication No. 06-682. 342. Shanhua, Taiwan

Shaul O, Hilgemann DW, de-Almeida-Engler J, Van Montagu M, Inzé D, Galili G (1999) Cloning and characterization of a novel Mg(2+)/H(+) exchanger. EMBO Journal 18: 3973–3980.

Shobha RN, Manish KP, Prasad GSV, Sudharshan I (2006) Historical significance, grain quality features and precision breeding for improvement of export quality *basmati* varieties in India. Indian Journal Crop Science 1 (1-2): 29-41.

Shrimpton R (1993) Zinc deficiency – is it widespread but under recognized? SCN News **9:** 24–27.

Singh DP, Ahlawat IPS (2005) Greengram and blackgram improvement in India: past, present and future prospects. Indian Journal Agricultural Science 75: 243–50.

Singh SP, Westermann DT (2002) A single dominant gene controlling resistance to soil zinc deficiency in common bean. Crop Science 42: 1071–1074.

Slaton NA, Gbur EE, Wilson CE, Norman RJ (2005a) Rice response to granular zinc sources varying in water soluble zinc. Soil Science Society of American Journal 69: 443-452.

Somta Prakit, Peerasak Srinives (2007) Genome Research in Mungbean [V*igna radiata* (L.) Wilczek] and Blackgram [*V. mungo* (L.) Hepper] Science Asia 33: 69-74 Supplement 1.

Srivastava PC, Gupta UC (1996) Trace Elements in Crop Production. Science Publishers, Enfi eld.

Thavarajah D, Thavarajah P, See Chai-Thiam, Vandenberg A (2010) Phytic acid and Fe and Zn concentration in lentil (*Lens culinaris* L.) seeds is influenced by temperature during seed filling period. Food Chemistry 122: 254-259.

Tryphone GM, Msolla SN (2010) Diversity of common bean (Phaseolus vulgaris L.) genotypes in iron and zinc contents under screen house conditions. African Journal of Agricultural Research 5 (8): 738-747.

Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyper accumulation in plants. New Phytologist 181: 759-776.

Vert G, Briat JF, Curie C (2003) Dual regulation of the *Arabidopsis* high-affinity root iron uptake system by local and long-distance signals. Plant Physiology 132: 796–804.

Vijayalakshmi P, Amirthaveni S, Devadas RP, Weinberger K, Tsou SCS, Shanmugasundaram S (2003) Enhanced bioavailability of iron from mungbeans and its effects on health of school children. AVRDC Technical Bulletin No. 30 Shanhua, Taiwan

Welch RM (2002) The impact of mineral nutrients in food crops on global human health. Plant and Soil 247: 83-90.

Welch Ross M, Graham RD (2002) Breeding crops for enhanced micronutrient content. Plant and Soil 245: 205–214.

Welch Ross M, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany 55 (396): 353-364.

Weinberger K (2003) Impact analysis on mungbean research in south and south-east Asia. AVRDC Processing No. 99.9117.5, Shanhua, Taiwan

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends in Plant Science 10 (12).

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytologist 182: 49–84.

World Health Organization (WHO) (**2008)** http://www.who.int/ith/en/

World Hunger Facts: 2011. Why Are People Hungry? World Hunger Education. http://www.worldhunger.org/articles/Learn/world%20hunger%20facts%202002.ht

Zimmermann MB, Hurrell RF (2002) Improving iron, zinc and vitamin A nutrition through plant biotechnology. Current Opinion in Biotechnology 13: 142–145.

CHAPTER 3

Genetic Diversity of Mungbean (*Vigna radiata* L.) in Northern India

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Abstract

Mungbean (*Vigna radiata* L.) is one of the most important pulses in Indian agriculture. This paper describes the importance of participatory varietal selection in the development of suitable varieties of mungbean. With participatory varietal selection farmers have selected varieties with good agronomical traits. A conventional survey strategy, in combination with participatory varietal selection, molecular markers and chemical analysis was used. The genetic diversity in the selected genotypes was studied using 22 inter simple sequence repeat (iSSR) primer sets which showed a genetic diversity ranging from 65 to 87 percent. The selected mungbean genotypes had only a moderate amount of iron (1.76 - 6.58 mg/100g) and zinc (1.54 - 3.81 mg/100g). Farmer's preference, resistance levels and micronutrient contents must be considered while designing future breeding programs.

Keywords: mungbean, diversity, inter simple sequence repeat, micronutrients, participatory varietal selection

Introduction

Mungbean is a widely grown food grain legume in the developing world. It is cultivated almost in all seasons (Feb-April; April-June; June-Sept & July-Sept.) somewhere in India. It is thought to have its primary centre of diversity in the Indian subcontinent (de Candolle 1884; Vavilov 1926) with maximum diversity in the upper Western Ghats and Deccan hills and the Indo-Gangetic plains. From the standpoint of production, dry beans including mungbean (19.7 mt), field pea (10.4 mt), chickpea (9.7 mt), cowpea (5.7 mt), lentil (3.6 mt) and pigeon pea (3.5 mt) are the most important pulses (Murleedhar *et al.* 2013). The major mungbean growing states are Orissa, Maharashtra, Andhra Pradesh, Rajasthan, Madhya Pradesh, Bihar, Karnataka, Uttar Pradesh and Haryana. Out of the sixteen essential nutrients, seven are classified as micronutrients or trace elements. Two of these, iron and zinc play a vital role in human, animal and plant health and development. Mungbean is an important food crop for vegetarians, being a major source of protein. It contains 25-31 percent of crude protein (Anwar *et al.* 2007), 4-6mg/100g of iron (Vijayalakshmi *et al.* 2001), 1486-1570 KJ/100 g of energy, and 1-5 percent crude fibre (Shanmugasundaram 2002). Resource-poor farmers grow mungbean because it only requires moderate irrigation and other inputs. Mungbeans restore soil fertility through symbiotic nitrogen fixation (Ashraf *et al.* 2003) and can withstand high temperatures (average 35.3°C).

In order to feed the increasing population of the world the green revolution played a very important role. It prevented the world from massive starvation by raising the production of staple food crops (wheat & rice). The downside was that the green revolution has also lead to lower intake of micronutrients. Despite the fact that this can be compensated by eating more legumes the production and productivity of a legume like mungbean in India is static and the per capita availability has even declined over the past decades (IASRI 1999; DOES 2000; Vijayalakshmi *et al.* 2003). This decline is a major concern and difficult to understand since mungbean is for growers profitable and for the consumers nutritious and potentially rich in minerals.

Abiotic and biotic stresses limit mungbean production in India. One of the main biotic stresses is mungbean yellow mosaic virus (MYMV) this virus can inflict heavy yield losses (30-70%) (Maiti *et al.* 2011). Farmers try to get good seeds from private companies and government agencies. Different mungbean varieties are often mixed and the impact of MYMV on individual homozygous genotypes is not well understood. An assessment of the genetic diversity of pulses is an important first step in a program to improve crop yield.

The objective of the present study were: (i) to understand the indigenous knowledge of farmers in respect to mungbean yellow mosaic virus (MYMV) and other related traits and what role it plays in participatory varietal selection (PVS), (ii) to analyse the genetic diversity and range of the concentration in mineral micronutrients (Fe & Zn) of the genotypes selected by the farmers.

Material and Methods

The district of Hisar in the Haryana state was selected for the study (Figure 1). There are three growing seasons for mungbeans in Haryana, *kharif* (July to September) is the most preferred season for the farmers as monsoon favours a good yield.

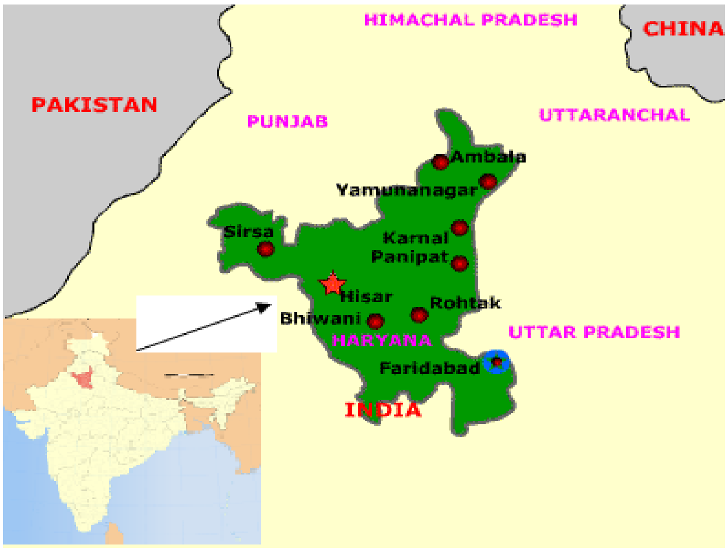


Figure 1 Location of the district Hisar within Haryana state used for the survey study

Due to the high level of local biodiversity the Hisar district in Haryana state was selected for our study. The district falls under the hot-arid western plane eco-region. Two major mungbean producing blocks of Hisar were selected, and from these blocks two villages, Mangali and Dhiktana (one each from a block) were selected because of the production-consumption pattern of mungbean (Table 1).

Table 1 Production, area and volume of mungbean in Hisar and Haryana

	Area (x10^3 Hectares)			Production (x10^3 Tonnes)			Yield (kg/ha)		
	03-04	04-05	05-06	03-04	04-05	05-06	03-04	04-05	05-06
Hisar	4.7	11.5	8.3	1.2	2.3	3.3	256	196	400
Haryana	18.3	25.2	16.0	2.8	4.0	6.1	159	158	384

Source: Statistical Abstract of Haryana, Director of Agriculture, Haryana, 2003, 2004, 2005 and 2006.

Sampling and data analysis

Diagnostic survey

A questionnaire (appendix I) was prepared and used for primary data collection. From two selected villages, a random sample of 100 farmers who were actively involved in farming, were selected. For the follow-up stage survey twenty active farmers were selected (ten from each village) and invited on the research field area at the pulse research station, Department of Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India for evaluation of advance genotypes during the *kharif* season 2008.

Genetic diversity: Level of virus resistance and markers

The genotypes selected by the farmers at the research fields were morphologically characterized at university experimental farms in Hisar. Hisar has a latitude of 29^{o}10'N, longitude of 75^{o}46'E and altitude 215.2 m above sea level and falls in the semi-tropical region of the Western Zone of India. The experiment was conducted in the *kharif* (July to September) season 2008, in a Random Block Design with spacing of 40 cm between rows and 15 cm between plants within the row. About two weeks before harvesting, the level of mungbean yellow mosaic virus (MYMV) damage was scored on a scale from 1 (completely resistant) to 9 (completely susceptible) (Singh *et al.* 1992).

The genetic diversity was determined in the selected genotypes using 22 inter simple sequence repeat primer sets at Wageningen UR Plant Breeding, the Netherlands. Leaves from 3-5 week old seedlings were collected and immediately stored at -80^{o}C until DNA isolation. Leaf tissue from each individual was ground to a fine powder using two grinding beads in a Shatter-box and total genomic DNA was extracted and treated with RNAse and Proteinase K (Raeder and Baroda 1985).

Concentrations were determined with ethidium bromide staining of gels and comparing with samples with a known DNA concentration**.**

Mineral (Fe and Zn) Analysis

Two gram of seeds was taken randomly from each variety, washed with sterile water and before grinding dried in an oven for 2 days at 45°C. Mineral analysis (Fe and Zn) was done on powdered sample using atomic absorption spectroscopy (AAS). Sample preparation for AAS involved digesting 1 g flour with nitric/perchloric acid (5 ml of a 2:1 mixture of 65 percent nitric acid (HNO_3) and 70 percent per-chloric acid ($HClO_4$)) for 2 h followed by a heat treatment for 2 h and re-suspension in 25 ml of de-ionized water. The resulting samples were analysed in a mass spectrometer with acetylene flame **(**Lindsay and Norvell 1978**).**

Statistical Analysis

The survey data were analysed by using frequency, percentage and rank. Statistical software SAS (for survey/field data) & SPSS 12.0 (chemical analysis) was used to examine interrelationships and frequencies while for the molecular data, the ISSR bands were scored as present (1) or absent (0), each of which was treated as an independent character while, faint bands were not considered for final scoring. Molecular weights of the bands were estimated by using the Gene Ruler 1 kb DNA ladder (MBI Fermentas, UK). Data analyses were performed using the NTSYS-pc 2.01b (Numerical Taxonomy and Multivariate Analysis System, Applied Biostatistics Inc. 1986–1997, Rohlf 1990). The SIMQUAL program was used to calculate the Jaccard's coefficient, a common estimator of genetic identity and was calculated as follows:

$$\textit{Jaccard's coefficient} = N_{AB} / (N_{AB} + N_A + N_B)$$

Where N_{AB} is the number of bands shared by samples, N_A represents amplified fragments in sample A, and N_B represents fragments in sample B. Similarity matrices based on these indices were calculated. Similarity matrices were utilized to construct the UPGMA (unweighted pair group method with arithmetic average) dendrogram. Marker index was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula:

$$PIC = 1\text{-}\Sigma pi\ 2,$$

Where, *pi* is the frequency of the i^{th} allele (Muthuswamy *et al.* 2008).

Results and Discussion

Farmer's perspective in relation to mungbean - A survey study

All the farmers were male and literate, middle-aged and experienced. Majority of them were in the age group of 30-45 (Figure 2).

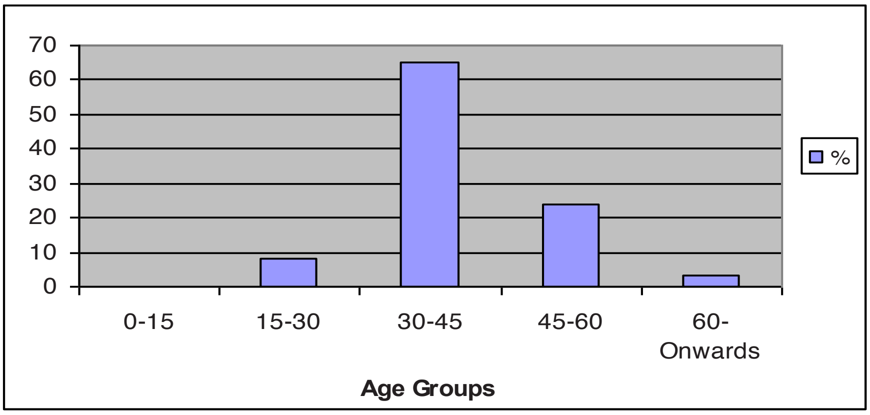


Figure 2 Percentage distribution of farmers on basis of age (N= 100)

About 50 percent of the farmers from Mangali and Dhiktana villages thought that yield and the lack of mungbean yellow mosaic virus (MYMV) resistance were the major limiting factors in the choice to grow mungbeans. Majority of the farmers (>80 percent) in both villages were willing to take advice from regional agricultural institutes reflecting their willingness to incorporate better farming techniques. Only few farmers considered the lack of drought resistance, uniform maturating etc. to be the most important factors not to grow mungbean (Table 2).

Table 2 Benefits and constraints of mungbean as assessed by 100 farmers in two villages (*Mangali*, *Dhiktana*) in the Hisar district.

Variable	Description	Response (N= 100)
Market value	Good Average Poor	33 67 -
Household use	Frequently used Less frequent use	35 65
Used as rotational crop	Yes No	18 82
Impact on soil health	Good impact Average impact Poor impact	16 84 -
Drought resistance required	Yes No	12 88
Is MYMV susceptibility a major constraint	Yes No	40 60
Low yield is a limiting factor	Yes No	48 52
Scientific assistance required	Yes No	88 12

Observations of agronomic traits by farmers during field visit

Participatory Varietal Selection (PVS) was used to identify the selection criteria of farmers and to evaluate advanced varieties after 50% pod development (Figure 3). By seeing the farmer's personal interest and funding limitations, only twenty farmers (ten from each village) were invited for the PVS at the research fields. In informal discussions the farmers expressed their opinion, identified important target traits and made selections on the basis of these traits (Table 3). Farmers prefer medium height varieties and long sized pods. This is directly related to the number of seeds (yield). Medium sized seeds are preferred because large seeds take more cooking time and are therefore not popular.

Figure 3 Involvement of farmers in mungbean development program

Table 3 Perception of farmers on quality traits of mungbeans (N= 20)

Characteristic	Category	Farmer's Preferences (N=20) Frequency	%
Plant height	Tall	2	10
	Medium	14	70
	Dwarf	4	20
Plant growth	Vigorous in growth	4	20
	Not vigorous	16	80
Pod length	Long	12	60
	Medium	4	20
	Dwarf	4	20
Disease resistant	Yes (MYMV, leaf curl)	20	100
	No (MYMV, leaf curl)	-	-
Size of seed	Large seeded	2	10
	Medium seeded	15	75
	Small seeded	3	15
Color of seed	Yellow	-	-
	Green	17	85
	Light green	3	15
Pod Maturity	Uniform	20	100
	Non- uniform	-	-
Number of seeds/pod	5-6	-	-
	6-7	7	35
	8-9	13	65

Yield and quality were considered the most important agronomic traits**.** The yield of the selected varieties is given in Table 4. On average, the varieties which were selected by the farmers had a yield minimum of about 1000 kg/hectare (kg/ha). Mungbean Yellow Mosaic Virus (MYMV) is a limiting factor and on basis of their experience, the farmers selected some genotypes which were more or less tolerant to MYMV (Table 4). Farmers identify the disease in the early stages of plant development. Farmers prefer early maturing mungbean varieties (~ 65 days) so that they can incorporate it in rice-wheat system and grow three crops in a year. This is the most profitable and simultaneously the mungbean production improves soil fertility which is good for wheat production.

Table 4 Top five varieties selected on basis of Participatory Varietal Selection

Varieties	Preferred Characteristics	Maturity Duration (Days)	Potential Yield (kg/ha)**	Comments	Rank	Zn±S.E+ mg/100g	Fe±S.E+ mg/100g
MH 125 *(Basanti)*	Shiny green attractive seeds Medium sized MYMV resistant (3)* Tall variety (80-90 cm)	65	1500	Suitable for both autumn and spring cultivation	I	2.3±0.2	3.3±0.1
MH 318	Shiny green attractive seeds, Medium sized MYMV resistant (3)* Dwarf variety (60-65 cm)	59	1420 (2300)**	Start maturing after 55 days	II	3.2±0.12	5.0±0.8
MH 421 (*Bharpai*)	Shiny green attractive seeds Medium sized MYMV resistant (2)* Dwarf variety (60-65 cm)	60	1500 (2300)**	Start maturing after 55 days	III	2.7±0.1	3.8±0.1
MH 215 (*Sattya*)	Shiny green attractive seeds, MYMV resistant (4)* Medium height (70-80 cm)	67	1650	Suitable for autumn season	IV	2.4±0.2	3.3±0.1
MH 96-1 *(Muskan)*	Shiny green attractive seeds Small sized seed MYMV resistant (4)* Tall variety (80-90 cm)	77- 80	1200	-	V	4.6±0.2++	5.5±0.2++

* Score of MYMV on 1-9 Scale (1= resistant & 9= susceptible). **The yield varies with change in inputs and climate. Above yield is average yield. ** If a second flush of flowering is allowed the yield goes up to 2300 kg./ha; Name in parenthesis are the local names. +, ++from appendix I chapter 5

The genetic diversity of thirty varieties including those selected by farmers was determined with 22 ISSR primer sets. This was a subset of 32 primer sets that were tried out. The same genotypes were analysed for mineral micronutrient (Fe & Zn) content (Table 5). The seed iron content varied from 1.76 ± 0.11 mg/100g to 6.58 ± 0.21 while, zinc content ranged from 1.54±0.29 mg/100g to 3.81 ± 0.41 mg/100g.

Table 5 Origin (if known) and micronutrient content of 30 mungbean varieties

S.No.	Genotypes	Origin	Mean value of Zn ± S.E mg/100g	Fe ± S.E mg/100g
1	ML-803	Ludhiana (Punjab)	3.8 ± 0.4	5.3 ± 1.2
2	MH-125*	Hissar (Haryana)	2.3 ± 0.2	3.3 ± 0.1
3	ML-5	nn	2.4 ± 0.3	2.8 ± 0.1
4	ML-735	Ludhiana (Punjab)	2.8 ± 0.1	6.6 ± 0.2
5	2KM 112	IARI (New Delhi)	2.7 ± 0.2	4.5 ± 0.6
6	ML-1108	nn	2.2 ± 0.1	3.8 ± 0.4
7	MI-3580	nn	2.4 ± 0.1	4.3 ± 1.1
8	ML-839	Ludhiana (Punjab)	1.8 ± 0.2	3.2 ± 0.4
9	L-24-2	Ludhiana (Punjab)	1.7 ± 0.2	1.9 ± 0.1
10	MH-421*	Hissar (Haryana)	2.7 ± 0.1	3.8 ± 0.1
11	2KM-139	Hissar (Haryana)	1.8 ± 0.1	2.9 ± 0.1
12	2KM 135	Sri Ganganagar (Rajasthan)	2.9 ± 0.1	3.2 ± 0.1
13	SMH-99-2	Hissar (Haryana)	2.5 ± 0.1	3.0 ± 0.1
14	2 KM-107	??	2.1 ± 0.1	3.9 ± 0.5
15	BG-39	??	2.6 ± 0.2	6.0 ± 0.4
16	ML-818	Ludhiana (Punjab)	3.2 ± 0.1	4.5 ± 0.4
17	ML-406	Ludhiana (Punjab)	1.5 ± 0.3	2.5 ± 0.4
18	2KM-151	Pant Nagar (Uttrakand)	2.0 ± 0.2	4.0 ± 0.2
19	2KM 155	??	2.9 ± 0.23	4.5 ± 0.9
20	2KM-138	Hissar (Haryana)	2.0 ± 0.5	2.4 ± 0.2
21	MH3-18*	Hissar (Haryana)	3.2 ± 0.12	5.0 ± 0.8
22	MH-124	Hissar (Haryana)	2.0 ± 0.1	3.0 ± 0.6
23	ASHA	Hissar (Haryana)	2.4 ± 0.2	4.1 ± 0.4
24	MH-215*	Hissar (Haryana)	2.4 ± 0.2	3.3 ± 0.1
25	SMH-99-DULL B	Hissar (Haryana)	3.1 ± 0.1	3.9 ± 0.1
26	PDM-9-249	IIPR Kanpur (Uttar Pradesh)	1.8 ± 0.2	2.6 ± 0.1
27	ML-759	Ludhiana (Punjab)	1.6 ± 0.1	2.6 ± 0.1
28	M 395	Ludhiana (Punjab)	1.6 ± 0.1	1.8 ± 0.1
29	PMB-14	Ludhiana (Punjab)	1.6 ± 0.2	2.0 ± 0.1
30	ML-506	Ludhiana (Punjab)	2.3 ± 0.1	4.8 ± 1.5

*selected genotypes (top 4) in PVS by farmers along with the advance mungbean material

Molecular Characterization

The criteria for selecting primer pairs were the quality of amplification and the presence of polymorphisms among the cultivars used for primer survey. Figure 4 is a representative ISSR pattern. A total of 200 amplification products were obtained of which 63.4 percent were polymorphic in the set of 30 mungbean genotypes. The genetic similarity ranged from 0.65-0.87. This shows the considerable amount of genetic diversity in the mungbean genotypes. ISSR techniques have been previously used in estimating the genetic relationships in genus *Vigna* (Ajibade *et al.* 2000) and in several other crops (Souframanien 2004). The mean PIC value is 0.218 and the highest and lowest PIC values are 0.67 (UBC820) and 0.05 (UBC864, UBC895) respectively (Table 6).

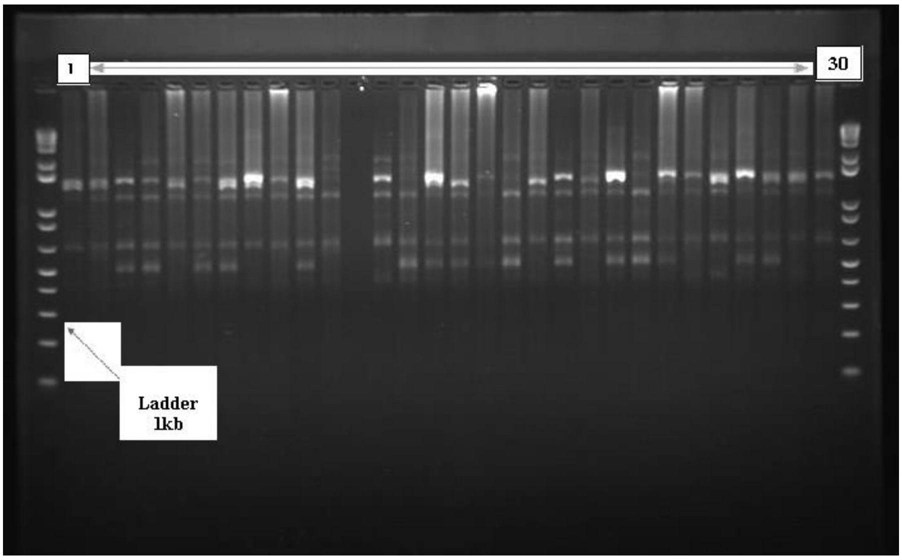
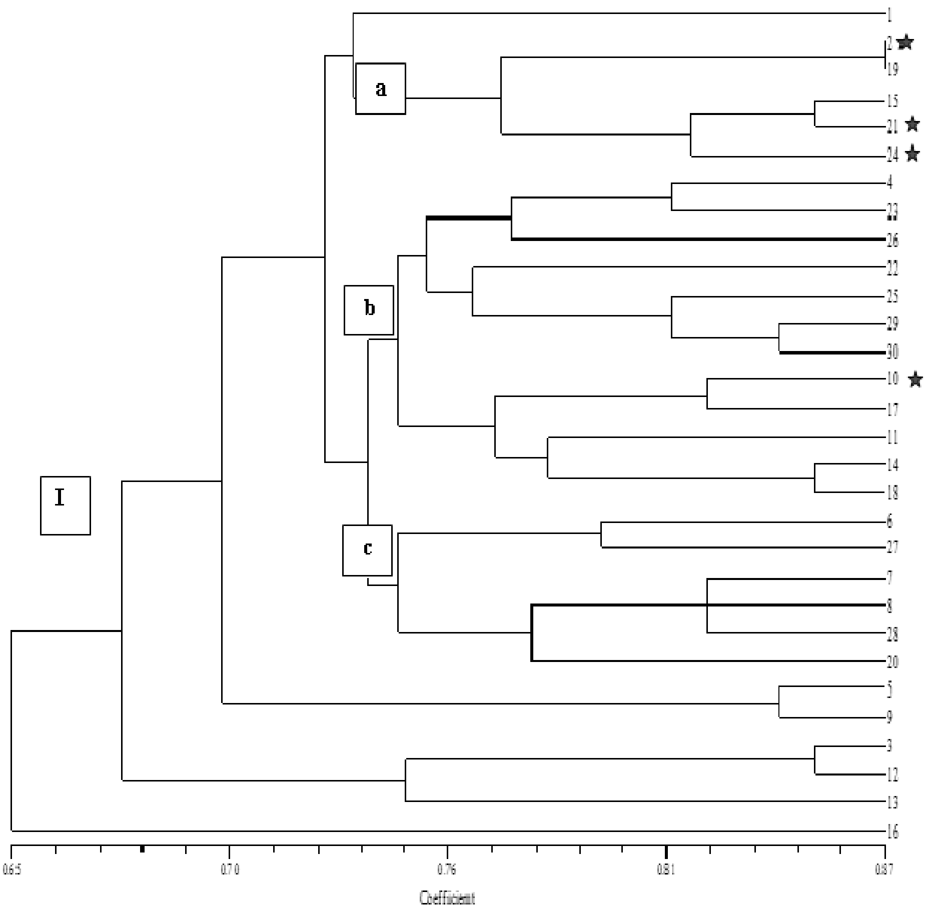


Figure 4 An ISSR pattern in mungbean (UBC821). Lane 1 & 32 are marker lanes.

Table 6 List of 22 primers and amplification products in 30 genotypes of mungbean

Marker	Primer sequence	Total no. of amplification products	No. of polymorphic products	Percentage of polymorphism	PIC value
UBC820	GTGTGTGTGTGTGTGTC	2	1	50	0.67
UBC836	AGAGAGAGAGAGAGAGYA	6	4	66.6	0.39
UBC821	GTGTGTGTGTGTGTGTA	5	4	80	0.63
UBC844	CTCTCTCTCTCTCTCTRC	4	1	25	0.59
IS 61	GAGAGAGAGAGAGAGAT	4	2	50	0.28
IS 65	AGAGAGAGAGAGAGAGT	4	2	50	0.17
UBC811	GAGAGAGAGAGAGAGAC	3	1	33.3	0.24
IS 63	AGAGAGAGAGAGAGAGC	3	1	33.3	0.26
UBC849	GTGTGTGTGTGTGTGTYA	4	2	50	0.07
UBC855	ACACACACACACACACYT	3	1	33.3	0.12
UBC857	ACACACACACACACACYG	7	4	57.1	0.07
UBC848	CACACACACACACACARG	7	5	71.4	0.11
UBC846	GAGAGAGAGAGAGAGAA	5	4	85.7	0.09
UBC864	ATGATGATGATGATGATG	7	6	85.7	0.05
UBC880	GGAGAGGAGAGGAGA	6	4	66.6	0.17
UBC812	GAGAGAGAGAGAGAGAA	3	1	33.3	0.31
UBC862	AGCAGCAGCAGCAGCAGC	3	1	33.3	0.06
URP 6F	GGCAAGCTGGTGGGAGGTAC	10	8	80	0.12
UBC835	AGAGAGAGAGAGAGAGYC	5	4	96	0.14
UBC859	TGTGTGTGTGTGTGTGRC	4	2	50	0.08
UBC895	AGGTCGCGGCCGCNNNNNNAT	7	6	85.7	0.05
URP 13R	TACATCGCAAGTGACACACC	6	4	66.6	0.13
	Total	108	68		
	Average per primer	4.91		58.31	0.218

Jaccard's similarity coefficient values were calculated and the diversity ranged from 0.65 to 0.87 with an average of 0.76. The UPGMA dendrogram (Figure 5) shows that four mung bean varieties are not in the main cluster (cluster I). This main cluster is divided in three subclusters: a, b and c. Three of the four preferred genotypes by farmers (based on visible traits) are in cluster I-a and the remaining one in I-b. In group I-a the varieties were mostly early maturing, dwarf varieties with resistance to MYMV while in group I-a early maturating, medium sized genotypes with a moderate resistance to MYMV are present.



*selected genotypes (top 4) in PVS by farmers along with the advance mungbean material

Figure 5 Dendrogram of genetic relationships of the thirty genotypes based on ISSR

Conclusion

The data presented show the importance of the perspective of farmers in relation to production. According to Bunders *et al.* (1996), farmers should be considered an important research partner because of their indigenous knowledge and capacity to innovate. Genotypes selected by farmers possesses most preferred quality traits; early maturing, MYMV disease resistance, high yield, shiny, green, medium sized, high protein and iron content grains. They have capacity to utilize available genetic resources to manage disease resistance and also they are aware of the integrated management approaches to control MYMV. Farmers can be helpful in participatory technology development especially breeding (Quaye *et. al.* 2011). Since farmers tend to save seeds, it is not sufficient to release varieties which meet the desires of farmers, but also to train them in how to maintain quality in seed conservation (Bains *et al.* 2004).

The chemical studies show the variation for iron and zinc content in the selected germplasm. As genotypes vary in their nutritional components it is important to incorporate this knowledge in a breeding programme to breed varieties that contain high levels of micronutrients along with high potential yield and MYMV resistance and other preferences from farmers. Future research should consider the following important four aspects before designing any interdisciplinary programme. First, farmers preferences, second, correlation between micronutrients and yield, third association between the mineral concentration and anti-nutrients while the fourth is the bioavailability of these micronutrients.

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References

Ajibade SR, Weeden NF, Michite S (2000) Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111(1): 47-55.

Anwar F, Latif S, Przybylski R, Sultana B, Asraf M (2007) Chemical composition and antioxidant activity of seeds of different cultivars of mungbean. Journal of Food Science 72 (7): 503-510.

Ashraf M, Mueen-ud-din M, Warraich NH (2003) Production efficiency of mungbean as affected by seed inoculation and NPK application. International Journal Agriculture and Biology 5:179-180.

Bains TS, Brar JS, Singh G, Sekhon HS, Kooner BS (2004) Status of production and distribution of mungbean seed in different cropping seasons. In: Shanmugasundaram, S. (Ed.). 2006. Improving income and nutrition by incorporating mungbean in cereal fallows in the Indo-Gangetic Plains of South Asia DFID mungbean project for 2002-2004. Proceedings of the final workshop and final meeting, Punjab Agricultural University, Ludhiana, Punjab, India, 27-31 May 2004. AVDRC– The World Vegetable Centre, Publication No. 06-682.

Bunders J, HaverKort B, Hiemstra W (1996) Biotechnology: Building on farmers' knowledge. Macmillan Education Ltd. London and Basingstoke.

De Candolle AP (1884) Origin of cultivated plants. Kegan Paul, Trench, London.

DOES (2000) Directorate of Economics and Statistics, Ministry of Agriculture, Government of India. New Delhi. Agricultural Statistics at a Glance.

IASRI (1999) Indian Agricultural Statistics Research Institute (IASRI), Agricultural research data book, New Delhi.

Lindsay WL, Norvell WR (1978) Development of DTPA soil test for zinc, iron manganese and copper. Soil Science Society of America Journal 42:421-428.

Maiti S, Basak J, Kundagrami S, Kundu A, Pal A (2011) Molecular marker-assisted genotyping of mungbean yellow mosaic India virus resistant germplasms of mungbean and urdbean. Molecular Biotechnology 47(2): 95-104.

Murleedhar A, Chaturvedi SK, Mishra N (2013) Quality seed maintenance of pulses in view of resource conservation agriculture. Indian Institute of Pulses Research, Kanpur

Muthuswamy S, Kanagarajan S, Ponnuswamy S (2008) Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. Electronic Journal of Biotechnology 11 (3): 1-10

Quaye W, Adofo K, Buckman ES, Frempong G, Jongerden J, Ruivenkamp, G (2011) A socio-economic assessment of cowpea diversity on the Ghanaian market: implications for breeding. International Journal of Consumer Studies 1-9.

Raeder U, Baroda P (1985) Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1: 17–20.

Rohlf FJ (1990) NTSYS -pc. Numerical taxonomy and multivariate analysis system. Exeter Software, New York.

Shanmugasundaram S (2002) Improving income and nutrition by incorporating mungbean in cereal-fallows in the Indo-Gangetic Plains of South Asia—an overview. Paper presented in the Planning Workshop of the DFID/Mungbean Project, 23–25 May 2002, Andhra Pradesh, India.

Singh G, Sharma YR, Kaur L (1992) Methods of rating mungbean yellow mosaic virus in mungbean and urdbean. Plant Disease Research 7 (1): 1-6

Souframanien, J. and Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in black gram genotypes using RAPD and ISSR markers. Theoretical and Applied Genetics, **109:** 1687-1693.

Statistical abstracts of Haryana 2003, 2004, 2005 & 2006. Annual reports Nehru library, CCS HAU, Hisar.

Vavilov NI (1926) Studies on the origin of cultivated plants. Inst. Bot. Appl. et d' Amelior. des Plantes. State Press, Leningrad. (In Russian and English).

Vijayalakshmi P, Amirthaveni M, Devadas RP (2001) Possibilities of increasing bioavailability of iron from mungbean and study on the effects of its supplementation on children and women. Project Report. Coimbatore, India: Avinashilingam Institute for Home Science and Higher Education for Women.

Vijayalakshmi P, Amirthaveni S, Devadas RP, Weinberger K, Tsou SCS, Shanmugasundaram S (2003) Enhanced bioavailability of iron from mungbean and its effects on health of school children. Asian Vegetable Research and Development Centre. Shanhua, Tainan, Taiwan 741, Republic of China (ROC). Technical Bulletin No. 30:3.

Appendix I

Questions (part I & II) for the appraisal study of the mungbean agronomical, breeding, and MYMV situation in Hissar, Haryana.

Part I: General questions related to principle crops with emphasis on mungbean.

1. What are the principle crops that you cultivate?

   Cereals ….. Legumes …. Fruits ….. Flowers ……. Other ……

   (*If mungbean is mentioned, ask the following*

2. If mungbean, what benefits do you get from this crop?

3. Are there other benefits you can derive from this crop but not getting now?

   Why is it so?

4. What varieties do you have and how do you use them for your farming activities?

5. What special characteristics do they have?

6. To what extent has the environment affected your cultivation of the crop?

7. Are there any undesirable traits you find with these desired varieties?

   (*If low productivity is mentioned then ask*)

8. Can you let us know the main cause of low productivity of mungbean?

   Biotic stress …… abiotic stress …. Soil ….. No irrigation ……

   Earliness …… No good quality seed ………

   No technical assistance …….. More than one mentioned before ……

   Others (specify) ………..

9. Mungbean yield is severely constrained by?

   a. low yield b. Mungbean yellow mosaic virus (MYMV)

c. lack of better variety

Part II: As mungbean yellow mosaic virus (MYMV) is one of the most important causes of crop loss in mungbean, therefore few questions related to MYMV are as follows:

1. Considering the items of question 10, you rank the two more important reasons of the low productivity of mungbean?

   a …………………….. b ……………………..

2. Do you know, what is the cause of MYMV?

   Yes ………………….. No ……………………

3. Where do you find the MYMV on the mungbean plant?

   Stems ……. Pots …… Leaves ….. Seeds …… Others ……….

4. Do you know how to identify MYMV resistant varieties?

   Yes ………………….. No ……………………

   If yes, How ……….

5. Do you know how to bread MYMV resistant mungbean varieties?

   Yes ………………….. No ……………………

6. Do you know how to manage the MYMV of mungbean?

a. Good mungbean variety

b. Pesticides

c. Integrated pest management (IPM)

d. Others

e. No management f MYMV

7. Do you have any other characteristics you would want to be in your desired varieties?

8. What are the challenges you encounter in your cultivation of the crop beside MYMV?

9. Are there any hopes for you to overcome these challenges by yourself?

CHAPTER 4

How Quality Traits of Mungbean can enhance Food Sovereignty

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Abstract

The study was conducted in 2007 in the Hisar district, Haryana India by an interdisciplinary team of a social scientist, a plant breeder, a food technologist and a nutritionist. The local perspective (farmers, consumers and processors) was given utmost importance in defining quality traits for mungbean in relation to food sovereignty. The results show that farmers prefer high yielding, early maturing and mungbean mosaic virus (MYMV) resistant varieties. Consumers prefer shiny, green, medium sized beans with a short cooking time and a good storability. Processors prefer shiny, green beans with a good soaking capacity and consistency. As mungbean cultivar MH 125 (*Basanti)* possesses most of the preferred quality traits (table 4, chapter 3) the use of MH 125 should be promoted along with training in, how to maintain seed quality and what the best processing methods are.

Keywords: Quality, Mungbean, Local perspective, Food sovereignty

Introduction

India is the largest producer and consumer of pulses in the world. According to FAO 2008, India produces about 25 % and consumes 27 % of the global production. Among pulses, mungbean (*Vigna radiata*), also known as green gram is one of the most important crops in India and it is widely cultivated throughout the country. Resource poor farmers produce mungbean because it only requires moderate irrigation and other inputs. It also restores soil fertility through symbiotic nitrogen fixation and it is a suitable crop for dry land and it can withstand high (average 35 °C) temperatures. Mungbean is important for the vegetarian population of the country, being a major source of protein. It contains 25-31 % of crude protein (Anwar *et al.* 2007), 4-6 mg/100 g of iron (Vijayalakshmi *et al.* 2001), 355-375 Kcal /100 g of energy, and 1-5 % crude fibre (Shanmugasundaram 2004).

Despite its importance and the large numbers of varieties and agro techniques, the production and productivity of mungbean in India is static and the per capita availability has even declined over the past decades (IASRI1999; DOES 2000; Vijayalakshmi *et al.* 2003). This is a major concern since mungbean is nutritious and potentially rich in minerals. Minerals like iron and zinc are important and anaemia due to iron deficiency is a serious threat. In India the occurrence of anaemia is approximately 74 % in children below 3 years, 85 % in expectant mothers and 90 % among adolescent girls of 10-16 years (MOHFW 1998-1999; ICMR 2001; Sharma 2003). In 2007 a survey was conducted on the prevalence of iron deficiency anaemia in relation to dietary intake pattern of local communities in Hisar-1 and Barwala block of Haryana state (Rani *et al.* 2009). Fifty eight percent of the school children in these communities were anaemic of which 49 % lacked enough iron. The quality of food in these communities was poor, particularly low iron bioavailability which ranged between 3.1 - 4.6 % in comparison to healthy adult iron absorption i.e. 10 -15%. The bioavailability of the micronutrients in food can change during processing and storage. Interdisciplinary research linking plant properties, food technology, nutritional characteristics and social sciences is needed in order to benefit as much as possible of the consumption of mungbean.

Methodology

The study was conducted in year 2007 by an interdisciplinary team consisting of four researchers in the field of plant, food, nutrition and social sciences. The researchers have been working with farmers, food processors and consumers with the main objective to apply the 'Science in Society' approach. Following this approach, research into the enhancement of varieties from agronomic, processing, and nutrition perspectives, has been done within local communities.

The research was executed in the Haryana state of India where a high level of mungbean biodiversity is present and in which the Hisar district is an important mungbean production area (Figure 1 and Table 1, chapter 3 (this thesis)). Two representative major mungbean producing blocks of the district, i.e., Hisar-I and Barwala, were selected for the study. Two villages, *Mangali* and *Dhiktana* were selected from Hisar-I and Barwala blocks. One hundred farmers, 150 consumers, 100 processors and 116 rural women from each village were selected randomly for the survey studies. While, for the participatory varietal selection (PVS) programme at the research fields only actively participated farmers were invited. Data were obtained through intensive observation and interaction with farmers, processors, and consumers of mungbean using structured questionnaires (Appendix I, chapter 3).

Results have been interpreted against the background of food sovereignty. Food sovereignty has been defined as the right of people and local communities to define their own food and agriculture; to protect and regulate domestic agricultural production and trade in order to achieve sustainable development objectives (People's Food Sovereignty Network 2004). We have identified definitions of quality from different local perspectives (farmers, processors, consumers) to define one's own agriculture and understand their perceived needs for production, processing, consumption, and nutritional aspects, which ultimately will be of significance to their food sovereignty.

Our focus on production and consumption networks in two villages is in conjunction with the conclusion. A synthesis report (2007) from the Nyeleni Forum states that "the majority of the world's food is still being produced or harvested at relatively small scales by local communities, based on local knowledge, using locally based technologies and locally available resources."

Results

Mungbean quality preferences

Production

For the majority of farmers Mungbean Yellow Mosaic Virus (MYMV) and low yield were the main constraints. After interviews and personal meetings with farmers, they were invited to experimental sites where mungbean was grown. In order to involve farmers in the selection procedure Participatory Varietal Selection (PVS) was carried out to identify important target (quality) traits and to evaluate and comment on new varieties. Twenty farmers from both villages were invited for informal discussions. During such visits they expressed their opinion, identified important target traits and made selections (Table 1).

Table 1 Most preferred quality traits by farmers in participatory varietal selection

No.	Plant characteristics	Comments
1	Plant height	Medium height plants
2	Plant growth	Less vigorous plants
3	Pod length	Pods must be long so that it can contain more seeds
4	Disease resistant	Disease resistance is one of the most preferred traits
5	Size of seed	Medium sized seeds (short cooking time is a must for consumers)
6	Colour of seed	There is a preference for green (better market acceptance)
7	Pod Maturity	Pod maturation should be uniform so that as many as possible pods can be harvested at the same time (less labour cost)
8	Number of seeds/pod	As many seeds as possible (higher yield)

Agronomic traits which were considered important by farmers were

Potential yield: The potential yield of mungbean without any disease pressure ranges from 12 Q/ha to 23 Q/ha i.e. 100 kg/10,000 square meters (Table 2). **Mungbean Yellow Mosaic Virus (MYMV)** is a limiting factor according to farmers since it causes heavy yield loss, varying from 30 to 100 % (Nene, 1972). Farmers can identify the disease in an early stage because infected plants are stunted in growth and usually mature late. Such plants produce very few flowers and curled small pods and consequently shrivelled seeds. Farmers could select lines which were more or less resistant to MYMV (Table 2). A **short duration** of the growing season is another important factor which influences the preference of farmers. Farmers preferred early maturing mungbean varieties (~ 65 days) so that they can incorporate it in a rice-wheat system and grow all three crops in a year. An additional advantage is that mungbean production prior to wheat production improves soil fertility as mungbean fixes atmospheric nitrogen.

Table 2 Top five varieties selected on basis of participatory varietal election*

Varieties	i. MH 125 *(Basanti)* ii. MH 215 (*Sattya*) iii. MH 318 iv. MH 421 (*Bharpai*) v. MH 96-1 *(Muskan)*

*table 4, chapter 3 (this thesis)

Mungbean is eaten separately or in combination with cereals. After saltening and sweetening mungbeans are eaten as *dhal* (thick spiced soup), while mung pudding called '*halwa*' and *'ladoo'* is commonly served as dessert on festivals and other special occasions. Rural consumers prefer non broken, medium sized mungbeans with a shiny, green colour, and without traces of disease (Table 3). Furthermore, a **short cooking time** is highly appreciated. Consumers knew that shiny, green coloured and medium sized mungbeans have a shorter cooking time compared to the light green small sized mungbeans. Sixty six percent of the rural consumers cooked mungbeans without soaking, sprouting and fermentation.

Table 3 Consumers' preferences for quality traits of mungbean (N=300)

Attributes	**N***	**(%)**
Shiny, green, medium sized	297	99
Short cooking time	270	90
Storability	180	60

* Multiple responses possible

Storability is another important quality trait of mungbeans for a majority of the consumers (60 %), mungbean is seasonally produced (mainly during *kharif* season July–September) but consumed throughout the year. After harvest mungbeans are solar dried for 8-10 hours to prevent insect infestation and then stored in mud bins, metal drums or gunny bags. Mud bins are cylindrical in shape and made of clay mixed with straw and animal dung, or from mud and bricks. Metal drums are cylindrical vessels made of iron sheets whereas gunny bags are made of jute. Activated charcoal, ash and sand are also used as endogenous method by the rural people to check insect infestation. Activated charcoal fills the space in between the grains thus making hard for insects to get enough oxygen while ash and sand scratches the outer skin of the insects resulting in water loss from the body of insects. The study revealed that the majority (90 %) of the consumers understood and preferred quality mungbeans.

Processing

It is also important to know the preferences of processors (Table 4). The **size of the grains** is important for processing and the majority (87 %) of the processors preferred medium sized grains for sprouting because the water soaking capacity of medium sized mungbean is higher. Whole mungbean dhal made from medium sized grains has a better consistency. Another trait is the **colour** because this directly relates to the colour of mungbean products. Processors prefer mostly a dark green colour of most of their products such as whole mungbean dhal, split mungbean dhal and whole mungbean *ladoo*. Yellow colour of split mungbean grains is preferred for dishes such as split dehulled mungbean *dhal* and mungbean *bhalle*.

Table 4 Influence of the quality parameters of mungbean on product quality

Quality Parameters	Influence on Products' Quality
Size	Consistency (*dhal, a mildly thick stew prepared with mungbean*)
Colour	Colour (*dhal, bhalle-mungbean dumplings*)
Texture	Consistency of mungbean products (*dhals*)
Moisture	Consistency and appearance of products (*mung dhal namkeen- salty crisp snacks)*
Surface shininess (appearance)	Appearance of mungbean products (*halwa* and *burfi- sweet dish), mungbean sprouts)*

Texture is another important characteristic of mungbean products. More brittle grains take less time to cook and were more easily processed into various mungbean products. Moreover, brittle grains give a better consistency of the products particularly in three types of dhals; whole, split and split dehulled mungbean dhal. **Surface shininess** of mungbeans is important for the appearance of mungbean products such as mungbean sweets like *halwa* and *burfi.* Similarly, shiny grains were preferred in case of *dhals* and mungbean sprouts. Too much **moisture** is not good in processing most of the mungbean products. For different products different characteristics are wanted (Table 5); soaking and cooking time (*dhals*), grinding, soaking and cooking (*halwa*), soaking and frying time (*namkeen*), consistency of mungbean paste (*dahi bhale, pakore)*, grinding and fermentation time (*wadi*), germination time (*sprouts*), drying period (*papad*). Sensory characteristics of some of the mungbean products is influenced by the quality of mungbean grains. Most important sensory parameters for the products are: consistency (Mungbean *dhal*), color, texture (*halwa*), taste and texture (*ladoo, papad* and *namkeen*), texture (*dahi bhale*), consistency (*khichadi*), texture (*wadi*), appearance and texture (sprouts).

Table 5 Different mungbean products along with their description

Mungbean Food Products	**Description**
Whole Dhal	Thick spiced soup of whole mungbean grain
Split Dhal	Thick spiced soup of split mungbean grain
Split dehulled Dhal	Thick spiced soup of split dehulled mungbean grain
Whole Ladoo	Round yellow sweet made from whole mungbean flour
Split dehulled Ladoo	Round sweet made from split dehulled mungbean
Burfi	Sweet made of dehusked mungbean paste
Halwa	Sweet pudding made of split dehulled mungbean paste
Namkeen	Fried split dehulled mungbean spiced snack
Papad	Spiced snack of mungbean dough sheets made after roasting
Bhalle	Fermented dehuked mungbean roundels
Pakore	Fried dehuked mungbean roundels
Wadi	Fermented and dried mungbean paste
Kichari	Mungbean cooked with rice
Sprouts	Germinated whole mungbean

Nutrition

Two hundred and thirty two women were asked for their quality preferences of mungbean (Table 6). More than 70 % of the women preferred a mungbean variety that is rich in protein content and therefore healthy. Sixty four of the women preferred a mungbean variety rich in iron content that keeps their body away from weaknesses. Further, the majority of women (85 %) desired a mungbean variety of which the nutritional compounds are well absorbed and with no or a low level of antinutrients**.**

Table 6 Rural women' perception for nutrition quality traits of mungbean; N=232

Description for variety preference in local language	N*	(%)	Inference
A variety that can help to maintain their body healthy	169	73	Food rich in protein like mungbean was preferred by the rural women
A variety that can keep weakness away from their body	148	64	They knew about the benefits of mungbean and preferred to have mungbean dhal in their diet
Is soaking of legumes prior to cooking good or not?	197	85	They knew soaking prior to cooking is good as it removes antinutrients and also reduces cooking time. Thus effort should be made to make a cultivar less in antinutrients

*Multiple responses

The five mungbean varieties grown on agricultural farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar were nutritionally analysed (Table 7). 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). The PA: Fe molar ratio among these five varieties ranged from 12.5 to 16.9, which means a good iron bioavailability.

Table 7 Content of protein, mineral micronutrients, phytic acid and polyphenols in selected mungbean varieties.

Variety	Protein (g/100g)	Zinc (mg/100g)	Iron (mg/100g)	Phytic acid (mg/100g)	Poly-phenols (mg/100g)	PA: Fe molar ratio
MH 96-1 ***(Muskan)***	24.20 ± 0.30	4.60 ± 0.17	5.52 ± 0.15	880 ± 18.8	265 ± 10.2	13.48
MH 125 ***(Basanti)***	24.39 ± 0.71	4.42 ± 0.88	5.63 ± 0.61	832 ± 10.6	294 ± 9.22	12.50
MH 215 **(*Sattya*)**	24.30 ± 0.22	3.78 ± 1.19	4.43 ±1.12	890 ± 10.0	207 ± 8.57	16.99
MH-318	24.02 ± 0.44	4.43 ± 0.18	5.11 ± 0.20	1043 ± 13.05	264.96 ± 10.00	17.27
MH 421 ***(Bharpai)***	24.68 ± 0.77	4.52 ± 0.34	5.43 ± 0.27	855 ± 12.4	237 ± 7.46	13.32

Values are mean ± SD of three independent determinations

Discussion

Significance of quality in food sovereignty

The data presented in this article show an unique perspective of local people on the meaning of quality of food (mungbean). Defining the desired traits of mungbean from a perspective of production, processing and consumption is a modest but important step in developing food sovereignty. In fact, the definition of problems and challenges, from a local perspective, is necessary for developing a research agenda needed for food sovereignty. This implies that farmers should play a role in research. By virtue of their creativity, their capacity to innovate, and their knowledge, they should be viewed as an valuable source of information (Bunders *et al.* 1996).

Farmers have a preference for early maturing mungbean varieties that are MYMV resistant and better yielding. The MH 125 *Basanti* variety scores high for these preferences: disease resistance (MYMV), high yield (1500 Q/ha) and early maturing (65 days). Since farmers tend to save seeds for sowing, it is not sufficient to release good varieties but also to train farmers in good seed conservation (Bains *et al.* 2004).

This study reveals that consumers' knowledge and traditional practices play a critical role in their perception of mungbean quality traits. Consumers prefer shiny, green coloured and medium sized varieties. The MH 125 *Basanti* variety has the preferred quality traits. The beans are shiny, green and medium sized and have a short cooking time. Rural consumers just cook the mungbeans, it is important to train them with other methods of processing such as soaking, fermentation and sprouting to maintain a sufficient intake of mungbean. Consumers also prefer a longer storability of mungbean. Mungbean is consumed throughout the year and is susceptible to insect infestation. The processing technology of milling can increase storability.

Shiny, green and medium sized varieties are preferred by processors. These varieties have good water soaking capacity, better processing properties and their products have a good appearance and colour. The MH 125 *Basanti* variety also possesses the preferred characteristics for processors.

For breeding new good varieties of mungbean it is desirable to determine which variables of a variety influence the quality of the product. Apart from that it needs to be determined what the effect is of the different processing methods on sensory properties. The MH 125 mungbean variety is a good candidate to do sensory studies with local consumers or sensory experts with products made with this variety. The behaviour of the MH 125 *Basanti* variety in different processing steps needed to prepare different mungbean products needs also to be investigated.

Nutritional perceptions of local people in their food (mungbean) are important since their nutritional status is poor (Rani *et al.* 2009). The local people indicated their wish for high protein, high iron and less anti-nutrients containing mungbean varieties. The MH 125 *Basanti* variety contains relatively high protein (24.39 g/100g), iron (5.63 mg/100g) and zinc (4.42 mg/100g). The PA:Fe molar ratio of 12.50 indicates a good iron bioavailability. The use of the MH 125 *Basanti* variety in local diets will most likely improve iron and other nutritional status of local people.

Conclusion

Local people define their own quality traits of mungbean and needs of production, processing, consumption, and nutrition which may contribute in developing new mungbean varieties. MH 125 *Basanti* variety of mungbean possesses most preferred quality traits and incorporation of MH 125 *Basanti* variety might satisfy the local needs of production, processing, consumption and nutrition. Further quality improvements in MH 125 *Basanti* variety through production, processing and consumption would help food sovereignty. It is needed that the characteristics of MH 125 *Basanti* in different processing methods are investigated to determine the effect of the variety on quality of mungbean products. For their food sovereignty local people need also to be trained to maintain and use quality seeds for future mungbean cultivations.

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References

Anwar F, Latif S, Przybylski R., Sultana B, Asraf M (2007) Chemical composition and antioxidant activity of seeds of different cultivars of mungbean. Journal of Food Science 72 (7): 503-510.

Bains TS, Brar JS, Singh Gurdip, Sekhon HS, Kooner BS (2004). Status of production and distribution of mungbean seed in different cropping seasons. In: Shanmugasundaram, S. (Ed.). 2006. Improving income and nutrition by incorporating mungbean in cereal fallows in the Indo-Gangetic Plains of South Asia DFID mungbean project for 2002-2004. Proceedings of the final workshop and final meeting, Punjab Agricultural University, Ludhiana, Punjab, India, 27-31 May 2004. AVDRC– The World Vegetable Centre, Publication No. 06-682.

Bunders J, Bertus H, Wim H (1996) Biotechnology: Building on farmers' knowledge. Macmillan Education Ltd. London and Basingstoke.

DOES (2000) Directorate of Economics and Statistics, Ministry of Agriculture, Government of India. New Delhi. Agricultural Statistics at a Glance.

Food and Agriculture Organization of the United Nations (FAO) (2008) "FAOSTAT." http://apps.fao.org/default.htm. Accessed: October 2008.

IASRI (1999) Indian Agricultural Statistics Research Institute (IASRI), Agricultural research data book, New Delhi.

ICMR (2001) Indian Council of Medical Research. Micronutrient deficiency disorders in 16 districts of India. Part I. Report of an ICMR Task Force Study-District Nutrition Project. New Delhi.

Karunaratne AM, Amerasinghe PH, Sadagopa Ramanujam VM, Sandstead HH, Perera PAJ (2008) Zinc, iron and phytic acid levels of some popular foods consumed by rural children in Sri Lanka. Journal of Food Composition and Analysis 21**:** 481– 488.

MOHFW (1998-1999) Ministry of Health and Family Welfare. Key findings. National Family Health Survey -2. Mumbai, MOHFW, Government of India/International Institute of Population Studies.

Nene YL (1972) A survey of viral diseases of pulse crops in Uttar Pradesh, G.B. Pant University Agriculture and Technology., Pantnagar, Research Bulletein No. **4**.

People's Food Sovereignty Network (2004) Beijing Declaration from NGO/CSO Regional Consultation: From Agenda to Action' Follow-up to the NGO/CSO Forum for Food Sovereignty. www.peoplesfoodsovereignty.org/statements/new/23.htm

Rani V, Brouwer Inge, Khetarpaul N, Zimmerman MB (2009) Iron status and associated factors in rural school going children (5-8 years) of Haryana state, India., Abstract Book Wageningen Nutritional Sciences Forum March 4–6, 2009 European Journal of Clinical Nutrition 63 (3S):20

Sharma KK (2003) Improving bioavaibility of iron in Indian diets through food–based approaches for the control of iron deficiency anaemia. Food Nutrition and Agriculture (FAO) Alimentation 32: 51-61.

Shanmugasundaram S (2004) Improving income and nutrition by incorporating mungbean in cereal fallows in the Indo-Gangetic plains of south Asia. Proceedings of the final workshop and meetings, 27-31 May 2004. Punjab Agricultural University, Punjab India.

Synthesis Report: Nyeleni (2007) Forum for Food Sovereignty, February 23 - 27, 2007, Selingue, Mali, March 2007. http://www.nyeleni2007.org/; Accessed: November 2008.

Vijayalakshmi PM, Amirthaveni S, Devadas RP (2001) Possibilities of increasing bioavailability of iron from mungbean and study on the effects of its supplementation on Children and Women. Project Report, Coimbatore. India: Avinashilingam Institute for Home Science and Higher Education for Women.

Vijayalakshmi P, Amirthaveni S, Devadas RP, Weinberger K, Tsou SCS, Shanmugasundaram S (2003) Enhanced bioavailability of iron from mungbean and its effects on health of school children. Asian Vegetable Research and Development Centre. Shanhua, Tainan, Taiwan 741, Republic of China (ROC). Technical Bulletin No. 30: 32.

CHAPTER 5

Genetic variation and correlation studies between micronutrient (Fe & Zn) content, protein content and some quantitative traits in mungbean (*V. radiata* L.)

Renu Singh, Richard G.F. Visser, Ram Kumar and Adriaan W. van Heusden

Abstract

Mungbean can effectively contribute to the alleviation of iron, zinc and protein malnutrition as it is a source of micronutrients and protein. To achieve a faster improvement of the current bad situation it would help tremendously to improve mungbean cultivars which are even richer in micronutrients and protein. However in most breeding programmes to date most attention is being directed towards yield. Breeding mungbean for enhanced grain nutrients is still in its start-up phase. The present study was carried out to assess genetic variation for both quantitative as well qualitative traits. The correlation between important traits such as yield and high Fe, Zn and protein content was calculated. A positive correlation was found between iron and zinc content (r = 0.47) and no significant correlations were observed with yield. Breeding a cultivar which is nutritionally improved along with high yield seems therefore possible. A few promising cultivars with high micronutrient contents, protein content and overall yield were identified. These cultivars can be used in specific breeding programs aiming at nutrient-rich high yielding cultivars.

Keywords: Correlation, iron, mungbean, protein, quality traits, quantitative traits.

Introduction

Mungbean or greengram (*Vigna radiata* L) is one of the most important food legumes of the genus *Vigna*. Especially for the vegetarian population it is a good source of protein, carbohydrates, vitamins and minerals (Adel *et al.* 1980; Amarteifio and Moholo 1998). Proteins of grain legumes are rich in lysine and threonine but poor in methionine and tryptophan. In order to increase the nutritional value of meal, grain legumes should be eaten together with cereals. Regular consumption of pulses is an excellent method to overcome malnutrition, especially among growing children, pregnant women and nourishing mothers.

In developing countries cereal grains and food legumes are the primary source of calcium, iron and zinc but their intake nowadays is not high enough. Studies have shown that in developing nations 26% of the population is anaemic, while this is 10% and 11% in Europe and the US respectively. Studies by Barclay *et al.* (1996) and Rosado *et al.* (1992, 2007) have shown that anaemia is mainly caused by iron deficiency. Forty per cent of iron intake is coming from legumes and cereals. Besides iron, zinc is also an essential micronutrient for normal growth, appetite and immunity. It is an essential component of more than one hundred enzymes involved in digestion, metabolism and wound healing (Stauffer 1999). While iron deficiency has long been considered a major nutritional problem, zinc deficiency has only recently been recognized as a public health problem (Ranum 1999).

To start a breeding program to improve mungbean, variation is required for yield but also for iron and zinc content and other quantitative traits. Knowledge regarding the availability of such variation, the genetic background causing differences and response to different environmental conditions is important.

The majority of the breeding research has had its emphasis on yield and resistance against biotic and abiotic stress while little attention has been paid to nutritional value. It is known that concentrations of micronutrients vary in tissue or seed between cultivars and that this variation is partially genetically determined. Literature shows comparable ranges of mineral concentrations in most leguminous crop seeds like in common bean, peas, chickpeas, lentils etc. (Islam *et al.* 2002; Grusak and Cakmak 2005; Haq *et al.* 2007; Thavarajah *et al.* 2010). In the light of such variation in related leguminous crops a study was done to search for variation in mungbean (*V. radiata* L.). As for the producers (farmers) quantitative traits such as yield are of more importance the correlation was studied between some of these quantitative traits and micronutrient and protein content. Besides correlation studies to assess the direct and indirect contribution of the individual components towards other traits a path co-efficient analysis was carried out. Correlations among the variables were estimated to know the association, if any, among the variables. Association in terms of direction as well as magnitude. These correlations were used in path analysis to know the direct and indirect contribution of each independent variable (days to maturity, number of branches per plant etc.) towards dependent variables (like e.g. seed yield). The direction and magnitude of direct and indirect contribution of independent variables to seed yield helps in selecting the important variables for seed yield.

The overall objective of this study is to obtain better yielding cultivars with a considerable content of micronutrients and proteins. The availability of such cultivars will improve the status of the people depending on mungbean as the major source of protein and micronutrients.

Material and Methods

Plant materials

The experimental material comprised of ninety two elite mungbean cultivars along with three controls i.e. MH-215, MH-318 and MH-421 (appendix I). These cultivars were provided by the Pulses Section, Department of Plant Breeding, CCS HAU, Hisar (India).

Field trials and experimental design

The material was grown in two different years (July to September 2007 and 2008) at the university experimental farms of Hisar. Hisar is geographically situated at latitude 29^{o}10'N, longitude 75^{o}46'E and altitude 215.2 m above sea level and falls in the semi-tropical region of the Western Zone of India. The overall weather data during the course of experiment (2007, 2008 like temperature (maximum/minimum), relative humidity, rainfall, bright sunshine hours) was collected with the help of the meteorological observatory of the Department of Meteorology, CCS HAU, Hisar (appendix II; Figure A, B).

Recommended pesticides were used to protect the crop during both years. The overall mean maximum/minimum temperatures during the period of the study were 35.3/24.6 ^oC (2007), 34.5/24.4 ^oC (2008) , while the mean relative humidity (morning and evening) during the experiment was 86.5% / 87.6% (morning 2007, 2008), 56.5% / 58.9% (evening 2007, 2008) and rainfall 14.1 mm / 28.5 mm respectively. Data for some of the agronomic traits were taken at different time points like days to 50% flowering was when the first flower makes its appearance and days to maturity is when most (90%) of the pods on the plant became dark brown in colour. About two weeks before harvesting, the severity of mungbean yellow mosaic virus (MYMV) damage was scored on a scale from 1 to 9. (Singh *et al.* 1992). For yellow mosaic virus symptoms a score 1 stands for a completely resistant plant and 9 for a completely susceptible plant. At harvest five random plants were selected from each cultivar from each block and yield and quality parameters were measured. These includes plant height (cm), number of branches per plant, number of pods, MYMV severity, seed yield per plant (g), protein (%), iron (ppm) and zinc (ppm).

Statistical analysis

The mean values of yield and its components from the five random plants of each cultivar from each replication were subjected to statistical analysis. The data for each trait were subjected to analysis of variance of the randomized complete block design described by Panse and Sukhatme (1989).

$$Y_{ijk} = \mu + R_i + G_j + e_{ijk}$$

Where, Y_{ijk} is k^{th} observation on j^{th} G cultivar of i^{th} R replication; μ is overall general mean of the population; R_i is the effect of i^{th} replication (i= 1, 2, 3); G_j is the effect of j^{th} cultivar (i.e. j – 1, 2, 3…..92) and e_{ij} is random error.

For protein content, mineral micronutrient concentration (iron and zinc) and yield, correlation coefficients were calculated. Before calculating, the data recorded in percentage were subjected to angular transformation and the transformed data was subjected to statistical analysis.

Heritability was calculated according to the formula of Hanson et al. (1956): $h^2 = \sigma^2 g/ \sigma^2 p$, where genetic variance $\sigma^2 g$ = (MSg- MSe)/k where k is number of replication, variance due to error $\sigma^2 e$ = MSe and $\sigma^2 p = \sigma^2 g + \sigma^2 e$. genotypic correlation were computed using genotypic variance and genotypic co-variances obtained from the analysis of variance and co-variance in a manner used for heritability estimation as described by Becker (1975). To estimate the inter-relationships among the variables and their contribution to yield performance the data was subjected to correlation coefficient and path coefficient analysis (Dewey and Lu 1969).

Genetic diversity was determined following Mahalanobis's (1936) generalized distance (D^2) extended by Rao (1952). Clustering of genotypes was done according to Tocher's method (Rao 1952) and Principal Component Analysis for graphical representation of the genotypes. Since, Mahalanobis's (1936) played a fundamental and important role in statistics and data analysis, therefore this model is used in the present study. Different clusters were further arranged in order to their relative distance from each other and thus used to calculate inter and intra cluster distances. Average intra- and inter-cluster D^2 values were estimated using the formula: $\Sigma D^2{}_1/n$, where $\Sigma D^2{}_1$ is the sum of distances between all possible combinations (n) of mungbean cultivars included in a cluster. Significance of the squared distances for each cluster was tested against the tabulated $X2$ values at P degree of freedom at 5% probability level, where P represents the number of characters used for clustering genotypes. The intra-cluster distance was calculated to see the genetic diversity among the genotypes of different groups while the inter-cluster distance is used to see the divergence between the genotypes of a same group. Selection of parents from the highly divergent clusters is expected to manifest high heterosis and also wide variability in genetic architecture.

Mineral and Protein Analyses

A sub-sample of 2 g seeds were picked at random from the bulk seed harvest of each cultivar and washed with sterile water and then dried in an oven for 2 days at 45°C before grinding. Mineral analysis was then implemented on powdered sample using atomic absorption spectroscopy (AAS). Sample preparation for AAS involved digesting of 1 g flour with nitric/per-chloric acid (5 ml of a 2:1 mixture of 65% nitric acid (HNO_3) and 70% per-chloric acid ($HClO_4$)) for 2 h followed by a heat treatment for 2 h and re-suspension in 25 ml of de-ionized water. The resulting samples were analysed in a mass spectrometer with acetylene flame (Lindsay and Norvell 1978). While total nitrogen was determined using the Kjeldahl method (Pearson 1973) and protein content was calculated using conversion factors 6.25 (Altschul 1958) i.e.

Crude protein content (%) = nitrogen (%) X 6.2. The data of mineral micronutrient and protein means along with their standard error are presented in appendix I.

Results

Correlation and path analysis

All analysed traits are complex and influenced by several interdependent traits. Therefore selection for particular traits will not be effective unless the other traits influencing it directly and indirectly are taken into consideration. Therefore for all the seven traits the genotypic and phenotypic correlation coefficients were calculated (Table 1).

Table 1 Heritability (in parenthesis), Genotypic (rG), phenotypic correlation (rP) and environmental (rE) correlations among the six agronomic traits in mungbean (*V.radiata* L.)

Traits	r	DM (0.106)	PH (0.452)	BPP (0.207)	PPP (0.219)	MYMV (0.581)	SYP (0.243)
	G	0.503**	0.087	0.353**	0.075	0.190	-0.217
DF	P	0.238*	0.076	0.176	-0.032	0.114	-0.094
	E	0.056	0.064	0.094	-0.096	0.023	-0.021
	G		-0.030	0.077	0.126	-0.013	-0.095
DM	P		-0.042	0.067	0.098	-0.011	-0.008
	E		-0.053	0.064	0.086	-0.010	0.032
	G			0.401**	0.169	-0.186	0.009
PH	P			0.199	0.142	-0.132	-0.027
	E			0.095	0.133	-0.053	-0.055
	G				0.418**	0.157	0.242*
BPP	P				0.407**	0.065	0.238*
	E				0.404	0.009	0.237
	G					-0.261*	0.873**
PPP	P					-0.124	0.169
	E					-0.029	0.520
	G						-0.324**
MYMV	P						-0.157
	E						-0.035

DF= Days to 50% flowering, DM= Days to maturity; PH= Plant height (cm); BPP= No. of branches/plant; PPP= No. of pods/plant, MYMV score (1-9); SYP= Seed yield/plant (g). *Significance at 5%; **Significance at 1%

The estimates of the genotypic correlation (rG) are generally higher than those of the phenotypic correlation (rP). Number of branches/plant and pods/plant have a high genotypic correlation with seed yield/plant which shows the reliability of these traits in determining yield potential in mungbean.

Days to maturity genotypically and phenotypically correlated to days to flowering. Similarly, number of branches/plant showed a positive and high phenotypic and genotypic correlation with number of pods/plant. Since the pods were on the branches, the relationship between number of pods and branches was not unexpected. Another expected correlation was the negative correlation between seed yield and disease symptoms.

The direct and indirect effects of different traits on yield are presented in Table 2. Path coefficient analysis showed that the number of pods per plant had the largest direct effect (0.5456) with an indirect effect through reduction of branches per plant. Branches/plant (0.0884) also had a positive direct effect on seed yield with its largest indirect effect through days to maturity. Table 2 shows that day to maturity, number of branches and pods per plant have a direct positive effect on seed yield.

Table 2 Path coefficient analysis showing direct (diagonal) and indirect effects of seven traits in mungbean (*V. radiata* L.)

Traits	Direct effect on seed yield	Indirect effect on seed yield through					
		DF	**DM**	**PH**	**BPP**	**PPP**	**MYMV**
DF	-0.491	-	0.0494	-0.0101	0.0196	-0.0068	-0.0097
DM	0.1589	-0.0153	-	-0.0056	0.0204	0.0732	0.0128
PH	-0.1001	-0.0049	0.0089	-	0.0199	0.0828	0.0180
BPP	0.0884	-0.0109	0.0366	-0.0226	-	0.2265	-0.0025
PPP	0.5456	0.0006	0.0213	-0.0152	0.367	-	0.0163
MYMV	-0.1208	-0.0039	-0.0168	0.0149	0.0019	-0.0737	-

*Significance at 5%; **Significance at 1%; Residual effect = 0.7623

Genetic Diversity

The success of any crop improvement depends on the amount of diversity available in the crop. To know the spectrum of diversity, the assessment of divergence in the cultivar is essential. Knowledge about genetic variability and genetic divergence are of great value as they play a vital role in selecting the right parents for a successful breeding programme. As mentioned earlier Mahalanobis D^2 was used to access the diversity in population and clustering of genotypes was done according to Tocher's method (Rao 1952). Bhatt (1973) concluded that the application of D^2 statistics to study diversity and to choose parents is a more efficient method compared to choosing the parents based on eco-geography diversity.

Mahalanobis generalized distance (D^2) analysis-cluster analysis

The distance between two cultivars is calculated as the sum of squares of the difference between the mean of all the seven measured traits. The distances were used for the final grouping. Since each cultivar is compared to 91 other cultivars 4186 D^2 values were obtained for 92 cultivars including the 3 controls MH 215, MH 318 and MH 421.

Clustering pattern of cultivars

Treating the estimated D^2 values as the square of the generalized distance, 92 cultivars were grouped into 5 clusters following the method suggested by Tocher (Rao 1952). Clustering of genotypes was done based on the generalized distance (D^2) values using Tocher's method as described by Singh and Chaudhary (1985). Of these five clusters, cluster II had the highest number of cultivars (34) while cluster III is the smallest with only two cultivars (Table 3).

Table 3 Distribution of 92 mungbean cultivars into different clusters

Cluster	**I**	**23**	PMB-14, SMH-991-A, T-44, L-24-2, M-169, MH-318, MH-421, ML-515, ML-776, PDM-9-249, PLM-176, PLM-18, 2KM-151, 2KM-155, 2KM-161, AMP-36-10, AMP-36-4, ASHA, BDYR-2, CH-1355, CH-2103, GP182, GP32
	II	**34**	2KM-111, 2KM-112, 2KM-114, 2KM-116, 2KM-117, 2KM-135, 2KM-139, 2KMH-52, BDYR-1, BG-39, GANGA-8, GP149, GP150, GP248, GP69, GP78, GP861, PLM-62, PLM-65, SMH-991-D, SMH-99-2, SMH993A, HUM7, IC-39574, LM-10, M-1361B, MH-125, MH-98-1, ML-1108, ML-682, ML-759, ML-818, Muskan, P-105
	III	**2**	GP181, GP68B
	IV	**5**	SMH-99-3D, PM-827, ML-5, M-395, HUM-1
	V	**28**	MH419, MH961, MI3580, ML194, ML406, ML506. ML735, ML803, ML839, PLM116, SM99-1, SMH99-4, SML-668, MH 2-15, 2KM-107, 2KM-115, 2KM-138, AMP-36-1, CoGG-90, GP196, GP86, IC103196, IC39595, KM-92-11, M-516, M-605, MH-124, MH-215

Ninety two mungbean genotypes were grouped into five different clusters using clustering technique. A two dimensional scatter diagram was constructed using first canonical variable on X axis and second canonical variable on Y axis, reflecting the relative position of the genotypes (Figure 1). As per scatter diagram the genotypes were apparently distributed into 5 groups.

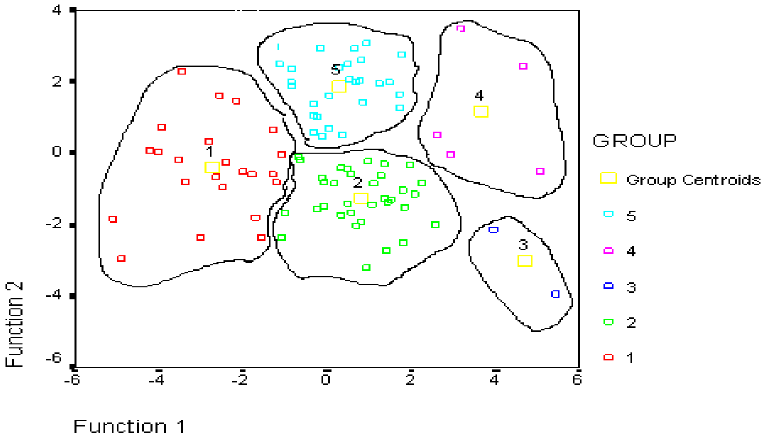


Figure 1 Scatter diagram presenting relationship among 92 mungbean cultivars as revealed by two dimensional plot along with cluster centres.

From the figure it can be seen that in cluster 1 there is more variability than in clusters 2 and cluster 5. Clusters 1 and 2 mainly comprise of early maturing cultivars. Cluster 3 comprise of tall cultivars with higher number of branches and yield per plant while cluster 2 and 5 had cultivars which show resistance to MYMV.

Intra- and intercluster average D^2 values and their relationship between clusters

The intra- and intercluster average value D^2 among the mungbean cultivars is shown in Table 4. Cluster III has the minimum intra cluster value (7.69) indicating that cultivars within the cluster were similar. While cluster IV showed the maximum intra cluster D^2 value (11.86), followed by cluster I (11.22) and cluster II (8.12) revealing the existence of diverse genotypes that fell in these clusters. The average intercluster value D^2 ranged from 19.29 to 48.68, this indicates that, cultivars included in clusters are genetically diverse and may give rise to high heterotic response.

Table 4 Average intra (diagonal) and intercluster D^2 values of mungbean cultivars

Cluster	1	2	3	4	5
1	**11.22**	22.27	48.68	40.34	22.92
2		**8.12**	26.91	22.89	19.29
3			**7.69**	26.76	40.76
4				**11.86**	21.37
5					**7.83**

The intercluster values represent the amount of diversity present among clusters, cluster 1 and cluster 3 show maximum intercluster distances (Table 4). Cluster 1 mostly includes short cultivars (< 60 cm) with fewer branches per plant while in cluster 3 the tall cultivars (> 100 cm) with high number branches per plant are grouped. In both clusters the cultivars took more than 65 days to mature.

Cluster means

Mean values, range, standard deviation and coefficient of variance were calculated for each character (Table 5) along with the cluster means of the five clusters (Table 6).

Table 5 Range, mean with standard error and coefficient of variance for each character based on the agronomic traits in ninety two cultivars of mungbean (*Vigna radiata* L.)

Traits	DF	DM	NBP	NPP	PH	MYMV	SYP
Range	37-51	53-69	1.66-3.8	10.5-66.5	50.7-117	2-9	0.5-14.6
Mean ±	42.1±	61.8 ±	2.57 ±	39.38 ±	75.76 ±	5.02 ±	4.79
S.E	0.28	0.35	0.04	1.13	1.27	0.16	± 0.23
C.V.	6.29	5.48	16.53	27.55	16.08	2.22	45.99

Table 5 shows a coefficient of variation and range for four traits i.e., number of branches/plant, number of pods/plant, plant height and yield/plant. Coefficient of variation shows the degree of variation available in the genotypes for a particular trait. Thus, for these traits heritability estimates along with the coefficient of variation will help in improvement through selection. Table 6 represents the overall mean values along with their ranking for each cluster on the basis of the two year average performance.

Table 6 The mean value of seven traits in five clusters of 92 mungbean cultivars.

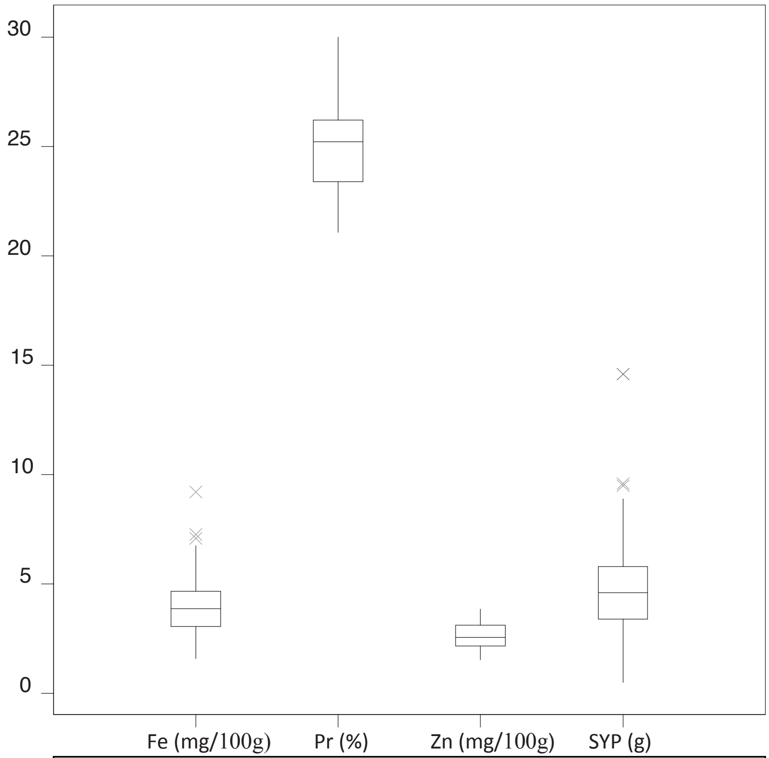
Cluster	No. of cultivars	Character						
		DF	DM	PH	NBP	NPP	SYP	MYMV
1	23	42.04 (4)	61.71 (4)	62.22 (5)	2.29 (5)	29.78 (5)	3.63 (4)	5.47 (1)
2	34	41.96 (5)	61.24 (5)	83.90 (3)	2.56 (4)	34.89 (4)	3.82 (3)	4.88 (3)
3	2	42.92 (2)	66.34 (1)	110.27 (1)	3.01 (1)	35.95 (3)	3.59 (5)	5.00 (2)
4	5	43.33 (1)	61.87 (3)	93.01 (2)	2.85 (2)	55.55 (1)	7.32 (1)	5.00 (2)
5	28	42.32 (3)	62.23 (2)	72.39 (4)	2.73 (3)	50.10 (2)	6.53 (2)	4.82 (4)

Value in parenthesis represents the ranking for each character among five clusters

Micronutrient content and their correlation with seed yield

The mean concentration (± standard error) of Fe, Zn concentration and protein content in the 92 cultivars of mungbean was determined (Figure 2). The seed protein content varied from 21.1% to 30.0% with a mean of 24.9 ± 0.2, the seed zinc concentration varies from 1.54 mg/kg to 3.85 mg/100g with a mean of 2.63 ± 0.1 and the seed iron concentration ranged from 1.59 mg/100g to 9.29 mg/100g with a mean of 4.03 mg/100g ± 0.1.

A considerable variation for protein content was found. Out of ninety two cultivars eight (2KM-107, 2KM-138, BDYR-1, GP-68B, ML-818, ML-803, ML-735 and GP-78) have quite high concentrations (> 28%) of protein. Six cultivars had seed Zn concentrations of more than 3.50 mg/100g. The highest values were observed in M-1361 B from Punjab (India) followed by KM-92-11 and GP-86 (Haryana). The top ranking cultivars for iron and zinc content were further used to study cultivar X environment (GXE) interactions in Chapter V of this thesis. Sixteen cultivars had seed Fe concentrations of more than 5.00 mg/100g (with KM-92-11 and MH-98-1 as the highest).



	Fe (mg/100g)	Pr (%)	Zn (mg/100g)	SYP (g)
Mean:	**4.03**	**25.23**	**2.63**	**4.79**
CV:	**33.44**	**8.39**	**22.88**	**45.99**
h^2:	**0.259**	**0.986**	**0.727**	**0.243**

Figure 2 Seed yield, protein & Fe and Zn micronutrients content in 92 mungbean (*Vigna radiata* L.) cultivars.

Coefficients of variation (CV) show that the variability was high for iron, zinc and seed yield. Therefore, heritability along with the CV provides an opportunity to select materials with high mineral nutrient content. The correlation coefficient among the few important agronomic traits, protein and mineral content were analysed and data was presented in Table 7. A significant positive correlation was found between seed yield and number of pods and between seed yield and number branches per plant correlation coefficients (*r*) of 0.656 and 0.201 respectively. Negative associations were seen between seed yield and days to 50% flowering, days to maturity, protein and zinc content.

Table 7 Correlation coefficients between the agronomic traits, protein and micronutrients in 92 cultivars of mungbean (*V. radiata* L.)

	DF	DM	MYMV	NBP	NPP	PH	Protein	SYP	Fe
DF	-								
DM	0.39**	-							
MYMV	0.119*	-0.035	-						
NBP	0.226*	0.069	0.097	-					
NPP	-0.016	0.093	-0.159*	0.441**	-				
PH	0.106	-0.015	-0.136*	0.304**	0.167*	-			
Protein	-0.017	-0.001	-0.031	-0.106	-0.116*	0.058	-		
SYP	-0.143	-0.065	-0.261**	0.201*	0.656**	-0.002	-0.149*	-	
Fe	-0.115	-0.228**	0.067	-0.106	-0.046	-0.135*	0.019	0.022	-
Zn	-0.124	-0.081	0.152*	-0.091	-0.174*	-0.074	0.105	-0.181*	0.469**

*Significance at 5%; **Significance at 1%

Discussion

Iron and zinc are needed for a healthy development of humans. Micronutrient dense staple crops can be a source of iron and zinc. Plant breeding should aim at high yielding cultivars with higher concentrations of iron and zinc. This is only possible if there is genetic variation for Fe and Zn content. Such to be developed cultivars will be important in fighting against 'hidden hunger', and will be beneficial to all age groups but especially to infants, children and women. Although legumes are often considered as complementary to cereals for specific amino acids they are also particularly important for a sufficient intake of micronutrients (Blair *et al.* 2009). Sarker *et al.* (2009) reported variation for iron content in lentils in the range of 4.1-13.2 mg/100g, for chickpea 4.4-13.5 mg/100g and in common bean up to 10 mg/100g. Cultivars of common bean show also variability for zinc concentrations ranging from 2 – 6 mg/100g (House *et al.* 2002; Hacisalihoglu *et al.* 2004).

Seed yield is another important trait and in order to use simply inherited traits like number of pods, number of branches to predict yield there must be a correlation between these traits and yield. In our study, the genotypic correlation coefficients were higher than the corresponding phenotypic correlations (Table 1). This indicates that the traits were at least partial under genetic control. Gill *et al.* (1995) in green gram and Byre *et al.* (1996) in pigeon pea had similar results. Table 2 shows that the number of pods per plant had a significant positive and direct effect on seed yield. Khan (1988), Khan and Ahmed (1989) and Gill *et al.* (1995) also reported that selection for high number of pods per mungbean plant leads to higher yield. The number of branches/plant had also a significant positive genotypic and phenotypic correlation with pods/plant and therefore with yield. This has also been reported in green gram (Khan 1988; Maht and Mahto 1997) and black gram (Santha and Veluswamy 1997). The information derived from the correlation coefficients can be augmented by partitioning correlation into direct and indirect effects by path analysis. Yield is a complex character and the result of diverse and interrelated developmental processes. To get the highest possible yield traits like maturity, number of branches and pods per plant are traits which have to be selected for. Shansuzzaman *et al.* 1983; Singh *et al.* 1995; Gartan and Sood 1996; Paul *et al.* 1996 and Santha and Veluswamy 1997, observed similar results in mungbean, black gram and pigeon pea.

The cluster analysis grouped the ninety two cultivars into five clusters (Table 3). The mungbean cultivars show hardly variation for days to 50% flowering and days to harvest but show considerable variation for traits like pods per plant, plant height and seed yield. Clusters 2 and 5 include cultivars higher in yield and more MYMV resistant. Table 6 shows that the cultivars of Cluster 1 are early maturing, dwarf cultivars with medium to low yield whereas cluster 2, includes cultivars which are early maturing but with medium length and with medium disease resistance and yield. Cluster 3 comprises only of two cultivars which are late maturing, tall, low yielding with maximum branching. Cluster 4 comprises of medium to tall cultivars with high yield and takes about sixty two days (medium) to mature while Cluster 5 comprises dwarf resistant cultivars with high yield.

Data for micronutrients show a considerable range for both micronutrients in the mungbean cultivars. Among the micronutrients and protein content, a significant positive correlation was found between Fe and Zn (r = 0.469). These results imply that a high Fe content can be accompanied by high Zn content. Both these micronutrients exhibit a low but positive correlation with protein content (r (Fe) = 0.019; r (Zn) = 0.105). This was consistent with findings of Tryphone and Masolla (2010) in common bean, Thavarajah *et al.* (2010) in lentils, Dixon *et al.* (2000) in maize, Anandan *et al.* (2011) in rice, Velu *et al.* (2011) wheat etc. In our study a non-significant correlation was observed between micronutrient content and yield, making it possible to develop cultivars with high micronutrient concentrations in combination with high yield. To obtain the potential highest yield of a particular cultivar there should be no disease pressure. Mungbean yellow mosaic virus (MYMV) is the most treat in mungbean cultivation therefore it is important to screen for the highest possible levels of resistance to MYMV. A number of potential mungbean cultivars for further breeding are listed in Table 8.

Table 8 Potential mungbean cultivars for further breeding

Cultivars	Potential Yield (kg/ha)*	Maturity Duration (Days)	Characteristics
MH 125	1500	65	Shiny green attractive seed, Medium sized, **MYMV resistant**
MH 215	1650	67	Shiny green attractive seeds, MYMV resistant
MH 318	1420 (2300)**	59	Shiny green attractive seeds, Medium sized, Dwarf cultivar, MYMV resistant
MH 421	1500 (2300)**	60	Shiny green attractive seeds, Medium sized, Dwarf cultivar, MYMV resistant
MH 96-1	1200	77- 80	Shiny green attractive seeds, Small sized seed, MYMV resistant

* The yield varies with change in inputs and climate. Above yield is average yield. ** If a second flush of flowering is allowed the yield goes up to 2300 kg

The top ten cultivars with a combination of high yield and high micronutrient content are listed in Table 9. These cultivars can be used in cultivar x environment studies, in breeding programs and/or may be the parents in the generation of mapping studies in order to do genetic studies and to find quantitative trait loci (QTL) for Fe/Zn content, protein content and high yield (Chapter VI).

Table 9 Selected mungbean cultivars with high micronutrient content and their protein content and yield

S.No.	Cultivar	Fe (mg/100g)	Zn (mg/100g)	Protein (%)	Yield/plant (g)
1	KM-92-11	9.22	3.57	22.7	6.2
2	ML-776	7.27	3.32	23.6	2.9
3	MH-98-1	7.07	2.92	25.4	4.3
4	CoGG902	6.75	3.13	26.2	5.1
5	ML-735	6.58	2.82	28.1	8.8
6	PM-827	6.14	2.72	21.8	5.2
7	BG-39	6.02	2.64	26.2	2.6
8	GP-149	6.02	2.85	22.4	4.3
9	GP-182	5.71	3.34	22.7	1.7
10	ML-515	5.65	3.35	22.7	1.9

Before including micronutrient content in a breeding programme it is important to consider whether micronutrient content is influenced by different environments (Genotype x Environment), and differences in cultural practices. Potential associations with anti-nutritional factors (ANFs) such as tannins, saponins, phytates, lectins etc. These ANFs are the potent inhibitors of iron and zinc (Enneking and Wink 2000). Therefore these ANF should also be considered while planning experiment. The present study will help in making choices in the conversation of genetic resources and in choosing the best possible cultivars for future breeding programmes.

References

Anandan A, Rajiv G, Eswaran R, Prakash M (2011) Genotypic Variation and Relationships between Quality Traits and Trace Elements in Traditional and Improved Rice (*Oryza sativa* L.) Cultivars. Journal of Food Science 76 (4): 122-130.

Adel A , Shehata Y, Mohammed Thannoun A (1980) Chemical and amino acid composition of Iraqi mung beans. Z. Lebensm. Unters. Forsch 171: 360-362.

Altschul A (1958) Processed Plant Protein Foodstuffs, Edition 1958 Academic Press, New York, N.Y.

Amarteifio JO, Moholo D (1998) The Chemical Composition of Four Legumes Consumed in Botswana. Journal of Food Composition & Analysis 11 (4): 329-332.

Barclay DV, Heredia L, Gil-Ramos J, Montalvo MM, Lozano R, Mena M, Dirren H (1996) Nutritional status of institutionalized elderly in Ecuador. Arch Latinoam Nutrition 46:122-127.

Becker WA (1975) Manual of quantitative genetics 3rd edition. Students Book Corporation Rullman, Washington, USA.

Bhatt GM (1973) Comparison of various methods of selecting parents for hybridization in common wheat. Australian Journal of Agriculture Research 24: 457-467.

Blair MW, Giraldo MC, Buendía HF, Tovar E, Duque MC, Beebe SE (2009) Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics 113: 100-109.

Byre Gowda M, Shambulinggappa KG, Shanthala J, Chandran Prakasn J (1996) Relationship among quantitative traits and its implication in breeding for yield in pigeonpea. Mysore Journal of Agriculture Sciences 30: 64-68.

Dewey DR, Lu KH (1959) A correlation and path coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal 51: 515–518.

Dixon BM, Khing GJ, Menkir A, Dixon A (2000) Genetic variation in total carotene, iron and zinc contents of maize and cassava cultivars. Food Nutrition Bulletin 21: 419-422.

Enneking D, Wink M (2000) Towards the elimination of anti-nutritional factors in grain legumes. Linking research and marketing opportunities for pulses in the 21st Century. Kluwer Academic Publishers 671-683.

Velu G, Ortiz-Monasterio I, Singh RP, Payne T (2011) Variation for Grain Micronutrients Concentration in Wheat Core-collection Accessions of Diverse Origin. Asian Journal of Crop Science 3 (1): 43-48.

Gartan SL, Sood BC (1996) Relative contribution of various yield components in urd bean. Indian Journal of Pulses Research. 9(1): 11-13.

Gill JS, Verma MM, Gumber RK, Balwant S (1995) Character association in mungbean lines derived from three interval crosses in mungbean. Crop Improvement 22 (2): 255-260

Grusak MA, Cakmak I (2005) Methods to improve the crop delivery of minerals to humans and livestock. In: MR Broadley, PJ White (eds) Plant Nutritional Genomics. CRC Press, Boca Raton 265–286.

Hacisalihoglu G, Osturk L, Cakmak I, Welch RM, Kochian L (2004) Genotypic variation in common bean in response to zinc deficiency in calcareous soil. Plant Soil 259:71–83.

Haq Zia Ul- M, Iqbal S, Ahmad S, Imran M, Niaz A, Bhanger MI (2007) Nutritional and compositional study of Desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. Food Chemistry 105: 1357-1363.

House WA, Welch RM, Beebe S, Cheng Z (2002) Potential for increasing the amounts of bio-available zinc in dry beans through plant breeding. Journal of the Science of Food and Agriculture 82:1452–1457.

Islam FMA, Basford KE, Jara C, Redden RJ, Beebe SE (2002) Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genetic Resources and Crop Evolution 49: 285–293.

Khan IA (1988) Path coefficient analysis of yield attributes in mungbean. Legume Research 11 (1): 41-43.

Khan IA, Ahmed R (1989) Relationship of yield and its component characters in mungbean. Legume Research 12 (4): 173-176.

Lindsay WL, Norvell WR (1978) Development of DTPA soil test for zinc, iron manganese and copper. Soil Science Society of America Journal 42: 421-428.

Mahalanobis PC (1936) On the generalized distance in statistics, Proceedings National Institute of Science, India. 12: 49-55.

Maht RN, Mahto JL (1997) Correlation and regression study in black gram for yield attributes under rainfed conditions. Environment and Ecology 15 (3): 556-558.

Panse VG, Sukhatme PV (1989) Statistical methods for agricultural Workers. Publication and Information division, Indian Council of Agricultural Research.

Paul PR, Singh RM, Raina R (1996) Character association and path coefficient analysis in hybrid pigeonpea. Madras Agricultural Journal 83 (1): 34-37.

Pearson D (1973) Laboratory techniques in food analysis. , John Wiley & Sons, London.

Rao CR (1952) Advanced statistical methods in biometrical research. John Wiley & Sons, Inc. New York

Ranum P (1999) Zinc enrichment of cereal staples. Cereal Foods World 44: 604-605.

Rosado JL, Loapez P, Morales M, Munaoz E, Allen LH (1992) Bioavailability of energy, nitrogen, fat, zinc, iron and calcium from rural and urban Mexican diets. British Journal of Nutrition 68:45-58.

Rosado JL, Lopez P, Morales M, Munoz E, Allen LH (2007) Bioavailability of energy, nitrogen, fat, zinc, iron and calcium from rural and urban Mexican diets. Nutrition 68: 45-58.

Santha S, Veluswamy P (1997) Character association and path analysis in black gram. Madras Agricultural Journal 84 (11/12): 678-681.

Sarker A, Aydogan A, Chandra S, Kharrat M, Sabaghpour SH (2009) Genetic enhancement for yield and stability. p. 102–120. I*n W*. Erskine, F Muehlbauer, A Sarker, B Sharma (ed.) the lentil: botany, production and uses. Cab Int., Wallingford, UK.

Shansuzzaman KM, Khan MRH, Saikh MAQ (1983) Genetic variability and character association in mungbean (*Vigna radita* L). Bangladesh Journal of Agriculture Research 8: 1-5.

Singh RK, Chaudhary BD (1985) Biometrical Methods in Quantitative Genetics Analysis (Rev. ed.). Kalyani Publishers, New Delhi- Ludhiana. 78.

Singh IS, Hue NTN, Gupta AK (1995) Association and cuase and effect analysis in some F_2 populations of green gram. Legume Reesrach 18 (3/4): 137-142.

Singh G, Sharma YR, Kaur L (1992) Methods of rating mungbean yellow mosaic virus in mungbean and urdbean. Plant Disease Research 7 (1): 1-6.

Stauffer JE (1999) Nutraceuticals. Cereal Foods World 44:115-117.

Thavarajah D, Thavarajah P, See Chai-Thiam, Vandenberg A (2010) Phytic acid and Fe and Zn concentration in lentil (*Lens culinaris* L.) seeds is influenced by temperature during seed filling period. Food Chemistry 122: 254-259.

Tryphone GM, Msolla SN (2010) Diversity of common bean (*Phaseolus vulgaris* L.) cultivars in iron and zinc contents under screen house conditions. African Journal of Agriculture Research 5 (8): 738-747.

Appendix I

List of 92 cultivars of mungbean varieties with their origin, zinc, iron and protein content.

V.No	Cultivars	Origin	Mean value of			
			Zn ± S.E mg/100g	**Fe ± S.E** mg/100g	**Protein ± S.E** %	**SYP ± S.E g**
1	2KM-107	-	2.107 ± 0.009	3.877 ± 0.484	28.067 ± 0.030	4.812 ± 1.389
2	2KM-111	Ludhiana	3.270 ± 0.031	4.667 ± 0.447	26.227 ± 0.056	3.383 ±0.818
3	2KM-112	IARI	2.680 ± 0.210	4.467 ± 0.592	21.360 ± 0.248	2.070 ±0.709
4	2KM-114	IARI	3.653 ± 0.035	4.437 ± 0.066	23.497 ± 0.092	3.989 ±1.461
5	2KM-115	Sri Ganganagar	2.033 ± 0.041	2.760 ± 0.211	26.173 ± 0.050	7.554 ±2.072
6	2KM-116	Ludhiana	2.333 ± 0.127	3.510 ± 0.012	21.780 ± 0.127	5.458 ±1.056
7	2KM-117	Varanasi	2.917 ± 0.018	4.000 ± 0.632	23.327 ± 0.161	4.701 ±0.963
8	2KM135	Sri Ganganagar	2.947 ± 0.041	3.243 ± 0.100	27.127 ± 0.066	3.943 ±0.891
9	2KM-138	Hisar	2.040 ± 0.477	2.393 ± 0.185	28.090 ± 0.040	6.586 ±2.280
10	2KM-139	Hisar	1.757 ± 0.144	2.917 ± 0.079	27.133 ± 0.049	2.164 ±0.545
11	2KM-151	Pant Nagar	2.040 ± 0.231	4.017 ± 0.181	26.273 ± 0.062	5.159 ±1.047
12	2KM-155	Ludhiana	2.943 ± 0.288	4.537 ± 0.872	27.133 ± 0.049	4.145 ±0.563
13	2KM-161	Ludhiana	1.820 ± 0.288	3.053 ± 0.028	26.153 ± 0.069	2.862 ±1.110
14	2KMH-52	Hisar	2.510 ± 0.012	3.703 ± 0.057	23.653 ± 0.064	4.602 ±0.793
15	AMP-36-10	Hisar	2.443 ± 0.299	2.653 ± 0.274	23.610 ± 0.012	5.679 ±2.099
16	AMP-36-18	Hisar	2.350 ± 0.062	2.680 ± 0.121	22.670 ± 0.046	9.585 ±3.089
17	AMP-36-4	Hisar	2.380 ± 0.076	2.570 ± 0.111	25.227 ± 0.111	5.588 ±1.324
18	ASHA	Hisar	2.363 ± 0.177	4.117 ± 0.351	25.370 ± 0.026	3.422 ±0.890
19	BDYR-1	Bangladesh	2.503 ± 0.052	4.527 ± 0.113	28.550 ± 0.282	4.363 ±1.045
20	BDYR-2	Bangladesh	2.490 ± 0.095	4.660 ± 0.70	22.670 ± 0.046	6.441 ±1.780

21	BG-39	-	2.640 ± 0.191	6.023 ± 0.405	26.200 ± 0.036	2.643 ±1.026
22	CH1355	-	3.450 ± 0.131	3.860 ± 0.061	24.503 ± 0.012	0.533 ±0.255
23	CH2103	-	3.130 ± 0.064	4.620 ± 0.488	26.250 ± 0.035	0.565 ±0.160
24	CoGG902	-	3.133 ± 0.050	6.750 ± 2.902	26.217 ± 0.018	5.091 ±1.016
25	GANGA-8	Sri Ganganagar	2.837 ± 0.636	2.390 ± 0.604	25.383 ± 0.003	2.934 ±0.771
26	GP-149	Varanasi	2.853 ± 0.081	6.017 ± 1.523	22.367 ± 0.209	4.255 ±0.589
27	GP-150	-	3.273 ± 0.027	3.603 ± 0.314	24.440 ± 0.060	4.040 ±0.812
28	GP-181	Hisar	3.560 ± 0.040	4.923 ± 0.369	21.770 ± 0.059	4.192 ±1.0101
29	GP-182	Hisar	3.340 ± 0.036	5.713 ± 1.988	22.723 ± 0.018	1.742 ±0.609
30	GP-196	Hisar	2.743 ± 0.116	4.440 ± 0.301	26.263 ± 0.024	4.437 ±1.184
31	GP-248	-	2.157 ± 0.037	3.117 ± 0.112	27.117 ± 0.058	5.529 ±1.469
32	GP-32	-	2.903 ± 0.369	4.627 ± 0.470	22.717 ± 0.024	2.032 ±0.269
33	GP-68B	-	2.753 ± 0.035	5.097 ± 0.871	28.093 ± 0.069	2.979 ±0.361
34	GP-69	Hisar	3.287 ± 0.015	3.260 ± 0.546	24.513 ± 0.015	4.756 ±0.542
35	GP-78	Hisar	3.070 ± 0.025	4.600 ± 0.226	30.003 ± 0.450	5.604 ±0.232
36	GP-86	Hisar	3.570 ± 0.032	3.890 ± 0.767	23.577 ± 0.035	5.554 ±0.691
37	GP-86-1	Hisar	3.290 ± 0.046	4.830 ± 1.182	24.510 ± 0.017	4.093 ±1.251
38	HUM-1	Varanasi	2.010 ± 0.115	1.597 ± 0.234	25.327 ± 0.027	4.545 ±0.685
39	HUM-7	Varanasi	2.210 ± 0.017	2.707 ± 0.250	25.277 ± 0.129	5.615 ±1.881
40	IC103196	NBPGR	2.550 ± 0.035	5.253 ± 1.840	24.510 ± 0.017	4.313 ±1.748
41	IC39574	NBPGR	3.363 ± 0.059	4.060 ± 0.464	21.797 ± 0.056	2.949 ±0.858
42	IC39595	NBPGR	2.287 ± 0.018	4.950 ± 0.955	24.510 ± 0.012	5.865 ±1.380
43	KM-92-11	-	3.570 ± 0.049	9.223 ± 1.747	22.673 ± 0.113	6.218 ±1.387
44	L-24-2	Ludhiana	1.723 ± 0.241	1.920 ± 0.142	21.820 ± 0.035	2.197 ±0.109

45	LM-10	Ludhiana	2.927 ± 0.074	3.473 ± 0.263	24.487 ± 0.015	4.168 ±0.675
46	M-1361B	Ludhiana	3.853 ± 0.043	5.107 ± 1.112	26.220 ± 0.046	4.925 ±0.808
47	M-169	Kanpur	2.057 ± 0.027	3.833 ± 0.276	25.390 ± 0.199	5.033 ±0.651
48	M-395	Ludhiana	1.607 ± 0.045	1.757 ± 0.111	22.670 ± 0.053	5.401 ±0.688
49	M-516	Kanpur	1.910 ± 0.085	2.253 ± 0.168	21.777 ± 0.055	6.423 ±0.960
50	M-605	-	2.257 ± 0.035	3.007 ± 0.294	26.297 ± 0.101	6.031 ±1.250
51	MH-124	Hisar	1.957 ± 0.039	3.047 ± 0.641	23.610 ± 0.012	6.671 ±1.612
52	MH-125	Hisar	2.323 ± 0.184	3.267 ± 0.135	26.217 ± 0.018	5.520 ±0.763
53	MH-215	Hisar	2.433 ± 0.219	3.283 ± 0.133	21.057 ± 0.033	5.723 ±1.167
54	MH-318	Hisar	3.153 ± 0.178	5.022 ± 0.768	23.554 ± 0.052	7.707 ±1.600
55	MH-419	Hisar	2.550 ± 0.136	3.790 ± 0.583	23.637 ± 0.058	8.850 ±0.261
56	MH-421	Hisar	2.725 ± 0.083	3.803 ± 0.058	25.325 ± 0.036	5.939 ±0.706
57	MH-96-1	Hisar	2.263 ± 0.098	3.113 ± 0.227	24.457 ± 0.035	6.655 ±1.687
58	MH-98-1	Hisar	2.923 ± 0.072	7.097 ± 3.820	25.440 ± 0.119	4.299 ±0.774
59	MI-3580	-	2.393 ± 0.043	4.257 ± 1.054	27.133 ± 0.043	6.845 ±2.003
60	ML-1108	-	2.227 ± 0.046	3.760 ± 0.394	23.550 ± 0.044	4.346 ±0.645
61	ML-194	Ludhiana	3.050 ± 0.031	4.407 ± 1.176	22.640 ± 0.078	6.909 ±1.231
62	ML-406	Ludhiana	1.537 ± 0.290	2.523 ± 0.361	21.830 ± 0.036	5.035 ±1.278
63	ML-5	-	2.413 ± 0.280	2.780 ± 0.096	27.153 ± 0.034	6.812 ±1.505
64	ML-506	Ludhiana	2.267 ± 0.026	4.800 ± 1.481	24.457 ± 0.035	4.765 ±1.218
65	ML-515	Ludhiana	3.350 ± 0.061	5.650 ± 1.005	22.730 ± 0.053	1.992 ±0.467
66	ML-682	Ludhiana	3.189 ± 0.146	3.303 ± 0.108	25.627 ± 0.135	3.463 ±0.985
67	ML-735	Ludhiana	2.820 ± 0.080	6.580 ± 0.206	28.053 ± 0.034	8.765 ±1.295
68	ML-759	Ludhiana	1.560 ± 0.076	2.643 ± 0.071	25.283 ± 0.128	2.460 ±0.671

69	ML-776	Ludhiana	3.323 ± 0.384	7.270 ± 1.939	23.587 ± 0.026	2.926 ±0.760
70	ML-803	Ludhiana	3.807 ± 0.414	5.340 ± 1.158	28.060 ± 0.032	5.695 ±0.424
71	ML-818	Ludhiana	3.187 ± 0.096	4.470 ± 0.354	29.743 ± 0.041	2.510 ±0.242
72	ML-839	Ludhiana	1.827 ± 0.299	3.237 ± 0.364	23.600 ± 0.021	7.571 ±1.553
73	Muskan	-	2.057 ± 0.029	2.717 ± 0.044	24.357 ± 0.163	4.383 ±0.760
74	P-105	IIPR Kanpur	2.753 ± 0.064	3.160 ± 0.518	26.210 ± 0.023	2.935 ±0.588
75	PDM-9249	IIPR Kanpur	1.833 ± 0.215	2.557 ± 0.128	25.327 ± 0.027	4.629 ±1.413
76	PLM-116	Ludhiana	3.647 ± 0.095	5.180 ± 1.061	25.363 ± 0.038	4.717 ±1.046
77	PLM-176	Ludhiana	3.533 ± 0.286	5.073 ± 0.826	27.140 ± 0.036	3.550 ±0.683
78	PLM-18	Ludhiana	3.250 ± 0.035	4.473 ± 1.360	25.340 ± 0.026	1.457 ±0.759
79	PLM-62	Ludhiana	1.830 ± 0.099	3.403 ± 0.255	25.330 ± 0.026	1.096 ±0.360
80	PLM-65	Ludhiana	2.713 ± 0.015	4.387 ± 0.688	23.603 ± 0.018	2.983 ±1.147
81	PM-827	-	2.717 ± 0.015	6.140 ± 1.050	21.823 ± 0.032	5.235 ±1.490
82	PMB-14	Ludhiana	1.603 ± 0.243	2.023 ± 0.052	23.533 ± 0.058	1.783 ±0.243
83	SM-99-1	Hisar	2.183 ± 0.068	4.843 ± 1.342	21.830 ± 0.026	7.395 ±1.210
84	SMH-99-1A	Hisar	3.143 ± 0.331	3.973 ± 0.339	28.103 ± 0.062	3.761 ±1.049
85	SMH-99-1DB	Hisar	3.110 ± 0.058	3.910 ± 0.120	27.133 ± 0.043	2.977 ±0.594
86	SMH-99-2	Hisar	2.487 ± 0.015	3.020 ± 0.100	25.483 ± 1.637	3.438 ±0.702
87	SMH-99-3A	Hisar	1.697 ± 0.316	3.403 ± 0.299	25.210 ± 0.098	4.426 ±0.966
88	SMH-99-3D	Hisar	1.657 ± 0.156	2.243 ± 0.054	21.590 ± 0.195	14.578 ±2.632
89	SMH-99-4	Hisar	2.743 ± 0.138	3.173 ± 0.097	27.190 ± 0.038	6.346 ±1.425
90	SML-668	Ludhiana	2.268 ± 0.260	4.012 ± 0.587	23.538 ± 0.095	8.827 ±1.983
91	Satya	Hisar	2.468 ± 0.102	4.060 ± 0.346	21.132 ± 0.043	9.537 ±1.919
92	T-44	-	3.177 ± 0.032	4.643 ± 0.527	26.220 ± 0.021	4.509 ±1.023

Appendix II

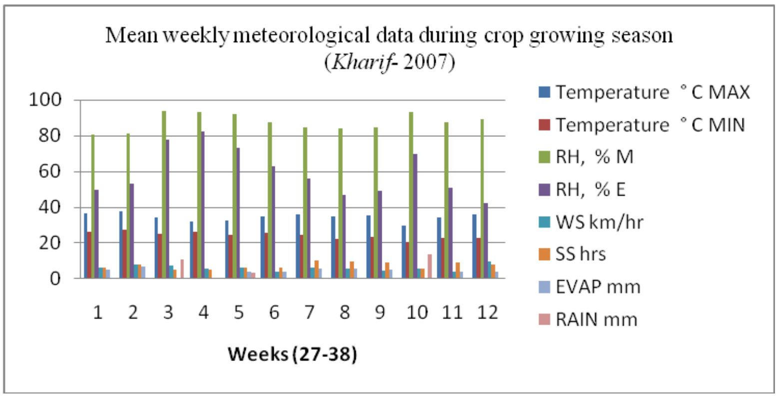


Figure A Mean weekly meteorological data during crop growing season (*Kharif* - 2007) recorded at the experimental station, CCS HAU, Hisar.

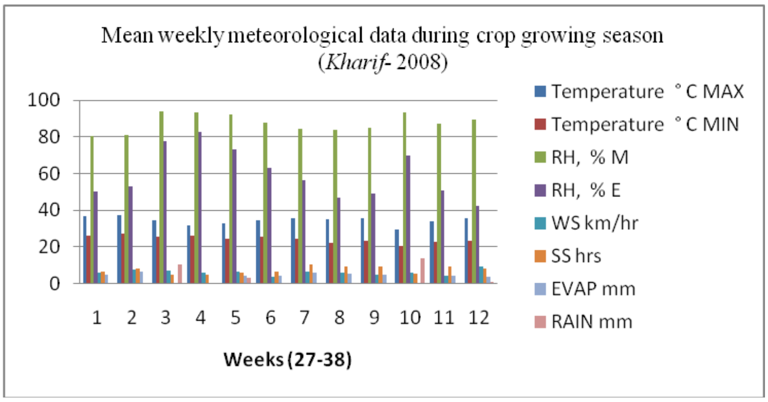


Figure B Mean weekly meteorological data during crop growing season (*Kharif* - 2008) recorded at the experimental station, CCS HAU, Hisar.

CHAPTER 6

Differential response of mungbean (*Vigna radiata* L.) varieties to changes in environmental conditions

Renu Singh, Ram Kumar, Richard G.F. Visser and Adriaan W. van Heusden

Abstract

One of the benefits of mungbean are its nutritional properties. Of the seven essential micronutrients, especially iron and zinc play a vital role in human and animal health. Breeding for varieties with a potential high concentration of micronutrients should be complemented with studies of environmental effects on the accumulation of micronutrients in seeds. GxE interactions were studied for plant height, number of pods and branches per plant, seed yield, iron and zinc seed content. Our major emphasis was to see the effect of iron and zinc supplementation on seed micronutrient content and other agronomic traits. Therefore, to study different compositions of the soil, six artificial conditions with different levels of micronutrients were created. Soil supplemented with $ZnSO_4$ or with $ZnSO_4$ and 0.5% $FeSO_4$ was beneficial for agronomic traits but not favourable for iron and zinc content of seeds. The main effects and interactions were statistically significant different. Three stability parameters (Mean, bi and S^2di) were calculated. No genotypes were found where the seed yield was not influenced by changes in environmental conditions.

Keywords: environmental index, GxE, Genotype x Fertilisation, micronutrients, stability.

Introduction

Mungbean is a widely grown food grain legume in the developing world. Mungbeans are locally grown and available for the local people. In India, there is always production in one state or the other. Dry beans, including mungbean (19.7 million tonnes (mt), field pea (10.4 mt), chickpea (9.7 mt), cowpea (5.7 mt), lentil (3.6 mt) and pigeon pea (3.5 mt), are important crops (FAO, 2010). The majority of population in India is vegetarian therefore dry beans and especially mungbean is a major replacement of animal proteins and micronutrients. Iron and zinc play a very important and vital role in the health and development of animals, humans and plants, therefore increasing their content in seed will may prove helpful in combatting micronutrient deficiency in a vegetarian society.

Major constraints in breeding pulses such as mungbean are the high genotype x environment (GxE) interactions and the low genetic diversity in the primary gene pool (Jitendra *et al.* 2011). Several other studies (Patil *et al.* 1996; Tiwari *et al.* 2000; Mehla *et al.* 2000) showed large GxE interactions which make it necessary to test new varieties over a large number of environments. Mather and Jinks (1982), Mukai (1988), and Wu and O'Malley (1998) describe two types of environmental variation: (1) micro environmental effects which can't easily be identified or predicted (e.g., year-to-year variation in rainfall, drought conditions, extent of insect damage) and (2) macro environmental variances which are known (e.g., soil type, management practices, controlled temperatures etc.). According to these investigators, the GxE interaction can only be estimated for the macro environmental effects. Breeding programs should aim at genotypes that perform well under as many conditions as possible. Therefore testing new varieties under varying local growing conditions is of utmost importance. Besides it is important to know the available germplasm and to know the relationship between different accessions; landraces and cultivars (see Chapter 3). Therefore in order to test the performance of the selected mungbean cultivars to the different soil types, a total of six artificial soil environments were created. These artificially created environments made it possibility to study the stability of mungbean cultivars for micronutrient content (iron and zinc) and important agronomic traits like yield.

Material and Methods

Plant material

The experiments were conducted with thirty elite genotypes growing in six different artificial soil types. The genotypes were selected on the basis of contrasts in micronutrient content and agronomic performance (Chapter 3, Table 5 & Chapter 5, this thesis) in 2007 and 2008.

Field trials and experimental design

Six different field environments were created at the pulses station, CCS HAU, Hisar, located at latitude of 29°10'N, longitude of 75°46'E and altitude 215.2 m above sea level. The experiment was conducted in the *kharif* (July to September) 2009, in a Random Block Design with spacing of 40 cm between rows and 15 cm between plants within the row. The different environments were created by adding different doses of micronutrients and fertilizer to the soil (Table 1). The recommended dose of fertilizers (RDF) was added in all soils. Single Super Phosphate (SSP) and zinc were added according to the recommendations of an experienced agronomist. Chelated iron can be applied directly as a foliar spray (Figure 1) to enhance uptake.

Table 1 Details of artificial field environments

Environment	Genotypes	No. of replications	Fertilizer doses
E1	30	3	RDF
E2	30	3	RDF + 0.5% $FeSO_4$
E3	30	3	RDF + SSP
E4	30	3	RDF + SSP + 0.5% $FeSO_4$
E5	30	3	RDF + SSP + $ZnSO_4$
E6	30	3	RDF + SSP + $ZnSO_4$ + 0.5% $FeSO_4$

RDF (Recommended dose of fertilizer) = 20 kg N/ha; 40 kg P_2O_5/ha; Fe = Foliar Spray ($FeSO_4$; 0.5%); Zn ($ZnSO_4$) = direct to soil (25kg/ha); SSP (Single Super Phosphate, Ca $(H_2OP_4)2.H_2O$) = contains 16% water soluble P_2O_5, 12% sulphur & 21% calcium.

An optimal supply of nitrogen (20 kg/hectare) ensures an optimal uptake of potassium as well as phosphorus, iron, zinc etc. (Ranade 2011). The mean maximum/minimum temperature during the period of the study was 36.1/24.8 ˚C, while the mean relative humidity was 81.7% (morning)/51.1% (evening). The soil of the present experimental field is from the Indo-Gangetic alluvium and is in texture loamy sand.

Before adding any fertilizer to the experimental fields, the physical-chemical characteristics of the soil were measured (Table 2). Soil samples were taken from 6 to 8 inches under the surface (the aerobic zone where most root growth and nutrient exchange happens). In total five samples were taken (from four corners and one from the centre of the field). Each sample was approximately equal in size and placed in a clean plastic bucket and mixed thoroughly and sends to the soil testing laboratory of the department of soil, CCS HAU, Hisar.

After foliar spray of iron (Figure 1) and soil supplementation with $ZnSO_4$ and RDF, in the middle of the growth season samples of soil were again analyzed.

Figure 1 Mungbean plant stage at which micronutrients were added.

To measure mungbean yield some yield related traits were measured. Five random plants were tested per row and plant height (cm), number of pods/plant, number of branches per plant, seed yield/plant (g) were measured. Along with these parameters, the iron and zinc content of the seed samples grown under different environments were analyzed.

Stability analysis

Statistical analysis was carried out with software OPStat (http://www.hau.ernet.in/link/spas.htm). The data for each trait were subjected to analysis of variance using the general linear model for RBD design. The appropriate 'F' values were obtained for testing the significance of genotypes against error mean square in accordance with the following model: $Yij = m + a_i + b_j + e_{ij}$ where, m is general mean, a_i is ith treatment level, b_j is jth replication level and e_{ij} is random error associated with treatment for jth block.

The mean values for different traits of all thirty genotypes in six environments as well as pooled over environments were used for analysis of variance for phenotypic stability. The stability parameters of individual genotype were computed, using an analytical approach suggested by Eberhart and Russell (1966). The model used in this approach is as follows: $Y_{ij} = \mu_i + b_i I_j + \delta_{ij}$ where, Y_{ij} is the variety mean of the ith genotype at the jth environment; μ_i is mean of the ith genotype over all environments; b_i is regression coefficient that measures the response of the ith genotype to varying environments; I_j is the environmental index obtained as the mean of the genotype at the jth environments minus the grand mean of overall genotypes and environments and δ_{ij} is the deviation from regression of the ith variety at the jth environment.

Results

The data in Table 2 show, that the loamy sandy soil of the experimental field is alkaline, low in available nitrogen (appendix I), medium in Fe, phosphorus, potassium, sulphur and Zn.

Table 2 Analysis of soil physicochemical characteristics.

Soil characteristics					
Macronutrients (ppm)					
pH	**Texture**	**Nitrogen (N)**	**Phosphorus (P)**	**Potassium (K)**	
7.80	loamy sandy	72	1.4	12.4	
Micronutrient (ppm) primary (Fe, Zn, Mn & Cu) and secondary (S)					
	Fe	**Zn**	**Mn**	**Cu**	**S**
Before supple-mentation and sowing	24.0	1.10	8.32	1.18	16.25
After supple-mentation (mid stage)	8.40	0.86	2.87	0.58	600.0
After harvest	5.05	2.02	1.39	0.44	520.0

Selecting the right percentage of iron for foliar application

In order to select the appropriate iron percentage a small experiment was conducted. Three concentrations i.e. 0.5%, 0.75% and 1.0% of $FeSO_4$ solution were tested (Figure 2). No visible signs were present with 0.5% but with 0.75% and 1.0% leaves were damaged (Figure 3).

Figure 2 Applying iron in mungbean field

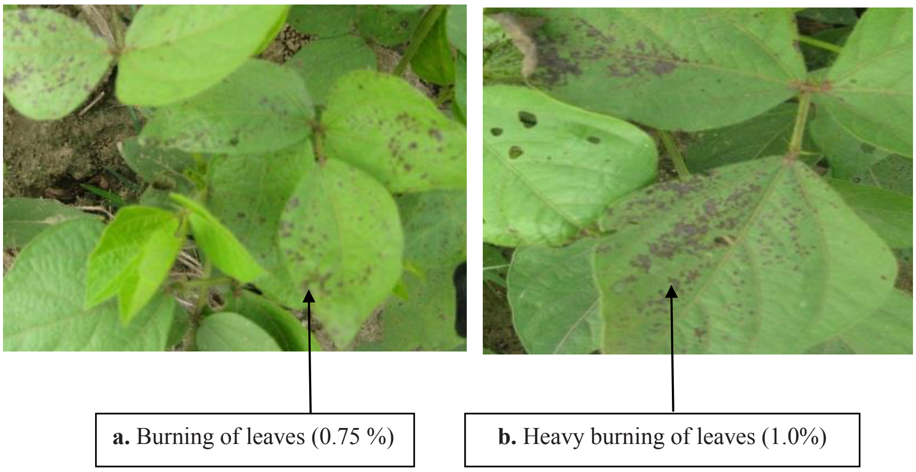


Figure 3 Effect of foliar application of iron ($FeSO_4$) on mungbean leaves

Effect of changes in the environment on performance genotypes

Analysis of variance

An analysis of variance (ANOVA), based on group variances and sample sizes tells, whether there is a statistically significant difference between group means (averages). A simple randomized block design analysis was carried out for different traits and six soil environments. ANOVA for different traits in all the environments was carried out to test the significance of phenotypic differences i.e. to see the presence of significant variation for a trait in different environments. In order to test the significance, F values were calculated by using the factor mean sum square against error mean square and further significance was tested against the tabulated values. It is evident from Table 3 that mean squares due to genotypes were significant in all the environments for all traits except for plant height which was non-significant in E_2. The critical difference values showed that enough sufficient genetic variation was present for most of the traits.

Table 3 Analysis of variance for six traits in mungbean under six different environmental conditions

Source of variation	d.f	Environments	PH***	NPP	NBP	SYP	Fe	Zn
Factor Block	2	E 1	30.56**	0.01	11.85	4.01	0.89	9.68**
		E 2	5.28	0.14	0.36	1.34	38.13**	3.84**
		E 3	1.06	0.16	7.79	1.23	34.70**	5.22**
		E 4	23.15**	0.03	7.04	0.20	10.52**	0.23
		E 5	16.06*	0.01	3.68	1.33	32.99**	0.23**
		E 6	0.30	0.02	6.80	10.96*	11.18**	4.87**
Factor Genotype	29	E 1	268.68**	0.75**	148.35**	85.01**	3.18**	0.71**
		E 2	290.88	0.58**	133.79**	75.89**	31.01**	12.16**
		E 3	235.34**	0.73**	106.09**	113.15**	30.54**	2.17**
		E 4	239.55**	1.02**	52.06**	76.12**	13.79**	1.35**
		E 5	241.57**	0.61**	44.42**	60.53**	92.62**	1.35**
		E 6	287.59**	1.29**	204.62**	126.36**	35.43**	36.91**
Error	58	E 1	4.07	0.08	10.62	2.65	1.09	0.33
		E 2	2.81	0.07	4.86	1.31	1.17	0.07
		E 3	4.38	0.09	3.47	1.19	1.25	0.12
		E 4	3.63	0.09	6.56	0.88	10.17	0.04
		E 5	5.46	0.06	2.69	1.28	1.16	0.04
		E 6	1.09	0.035	2.89	1.67	0.57	0.17
CD+ (5%)		E 1	4.69	0.67	7.59	3.79	2.45	1.34
		E 2	3.90	0.61	5.13	2.66	2.51	0.60
		E 3	4.87	0.71	4.33	2.53	2.61	0.82
		E 4	4.43	0.69	5.96	2.19	7.42	0.39
		E 5	5.41	0.58	3.79	2.63	2.51	0.39
		E 6	2.43	0.44	3.96	3.01	1.76	0.95

**Significant at P=0.01; * Significant at P= 0.05seeds; +CD=critical difference which depends on the MSE & the sample sizes; ***PH: Plant Height (cm), NBP: number of braches/plant, NPP: number of pods/plant, SYP: seed yield, Fe; iron seed content, Zn: zinc seed content

Estimation of environmental index (Ij) - grading of environments

The performance of a particular variety is the result of its genetic constitution and the environment in which it grows. In order to see which environment causes poor, fair or optimal growing conditions an environmental index can be estimated. The environmental index shows the suitability of an environment for the expression of a certain trait. The estimates of environmental index for all the six environments and traits are expressed as deviation from the mean of all the genotypes at a given location from the overall mean (Table 4).

Table 4 Environmental index of six traits of mungbean

Environment	Fertilizer doses	PH*	NBP	NPP	SYP	Fe	Zn
E1	RDF	10.02	0.15	5.93	1.89	-1.23	-0.34
E2	RDF + 0.5% $FeSO_4$	2.62	0.08	2.47	0.63	-0.47	0.87
E3	RDF + SSP	-1.28	0.11	-0.77	0.39	-0.61	0.24
E4	RDF + SSP+ 0.5% $FeSO_4$	-3.17	0.24	-0.44	0.81	-1.55	-0.74
E5	RDF + SSP + $ZnSO_4$	-2.37	-0.28	-3.81	-2.01	4.13	-0.75
E6	RDF + SSP + $ZnSO_4$ + 0.5% $FeSO_4$	-5.81	-0.29	-3.36	-0.44	-0.26	0.72

*PH: Plant Height (cm), NBP: number of braches/plant, NPP: number of pods/plant, SYP: seed yield, Fe; iron content in seed, Zn: zinc content in seed

A high environmental index shows the presence of negative GxE interaction and vice versa. Therefore, considering plant height, the environmental index Ij was highest in E1 and lowest in E6 meaning that E6 was the most favourable environment for plant height followed by E4, E5, E3 and E2. While the most unfavourable environment for this trait is E1. This might be because of the fact that in all other environments the soil was nutrient rich. For number of branches per plant, E6 and E5 are the most favourable environments, while the most unfavourable was E4.

For iron content, conditions in environment E5, the environment supplemented with only $ZnSO_4$, were not good. In E5 the Zn uptake is good but this condition was not different from the E4 results. On the basis of Ij values, the E5 and E6 environments are favourable for most of the traits but not for iron and zinc content.

Stability analysis

It is known that genotypes performing well under a particular environment may or may not perform well in other environments. It is therefore useful to study genotypes in different circumstances. The pooled analysis of variance shows that the mean squares due to genotypes were highly significant when tested against the pooled error (Table 5). This shows the presence of considerable variability among the genotypes for all traits. The model of Eberhart and Russell (1966) was used to calculate the magnitude of linear and non-linear components of variation, which provide information on predictable and unpredictable sources of variations. Highly significant mean squares due to environment plus genotype x environment (E + GxE) for all the traits suggest the presence of considerable interactions of genotypes with environmental conditions. Highly significant variances due to the environment (linear) indicate that mimicking environments by changing fertilizer doses is effective.

Table 5 Stability analysis of variance for different traits (Eberhart and Russell 1966)

Source of variation	d.f.	PH***	NBP	NPP	SYP	Fe	Zn
Genotypes (G)	29	340.9**++	0.8**++	90.3**++	79.3**++	14.7**++	3.1**
Environment (E)	5	949.6**++	1.6**++	407.2**++	54.1**+	129.7**++	15.2**++
G x E	145	36.1	0.2**	27.9**	19.9**	10.8**++	3.1**
Environments + (G x E)	150	66.5**++	0.2**++	40.6**++	21.1**	14.8**++	3.4**
E (linear)	1	4747.9**++	7.9**++	2036.1**++	270.2**++	648.5**++	76.1**++
G (linear) x E	29	57.1***++	0.3**+	36.2**	24.7**	25.9**++	4.2**+
Pooled deviation	120	29.8**	0.15**	24.9**	18.1**	6.9**	2.7**
Pooled error	348	3.6	0.07	5.2	1.5	2.6	0.1

*, ** = Significant mean square against pooled error at 5% and 1% probability level respectively

+, ++ = Significant mean square against pooled deviation at 5% and 1% probability level respectively

***PH: Plant Height (cm), NBP: number of braches/plant, NPP: number of pods/plant, SYP: seed yield, Fe; iron seed content, Zn: zinc seed content

Further partitioning of the GxE interaction into linear and non-linear (pooled deviation) components showed that both these components were significant for all the traits when tested against the pooled error. When the linear component of GxE interaction was compared with the pooled deviation (non-linear component) it was significantly different for plant height, number of branches per plant and for iron and zinc content in the seeds. The results indicate that the differences among genotypes will further be used to estimate the (bi) values. When the pooled deviation is highly significant it indicates that part of the variation of the genotypes is unpredictable. For two traits i.e. number of branches per plant and seed yield per plant, pooled deviations of mean squares was not significant suggesting that only predictable responses will be found for these traits.

From Table 5, it is clear that significant differences among genotypes, environments and interactions are detected for all studied traits. These results revealed that the mungbean genotypes responded differently to the different environmental conditions. For Fe and Zn this is obvious because all the environments have a different status of iron and zinc. The predictability of response to Fe and Zn content in soil will decide whether a particular genotype can accumulate more of these micronutrients or not. These aspects are given in table 8 and discussed hereafter.

Estimation of stability parameters for individual genotypes

Because overall GxE interactions were found to be significant, the next test can be to identify genotypes, which are less variable under different environmental conditions. Many models have been developed to identify stable genotypes but for the present study the Eberhart and Russell (1966) model was used. According to this model, a variety is stable when regression coefficient (bi) is equal to one and the deviation from regression (S^2di) is as close to zero as possible with a high mean performance. The above two measures of assessing the stability of genotype mean regression coefficient (bi) and the mean square deviation (S^2di) were used in assessing the stability of thirty genotypes (Appendix II, III & IV).

Plant height

The stability parameters (Appendix II) show that five genotypes had a significant bi and that all thirty genotypes had a significant S^2di. The performance of genotypes with a significant bi value can be predicted across environments. Both linear as non-linear components of the interactions were observed for five genotypes. The remaining twenty five genotypes had only the non-linear component as only their S^2di was significant. This means that the response in plant height across the environments cannot be predicted for these genotypes.

Number of branches per plant

The regression analysis of individual genotypes (Appendix II) shows that six genotypes (MH-125, 2KM-135, ML-406, 2KM-155, MH 215 and ML-506) had non-significant bi and S^2di values indicating the absence of GxE interactions for this character. One genotype, ML-759, had significant values for bi and S^2di meaning both linear and non-linear components of GxE interaction. One genotype (PDM-9-249) has a significant bi and no significant S^2di which shows presence of only linear portion of GxE interaction and therefore its performance can be predicted. Among the thirty genotypes (see chapter 3), only seven were found to be stable across the environments while the other 23 genotypes, with significant S^2di, were unstable in different environments. Only two genotypes, MH-125 and PDM-9-249 show above average response (bi $<$ 1.0) and were found to be stable in favourable environments i.e. environments supplemented with proper irrigation, spray and fertilizers. Two genotypes, ML-406 & ML-506, show below average response (bi$>$1.0) and were stable in poor environments while the other three i.e. 2KM-135, 2KM-155 and MH-215 were found to be stable in all types of environments.

Number of pods per plant

The test of significance for GxE interaction namely bi for individual genotypes with respect to number of pods per plant showed no linear component of GxE for one genotype: PDM-9-249 (Appendix 3). Twenty nine genotypes had a non-linear component showing that these genotypes are unstable and their response can't be predicted across different environments.

Seed yield per plant

Individual analysis of the behaviour of thirty genotypes (Appendix III) show that all the thirty genotypes show significant S^2di values, indicating their unpredictable behaviour across environments.

Iron content in seed

For iron content (Appendix IV) both stability values were significant in two genotypes, MI-3580 & 2KM-139 and only significant bi values were found in eleven genotypes. For these eleven genotypes the GxE interaction was linear and the performance of these genotypes can be predicted across the environments. Nothing could be predicted for MI-3580 and 2KM-139

Twelve genotypes were found unstable as their S^2di (2 with both bi & S^2di and 10 with only S^2di) were significant whereas the remaining 18 were found to be stable. Out of the eighteen stable genotypes, ten genotypes show above average response (bi >1.0) meaning good performance in favourable environments while the remaining 8 show average response and found to be stable across all kind of environments.

Zinc content in seed

For zinc content both stability parameters of two genotypes (2KM-138 and PDM-9-249) were non-significant therefore no GxE interactions in these genotypes (Appendix IV). Significant bi and S^2di values were found in KM-107 and ASHA while in ML-818 only the bi was significant. Twenty seven genotypes had a significant S^2di value, indicating that their response can't be predicted across the environments. Only three genotypes namely ML-818, 2KM-138 & PDM-9-249 were found to be stable with an average response (bi = 1).

Stability parameters for individual genotypes

Once the interactions were found to be significant, the next test was to identify stable genotypes, which interact less with differences in circumstances. A variety is said to be stable when the regression coefficient (bi) is equal to one, deviation from regression (S^2di) as close to zero as possible and a high mean performance. The linear regression (bi) could simply be regarded as the measure of response of a particular genotype and if it is of greater than one then the genotypes is sensitive to environmental changes but adopted to favourable environments. If it is lower than one it indicates above average stability. If this stability is accompanied by a high mean value, the genotype is said to be better adapted to widely differing conditions. On the other hand, deviation around the regression line is considered as a better measure of stability. With respect to the non-linear component of the GxE interaction, the genotype with the lowest standard deviation will be the most stable and vice-versa. The results of present study on stability parameter are discussed below (Table 6).

Table 6 Distribution of different genotypes on basis of different stability parameters for six traits in mungbean (*V. radiata* L)

Character	Predictable		Unpredictable	
	Both bi and $\bar{S}^2$di non-significant	Only bi significant	Both bi and $\bar{S}^2$di significant	Only $\bar{S}^2$di significant
PH	-	-	5	25
NBPP	6	1	1	22
NPPP	-	1	-	29
SYPP	-	-	-	30
Fe content	7	9	2	12
Zn content	2	1	2	25

For seed yield no genotype was found to be stable across different environments but for iron content the performance of eleven genotypes could be predicted across environments. For MH-125 and ML-5 both bi and $\bar{S}^2$di were non-significant therefore they are stable but their performance cannot be predicted. For zinc content only one genotype's performance namely ML-818 could be predicted.

The distribution of different stable genotypes for two traits i.e. iron and zinc content in seeds on basis of the three stability parameters (X, bi and S^2di) are presented in table 7; thirteen genotypes were found to be stable across the different treatments for iron content in seeds while only one genotype was stable for zinc content.

Table 7 Grouping of stable genotypes for iron and zinc content on basis of the three stability parameters

	Iron content in seeds of mungbean	Zinc content in seeds of mungbean	Environment conditions
Xi ≥ X bi > 1.0 S^2di = +	ML-1108, SMH-99-2, 2KM-138*, MH-124*, PDM-9-249,ML-759 (6, 13, 20*, 22*, 26, 27)	-	Good for favourable environment conditions
Xi ≥ X bi = 1.0 S^2di = +	ML-5*, 2KM-112, 2KM-135*, MH-215*, SMH-99-B (3*,5, 12* 24* 25)	-	Good for all type of environments
Xi ≥ X bi < 1.0 S^2di = +	MH-125*, ML-839*, MH-421*, ML-406*, MH-318*, M-395*, PMB-14* (2* 8* 10* 17* 21* 28* 29*)	ML-818 (16)	Good for poor environments

+ = non-significant; * = Xi < X; X = average mean; bi= regression coefficient; $\bar{S}^2di$= mean square deviation from the regression

Discussion

The genotypes studied in this chapter were selected from the first experiment based on *per se* performance, chemical analysis and diversity. Thirty genotypes were selected belonging to four separate clusters (Chapter 3 and 5). Analysis of variance of quantitative traits showed highly significant differences among the genotypes among all environments. This indicated that the chosen genotypes had sufficient variability. The pooled analysis of variance showed that mean sums of squares (MSS) due to genotypes were highly significant for all the traits indicating enough variation. The MSS due to environmental conditions were also significant for almost all the traits indicating the validity of conducting an experiment as we did in artificial environments. The interactions were significant for almost all traits indicating considerable interaction between genotypes and environments for the expression of traits. The MSS due to environment + (genotype x environment) and environment (linear) was significant for all the traits indicating that environmental effects are additive. The linear component of GxE interaction was also significant for all the traits under study indicating a significant role of the linear response of the genotypes to environmental changes. Singh *et al.* (1993), Singh and Kumar (1994) and Popalghat *et al.* (1999) also reported differential ranking in their studies in chickpea. The pooled deviation was also found significant for all the traits indicating that the non-linear component of GxE interaction was predominant. Similarly, joint regression analysis reveals that MSS due to genotype was significant and thus supports that there is presence of significant variation among the genotypes for all traits under study. Both heterogeneity between regression (GxE interaction linear) and remainder (non-linear) was found significant for all the traits when tested against the pooled error. It indicates that prediction will depend upon the relative magnitude of these two measures. Further, the prediction will be more reliable when only heterogeneity between regressions is significant against the remainder (Samuel *et al.* 1970). Therefore, in the present study a prediction for plant height, iron and zinc content in seeds of mungbean will be reliable.

Soil analyses during the course of experiment showed some expected and unexpected results. The uptake of copper can be explained by the fact that manganese helps in uptake of copper while after harvest the zinc concentration in the soil was found to be higher as before sowing which may be thought of that excess amount of iron results in decrease in zinc uptake (Ranade 2011) along with Cu presence which also reduces the availability of Zn. Sulphur concentration in soil after harvest had a thirty times higher concentration in comparison to the initial stage analysis which was thought to be caused by super sulphate, which was added in the soil during the course of experiment. An environmental index reveals the favourability/adaptability of an environment at a particular location. Breese (1969) pointed out that the estimates of the environmental index can provide the basis for the identifying the favourable environments for the expression of maximum potential of the genotype. As in the present study two major aspects were in consideration i.e. micronutrient (Fe and Zn) and yield and its attributes. Therefore, on basis of the results presented in Table 5, for yield and its related traits environment 1(E1) was found to be the most unfavourable. This may be related to the fact that this environment is not provided with single super phosphate which promotes the absorption of minerals from the soil. The second most unfavourable environment was E2 for all the traits except for iron content in seed. SSP was also not added in this environment which may be the reason for poor yield and its attributes. Adding SSP was found to be very important for pulses. Zinc uptake become lower after foliar spray of iron. For iron uptake E5 and E6 were unfavourable probably because of the added excess amount of $ZnSO_4$ which hinders the iron uptake (Ranade and Malvi 2011).

For yield E6 and E5, where both SSP and micronutrients were added, were found to be the most optimal. For iron content the most favourable environment was E4, which was expected as this environment SSP was added along with foliar spray of $FeSO_4$ and no excess of $ZnSO_4$. For zinc content E5 was the most favourable. Environment 6 is good for yield micronutrient uptake is lower showing that excess of micronutrients hinder their uptake (Ranade and Malvi 2011). In our study and in other studies it was noticed that there was a variable pattern of response for the different traits in different environments (Singh *et al.* 1990; Singh *et al.* 1991; Popalghat *et al.* 1999).

GxE limits the progress of crop improvement beyond the breeders' station. Nutritional quality of food legumes are subjected to variation caused by different environmental conditions. Dixon *et al*. (1991) define GxE interaction as the change in a cultivars relative performance over environments, resulting from differential response of the cultivar, to various edaphic, climatic and biotic factors. In the present study differential genotypic responses across the different created environments showed that the cultivars differ in response across different environments and thus this can complicate the evaluation and selection of cultivars for any specific purpose or trait like in the present study. This study shows the importance of studying the GxE interaction in mungbean improvement programs.

References

Ashraf M, Mueen-ud-din M, Warraich NH (2003) Production efficiency of mungbean as affected by seed inoculation and NPK application. International Journal Agriculture and Biology 5:179-180.

Breese EL (1969) The measurement of significance of genotype x environmental interaction in grasses. Heredity 24: 26-44.

Eberhart SA, Russell WA (1966) Stability parameters for comparing varieties. Crop Science 6: 36-40.

Jitendra K, Arbind KC, Ramesh KS, Aditya Pratap (2011) Towards marker-assisted selection in pulses: a review. Plant Breeding 130: 297-313.

Mehla IS, Waldia RS, Dahiya SS (2000) Phenotypic stability for some cooking quality attributes among kabuli chickpea (*Cicer arietinum* L.) genotypes. Journal of Genetics and Breeding **54:** 293-297.

Mukai T (1988) Genotype-environment interaction in relation to the maintenance of genetic variability in populations of *Drosophila melanogaster*. Proceedings of the 2nd International Conference on Quantitative Genetics (Editors: B. S. Weir, E. J. Eisen, M. M. Goodman, and G. Namkoong): 21-31.

Mather K, Jinks JL (1982) Biometrical Genetics. Chapman and Hall Ltd., London.

Patil VS, Deshmukh RB, Patil JV (1996) Genetic analysis of quantitative characters in mungbean. Indian Journal of Pulses Research 9: 132-136.

Popalghat GR, Patil JV, Deshmukh RB, Mhase LB (1999) Stability for yield and yield components in chickpea (*Cicer arietinum* L.). Legume Research 22:254-258.

Ranade UM (2011) Interaction of micronutrients with major nutrients with special reference to potassium. Karnataka Journal of Agricultural Sciences 24 (1):106-109.

Samuel CJA, Hill J, Breese EJ, Davis A (1970) Assessing and predicting environment response in *Lolium preme*. Journal of Agriculture Science 75: 1-19.

Singh O, Kumar S (1994) Phenotypic stability of yield and related characters in desi gram (*Cicer arietinum* L.). Indian Journal of Agricultural Science 64:815-820.

Singh KB, Bejiga G, Malhotra RS (1993) Genotype–environment interactions for protein content in chickpea. Journal of the Science of Food and Agriculture 63:87–90.

Singh G, Brar HS, Verma MM (1991) Genotype environment interaction and phenotypic stability in chickpea. Crop Improvement 18:135-140.

Singh KB, Williams PC, Nakkoul H (1990) Influence of growing season, location and planting time on some quality parameters of kabuli chickpea. Journal of the Science of Food and Agriculture 53: 429-441.

Tiwari S, Dwivedi VK, Tiwari S (2000) Stability studies in chickpea. Annals of Agricultural Research 21 (1): 114 - 118.

Wu RL, OíMalley DM (1998) Nonlinear genotypic response to macro- and microenvironments. Theoretical and Applied Genetic*s* 96: 669-675.

Appendix I

Limits for different macro and micronutrients in soil.

Macro/Micronutrient	Low	Medium	High
N	<250 kg/ha	250-500	>500
P	<10	10-20	>20
K	<125	125-300	>300
S	<10	10-20	>20
Zn	0.6 ppm	-	-
Cu	0.2 ppm	-	-
Fe	4.5 ppm	-	-
Mn	2.5 ppm	-	-

Appendix II

Estimates of stability parameters (Eberhart and Russell, 1966) for plant height and number of branches.

V.No		Plant Height			Number of branches per plant		
	Varieties	Mean	bi=1+ βi	S^2di	Mean	bi=1+ βi	S^2di
1	ML-803	48.9	0.84	44.82**	2.6	2.97	0.17**
2	MH-125	70.1	0.25*	8.09**	1.9	1.78	0.01
3	ML-5	75.5	0.88	66.87**	2.6	1.81	0.08**
4	ML-735	60.7	0.679	20.87**	2.0	1.65	0.04*
5	2KM 112	62.1	0.63	2.21*	2.2	0.29	0.04*
6	ML-1108	64.8	0.56	10.97**	2.5	1.75	0.19**
7	MI-3580	62.6	1.04	9.54**	1.7	0.55	0.07**
8	ML-839	61.1	1.24	57.33**	1.9	2.29	0.07**
9	L-24-2	71.6	1.07	10.07**	1.9	2.04	0.04*
10	MH-421	59.6	0.21*	8.55**	1.8	0.24	0.21**
11	2KM-139	75.9	0.28	21.82**	2.5	1.79	0.37**
12	2KM 135	70.5	1.01	23.42**	2.0	0.24	0.02
13	SMH-99-2	76.8	0.60	26.31**	2.8	0.85	0.14**
14	2 KM-107	63.8	0.29	40.82**	1.9	1.46	0.52**
15	BG-39	66.4	1.07	26.45**	2.1	0.19	0.10**
16	ML-818	72.2	0.43	27.29**	2.1	0.38	0.18**
17	ML-406	67.8	1.68	53.73**	2.5	-0.59	0.01
18	2KM-151	52.9	0.23	75.04**	1.7	0.73	0.07**
19	2KM 155	57.3	1.79	33.89**	2.1	0.32	0.01
20	2KM-138	66.2	1.14	13.34**	1.9	-0.82	0.17**
21	MH3-18	54.4	0.79	19.34**	2.3	-0.69	0.12**
22	MH-124	66.9	0.49*	3.65**	1.9	1.33	0.11**
23	ASHA	68.2	1.05	28.73**	2.3	0.35	0.06**
24	MH-215	63.6	1.07	7.42**	2.4	0.82	0.03
25	SMH-99-DULL B	79.1	1.94	112.73**	2.8	0.50	0.46**
26	PDM-9-249	66.8	1.46	12.66**	2.2	2.08*	0.01
27	ML-759	66.8	2.64	61.84**	2.4	2.87*	0.08**
28	M 395	69.8	1.81*	5.49**	2.4	1.58	0.15**
29	PMB-14	50.4	1.06	10.26**	2.9	1.26	0.08**
30	ML-506	63.4	1.83*	11.55**	3.1	-0.01	0.0
Mean		65.2	1.000		2.263	1.000	
S.E (±)		2.44	0.434		0.17	0.740	

*Significant at P= 0.05

Appendix III

Estimates of stability parameters (Eberhart and Russell, 1966) for number of pods and seed yield per plant in mungbean seeds

V.No		Number of pods per plant			Seed Yield per plant		
	Varieties	Mean	bi=1+ βi	$\bar{S}^2di$	Mean	bi=1+ βi	$\bar{S}2di$
1	ML-803	24.206	1.5486	67.850**	19.6	4.767	50.711**
2	MH-125	19.439	0.6266	7.808**	17.239	2.319	14.28**
3	ML-5	19.767	0.9433	6.159**	15.511	1.487	20.38**
4	ML-735	19.467	2.0925	16.863**	19.183	1.777	36.439**
5	2KM 112	17.817	0.4259	13.469**	15.072	0.800	28.532**
6	ML-1108	19.006	1.425	6.710**	14.65	1.661	21.437**
7	MI-3580	18.844	1.3197	14.11**	16.594	2.935	5.419**
8	ML-839	16.639	1.4375	3.0682*	13.994	3.624	10.78**
9	L-24-2	16.611	1.4559	20.972**	12.744	0.984	25.689**
10	MH-421	16.428	1.0676	17.562**	13.706	1.149	13.430**
11	2KM-139	19.683	0.463	12.864**	14.328	-0.308	7.593**
12	2KM 135	17.333	1.4707	170.188**	7.783	-0.581	8.833**
13	SMH-99-2	17.189	0.3429	23.141**	9.994	-0.537	7.427**
14	2 KM-107	11.9	0.0385	16.378**	8.95	0.479	5.684**
15	BG-39	16.622	1.0802	18.937**	11.2	-0.168	8.530**
16	ML-818	16.344	0.6801	5.148**	15.178	0.865	8.872**
17	ML-406	28.144	-0.6261	121.406**	18.978	-2.265	33.579**
18	2KM-151	13.189	0.5225	12.095**	14.117	-0.589	11.223**
19	2KM 155	13.944	1.1047	10.860**	11.372	0.995	9.834**
20	2KM-138	17.617	0.0074	13.556**	16.139	-2.168	6.136**
21	MH3-18	18.989	0.479	3.5976*	19.267	0.127	10.816**
22	MH-124	15.1	0.4724	8.981**	16	-0.771	13.296**
23	ASHA	18.778	1.36	6.285**	16.061	0.898	8.672**
24	MH-215	19.572	0.6465	8.874**	15.8	-0.939	22.086**
25	SMH-99-DULL B	23.789	2.25	16.185**	16.094	2.975	23.536**
26	PDM-9-249	21.372	2.7962*	23.337	18.178	2.502	38.429**
27	ML-759	14.161	1.5862	3.644*	8.756	2.868	10.109**
28	M 395	20.256	1.3538	13.089**	14.017	2.247	8.444**
29	PMB-14	15.206	0.1693	17.730**	11.611	0.709	46.251**
30	ML-506	28.85	1.4602	16.276**	24.267	2.159	11.733**
Mean		18.542	1.000		14.879	1.000	
S.E (±)		2.23	0.607		1.90	1.418	

*Significant at P= 0.05

Appendix IV

Estimates of stability parameters (Eberhart and Russell, 1966) for Iron & Zinc content in mungbean seeds

V.No		Iron content in seed			Zinc content in seed		
	Varieties	Mean	bi=1+ βi	S2di	Mean	bi=1+ βi	S2di
1	ML-803	5.6	-0.35	41.02**	2.9	0.57	0.69**
2	MH-125	2.9	0.17	0.90	1.6	1.02	0.35**
3	ML-5	3.5	0.96	-0.39	4.8	5.84	49.29**
4	ML-735	3.6	0.09	1.61*	2.2	0.07	0.29**
5	2KM 112	4.4	1.07	0.35	2.2	1.03	0.46**
6	ML-1108	5.1	1.83*	0.23	2.9	3.52	6.52**
7	MI-3580	4.1	2.33*	3.22**	1.6	-0.07	0.68**
8	ML-839	2.9	0.21*	0.71	1.8	0.01	0.39**
9	L-24-2	3.3	0.24	1.86*	2.0	1.59	0.91**
10	MH-421	3.4	0.03*	0.79	2.1	0.85	0.22**
11	2KM-139	9.1	4.68**	9.74**	1.7	0.92	0.13*
12	2KM 135	3.8	0.96	-0.63	2.6	2.53	3.67**
13	SMH-99-2	6.3	3.09**	1.21	1.6	1.02	0.32**
14	2 KM-107	6.6	0.59	7.01**	2.4	0.06*	0.12*
15	BG-39	7.9	1.08	46.63**	3.9	1.49	2.53**
16	ML-818	5.8	-0.23	17.04**	2.2	0.19**	0.08
17	ML-406	2.4	0.27**	-0.89	2.2	-0.82	0.33**
18	2KM-151	6.1	0.12	35.42**	1.6	0.26	0.19*
19	2KM 155	3.5	0.12	2.95**	3.2	2.56	4.45**
20	2KM-138	3.4	1.65*	-0.28	2.1	0.29	0.32
21	MH3-18	3.6	0.11**	-0.35	2.4	1.58	1.48**
22	MH-124	4.2	1.7*	0.04	1.5	-0.05*	0.09**
23	ASHA	4.4	1.98	3.52**	1.8	0.91	1.09**
24	MH-215	2.9	1.08	0.22	2.1	1.39	0.73**
25	SMH-99-DULL B	4.5	0.857	-0.63	2.3	0.46	0.30**
26	PDM-9-249	4.4	1.31*	-0.81	1.7	0.64	0.01
27	ML-759	4.9	2.09*	1.01	2.1	0.47	0.52**
28	M 395	2.7	0.06**	-0.62	1.9	0.62	0.52**
29	PMB-14	3.5	0.73	0.17	1.6	0.16	0.27**
30	ML-506	4.2	1.14	2.40**	1.9	0.88	0.29**
Mean		4.4	1.000		2.23	1.000	
S.E (±)		1.17	0.563		0.73	1.023	

*Significant at P= 0.05

CHAPTER 7

Towards Marker Assisted Breeding for Micronutrients (Fe and Zn) in Mungbean

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Abstract

Mineral micronutrient deficiency affects women and preschool children in every part of the world. One of the methods to mitigate micronutrient deficiency is to breed for food crop cultivars with higher levels of micronutrients. In mungbean a start has been made to do this. A molecular study was started in order to identify those chromosomal regions harbouring genes that play a role in the potential level of micronutrients in the plant. For this purpose two different recombinant inbred line populations (RILs) were made, 120 RILs per population were obtained after crossing contrasting parents and repeatedly selfing (single seed descent) until the F6 population. These RILs were phenotyped for micronutrient content and a start was made with genotyping. The content of iron and zinc varied between individuals of the populations: In cross 1 from 1.7 to 5.9 mg/100g (for Fe) and 2.0 to 3.8 mg/100g (for Zn). In cross 2: 2.1 to 8.2 mg/100g (Fe) and 1.0 to 8.4 mg/100g (Zn). In both crosses transgressive segregation was observed. Chemical analysis of the RILs showed as expected a positive correlation between iron and zinc content, however unfortunately in the marker analysis no significant association between the markers and the phenotypic traits was found in the RIL populations. A limited number of markers were run over 30 different cultivars where links to the micronutrient contents were found. One marker explaining 21% of the difference in iron content and 28 of the difference in the zinc content and another marker explaining 24 % of the difference in zinc content.

Keywords: Amplified fragment length polymorphism (AFLP), micronutrients, mungbean, recombinant inbred lines (RILs)

Introduction

Mungbean (Vigna radiata L.) also called green gram is one of the principal legumes and is a very nutritive crop. The seeds are used for human consumption, the plant as fodder for livestock and green manure. The seeds contain 25 to 28% protein, 1.0 to 1.5 % fats, 3.5 to 4.5% fibre, 60 to 65% carbohydrates and are rich in lysine, ascorbic acid, potassium, iron, phosphorus and calcium (Lambrides and Godwin 2007). Moreover, its seeds are more palatable, nutritive and digestible and non-flatulent in comparison to other pulses (Sadeghipour et al. 2010). Thus, mungbean is nature's gift to man in general and to children, pregnant or lactating women and the elderly people especially. It has the potential to be used as an economic food supplement to fight malnutrition. India is the largest producer and consumer of pulses in the world. In India, mungbean is grown on an area of 2.92 million ha, with a production of 1.42 million ton (486 kg/ha, Dixit 2005). As it is one of the major sources of protein and mineral micronutrients it is often traded and consumed locally.

Micronutrient malnutrition, and particularly Fe and Zn deficiencies (the so called 'hidden hunger'), affects over three billion people worldwide, mostly in developing countries (Welch and Graham 2004). Fortification of food is one step in combating these deficiencies but this is not always possible, for instance adding iron doesn't result in a stable product and makes the product unpalatable. To increase the concentration in the edible portions of crop plants it is necessary to incorporate micronutrient content in breeding programs.

Mungbean germplasm screening revealed genetic variation for the content of iron and zinc (ranging from 1.6–9.2 mg/100 g Fe and 1.5- 3.9 mg/100 mg Zn respectively). Iron and zinc concentrations in the seeds tend to be correlated (r = 0.47; this thesis Chapter 3), making it possible to screen for high concentrations of both. The content is substantially influenced by genotype (G) x environment (E) interactions. Recombinant Inbred Lines (RILs) make it possible to screen for high iron and zinc concentrations under different conditions and make it possible to screen for associations between DNA markers and high concentrations (Quantitative Trait Loci). These markers later on can be used in marker assisted selection (MAS) and in this way make the breeding more efficient. Mineral accumulation in higher plants appears to be under control of many genes (Blair *et al.* 2009). In *Arabidopsis thaliana* seed mineral accumulation was found to be quantitative and associated with various candidate genes, like for example 21 genes involved in ion homeostasis (Ding et al 2010).

As mungbean is primarily used as a food, extensive research is being done on seed quality traits such as size, shape, colour, hard-seededness, protein quality and quantity (Humphry *et al.* 2005; Lambrides and Godwin 2007) along with agronomic traits like drought resistance (Sholihin and Hautea 2002), virus resistance (notably MYMV) (Anjum *et al.* 2010). For micronutrient content several studies were conducted in common bean, peas, chickpeas, lentils etc. (Islam *et al.* 2002; Grusak and Cakmak 2005; Haq *et al.* 2007; Thavarajah *et al.* 2010) but till this date, not much effort has been made to locate genes/QTLs responsible for micronutrients in mungbean. With this in mind, the goals of this study were: (a) to choose the best parents to create mapping populations (recombinant inbred lines - RILs), (b) to determine the level of iron and zinc in the individuals of these RIL populations and (c) to analyse the segregation patterns of iron and zinc concentration in these RILs. After this it could be decided whether a more extensive QTL-mapping study will be a feasible follow up.

Materials and methods

Plant materials

120 Recombinant inbred lines (F6 RILs) obtained from a cross between BG 39 X 2KM 138 and 120 recombinant inbred lines from SMH 99-1 X BDYR1 were made through single seed descent (SSD) at the pulses research field, CCS HAU, Hisar (India). The parents used in both the crosses were contrasting in their micronutrient (Fe & Zn) content and agronomic characters (Table 1). The diversity analysis (Chapter 3 and 5 of this thesis) showed that the selected parents fall in different clusters. The 30 selected cultivars are the same as in Chapter 3, Table 4)

Table 1 Micronutrient content in RIL parents

No.	Crosses	Genotype	Characters	Cluster No.*
Cross 1	BG39 X 2KM138	BG39	High in Fe (6.0 mg/100 g), medium in Zn (2.6 mg/100 g)	II
		2KM138	Low in Fe (<2.4 mg/100 g), low in Zn (<2.0 mg/100 g)	V
Cross 2	SMH99-1 X BDYR1	SMH99-1	Medium in Fe (<3.9 mg/100 g), high in Zn (>3.1 g/100 mg)	I
		BDYR1	High in Fe (>4.5 mg/100 g), medium in Zn (>2.5mg/100 g)	II

*Chapter 3 and 5 (this thesis)

Experimental design and mineral analysis

120 recombinant inbred lines (RILs) of both crosses were sown in a randomized block design (RBD). Sowing was done in July 2010 and ten seeds of each RIL were sown in a four meter row, 45 cm apart with intra-spacing of 10 cm, CCS HAU, Hissar. Trials were managed with recommended fertilization rates and the plots were hand harvested to avoid any contamination. Each plant was threshed separately in a bag and used for chemical analysis. Phenotyping Fe and Zn content measurements were done using atomic absorption (AAS) analysis and was based on nitric/per-chloric acid digestion. Briefly, 1 g of each sample was acid digested with 5 ml of a 2:1 mixture of 65% nitric acid (HNO_3) and 70% per-chloric acid ($HClO_4$) in a 50 ml Taylor digestion tube for 2 h followed by a heat treatment of 200 ^oC for 2 h and a re-suspension in 25 ml of deionised water. With sample read on atomic absorption (Perkin Elmer Aanalyst 400 atomic absorption spectrophotometer) in the Shree Balaji Test Lab (SBTL).

Statistical analysis: Phenotypic/Chemical data

The phenotypic data obtained for iron and zinc content was analysed using t test ($t = \bar{X} - \mu / s/\sqrt{n}$). Where, s= standard deviation of the sample, n= number of observations, $\bar{X}$ = sample mean & μ = parent mean. Mean and range among the RILs in comparison with parents and correlation coefficients (r) among these traits were also estimated using the pooled data over environments (PE). The computation for the data was performed using the software package SAS.

Molecular marker analysis

Young leaves from 3 - 5 weeks-old seedlings were collected and immediately stored at -80^{o}C. The DNA isolation and molecular work was carried out at Wageningen-UR Plant Breeding, the Netherlands. Leaf tissue from each individual was ground to a fine powder using two grinding beads in a Shatter-box and total genomic DNA was extracted using 96 well plate automated DNA isolation machine (according to KingFisher® 96 manual; Thermo Labsystems). In the buffer solution RNAse and Proteinase K were added. The DNA concentration was estimated after gel electrophoresis by comparing with known λ DNA concentration standards. The Li-Cor AFLP Kit was used according to the recommendations of the manufacturer (Invitrogen). 100 ng DNA was digested with restriction enzymes *Eco*RI and *Mse*I and enzyme adapters were ligated to the digested DNA. The Restriction Ligation buffer (10mM Tris.HAc pH 7.5, 10mM MgAc, 50mM KAc, 5mM DTT, 50 ng/µl BSA) was suitable for both enzymes. After 10x dilution of the primary template in Tris-EDTA buffer the pre-amplification of the template was performed to generate the secondary template DNA that can be used in selective amplification. The selective amplification of restriction fragments was done with colour labelled primers with in total six selective nucleotides (Table 2). After selective amplification 5 µl of the reaction product was mixed with an equal volume formamide–loading buffer (98% formamide, 10mM EDTA pH 8.0 and 0.1% Bromo-phenol blue). The total mixture was carefully mixed and heated for 5 minutes at 94^{o}C in a hot-block and then quickly cooled on ice. From the 10 µl, 8 µl was loaded on a 6% denaturing polyacrylamide gel 1XTBE buffer. Li-Cor 4300 S DNA analyser.

Table 2 Primer combinations used to screen for the highest level of polymorphisms.

EcoRI		MseI		
Primers +0		Primers +0		
5'-GACTGCGTACCAATTCNNN-3'		5'-GATGAGTCCTGAGTAANNN-3'		
Primers +1	A-3	Primers +1		
E01	A-3	M02	C-3	
Primers +3				
E31	AAA-3	M47	CAA-3	
E32	AAC-3	M48	CAC-3	
E35	ACA-3	M49	CAG-3	
E36	ACC-3	M50	CAT-3	
E37	ACG-3	M51	CCA-3	
E40	AGC-3	M52	CCC-3	
E45	ATG-3	M53	CCG-3	
		M54	CCT-3	
		M55	CGA-3	
		M56	CGC-3	
		M57	CGG-3	
		M58	CGT-3	
		M59	CTA-3	
		M60	CTC-3	
		M61	CTG-3	
		M62	CTT-3	

Marker-Trait associations.

The software programs JoinMap® 4.1 (Van Ooijen 2011) were used to calculate linkage between markers and MapQTL® 6 (Van Ooijen 2009) was used to determine significant associations between markers and phenotypic traits.

To find markers related to iron and zinc content in the 30 cultivars, an uni-variate linear regression method was used.

Results

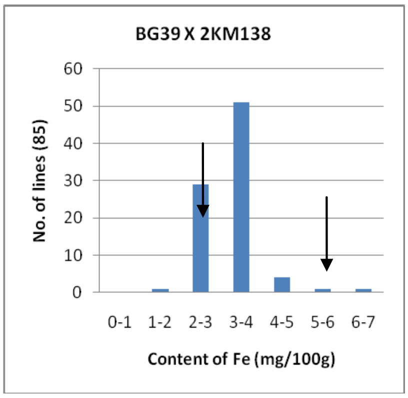
Phenotypic data analysis

The values of the individual lines in the RIL population in cross 1 ranged for iron from 1.7 mg/100g to 5.9 mg/100g and for zinc 2.0 mg/100g to 3.8 mg/100g while for cross 2 the range for iron is 2.1 mg/100g to 8.2 mg/100g and for zinc 1.0 mg/100g to 8.4 mg/100g (Table 3). A significant positive correlation was found between iron and zinc concentrations (r = 0.47) (Table 3). Frequency distribution of the pooled data for both the traits revealed in several cases transgressive segregation both on the negative and positive side (Table 3). Distributions in classes of the Fe and Zn content in both crosses are shown in Figure 1 (A-D).

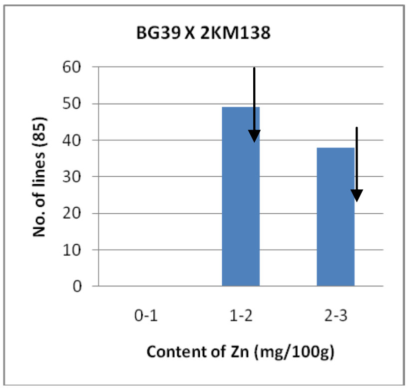
Table 3 Mean, range, standard deviation and correlation coefficients for iron and zinc in mungbean (*Vigna radiata* L).

Traits	Recombinant inbred lines			
	Parents Mean*	Mean	Range	St. Deviation
Cross I- Fe	4.2 (6.0 and 2.4)	3.2	1.7 – 5.9	0.56
Cross I- Zn	2.3(2.6 and 2.0)	2.9	2.0 – 3.8	0.32
Cross II- Fe	4.3(3.9 and 4.5)	4.1	2.1 – 8.2	1.05
Cross II- Zn	2.8(3.1 and 2.5)	2.9	1.0 – 8.4	0.78
		Pearson's Correlation Coefficient**		
		Fe	**Zn**	
Fe		1.000	0.038	
		0.0	0.474	

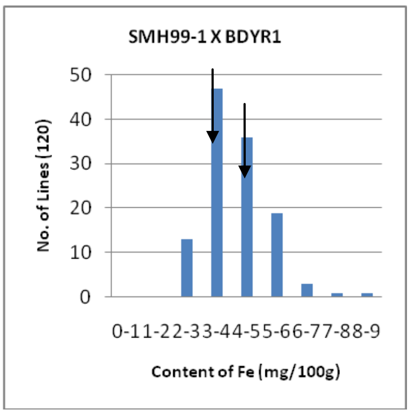
* Between brackets the values of the two parents; **overall correlation between iron and zinc



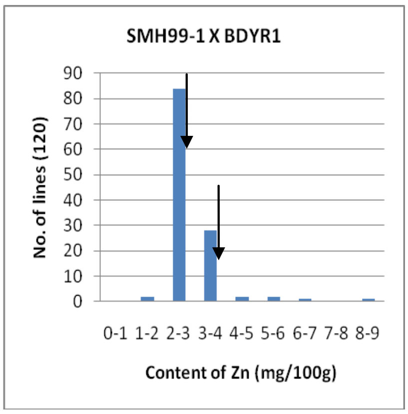
(A)



(B)



(C)



(D)

Figure 1 A. Distribution of Fe content in RIL population in cross 1; B. Distribution of Zn content in RIL population of cross 1; C. Distribution of Fe content in RIL population of cross 2; D. Distribution of Zn content in RIL population cross 2; Arrows show the parental values.

DNA polymorphism

Level of polymorphism determined by AFLP analysis

In order to choose the enzyme primer combinations with the highest level of polymorphisms the four parents were subjected to in total 96 different enzyme primer combinations. The average number of polymorphisms for the parents of cross 1 was 4.4, this was somewhat lower between the parents of cross 2 (3.7 polymorphisms per combination). See Table 4.

Table 4 The number of polymorphisms between the parents of both crosses with different primer combinations.

		M47	M48	M49	M50	M51	M52	M53	M54	M55	M56	M57	M58	M59	M60	M61	M62	
Pop 1	E32	8	6	1	8	6	3	7	6	1	4	1	2	3	4	x	x	60
Pop 1	E35	5	5	4	6	5	3	2	6	1	4	2	6	7	5	2	9	72
Pop 1	E36	4	7	4	9	8	5	8	3	4	4	3	5	4	6	4	7	85
Pop 1	E37	1	4	2	6	3	9	4	9	3	6	1	2	6	4	2	10	72
Pop 1	E40	7	7	1	7	7	3	5	4	1	2	2	4	6	0	6	8	70
Pop 1	E45	6	9	2	5	5	8	2	2	1	1	0	3	2	4	6	2	58
		31	38	14	29	34	31	28	30	11	21	9	22	28	23	20	36	417
Pop 2	E32	4	2	1	6	3	3	8	2	4	0	4	4	4	4	1	x	50
Pop 2	E35	6	5	0	5	2	2	4	8	3	2	4	1	4	2	3	8	59
Pop 2	E36	3	4	5	7	10	4	6	5	3	5	2	7	2	4	3	3	73
Pop 2	E37	4	2	5	7	4	7	3	8	3	3	3	2	3	4	3	8	69
Pop 2	E40	3	4	2	2	4	3	5	4	1	2	2	5	4	2	4	7	54
Pop 2	E45	3	7	4	9	4	5	3	1	1	2	2	0	1	1	3	4	50
		23	24	17	36	27	24	29	28	15	14	17	19	18	17	17	30	355

Screening RIL population of cross 2 for AFLPs

Due to limited time and technical constraints we decided to start with analysing the RIL population of cross 2 with the primer combinations E32M47 (8 polymorphisms), E32M48 (6 polymorphisms), E32M51 (6 polymorphisms), E32M53 (7 polymorphisms), E35M48 (5 polymorphisms), E35M51 (5 polymorphisms) and E40M47 (7 polymorphisms). The choice of enzyme primer combinations was based on the level of polymorphisms and the quality of the overall AFLP pattern. Of the expected 44 polymorphisms only 31 could be scored over the whole population. The other 13 were not clear enough for reliable scoring. This showed that only about four markers per combination were usable.

Mapping studies

The 31 segregating markers were used to calculate linkages between markers. Mungbean is diploid (2n=2x=22) and has a small genome size i.e. 0.60 pg/1C (579 Mbp) (Somta and Srinives 2007). With the software package JoinMap® 4.1 (Van Ooijen 2006) seven linkage groups were found and six unlinked markers. Most linkage groups were small (2 markers in 2 cM, 3 markers in 3 cM, 3 markers in 17 cM, 2 markers in 16 cM and 2 markers in 10 cM) but a few were formed by more markers (9 markers in 61 cM and 4 markers in 25 cM). The linkage groups with more than 2 markers are depicted in Figure 2. Fourteen markers had a significant skewed segregation, four towards parent 1 and ten towards parent 2.

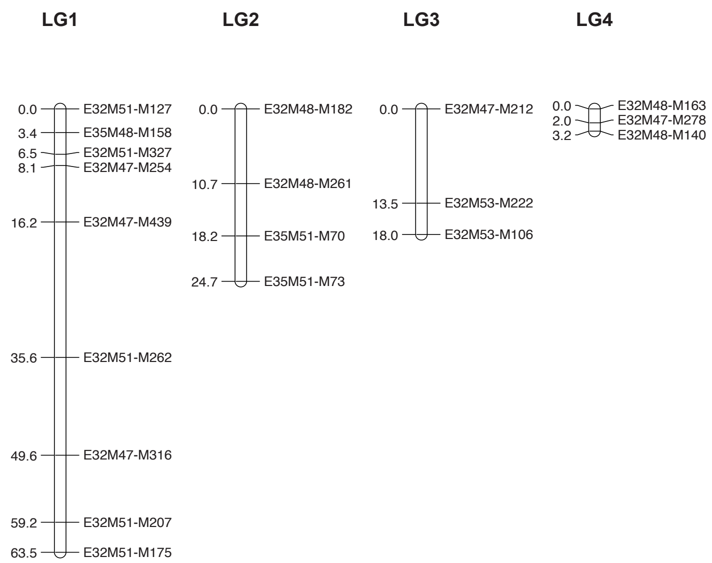


Figure 2 Four Linkage Groups with more than two markers based on the RIL population of cross 2

QTL analysis

All individual markers and the two traits were used for calculating marker-trait associations. The software package MapQTL6 (Van Ooijen 2009) was used to determine significant associations between markers and phenotypic traits. No significant or putative associations were found.

Association mapping

The 30 cultivars (Chapter 3, Table 4)were also genotyped with nine AFLP primer combinations (E32M51, E35M50, E35M48, E37M50, E35M59, E35M62, E32M47,E32M48, E32M49), in total 192 AFLP markers were present in part of the cultivars, an univariate linear regression resulted in the identification of one AFLP marker that was linked to a higher iron content. Twelve cultivars have this marker and eighteen not and the cultivars were divided over the phylogenetic tree. Genotypes with the marker (E32M48 M140) have an average Fe content of 4.1 mg/100g and the group without the marker had on average 3.4 mg/100g Fe content; this marker explains 21% of the variance and is present in genotypes 1, 2, 4, 7, 10, 11, 17, 19, 22, 23, 24, 30 (Chapter 3, Table 5). Due to the correlation of Fe and Zn content it was not unexpected that this marker was also associated with Zn content, and explained 28% of the variance. Another marker (E35M50 M204) explained 24% of the variance in Zn content.

Discussion

The main question we addressed in this chapter was: can we find DNA markers linked to genes that have a role in mineral uptake and can cause differences in the level of uptake. To find these markers or even the genes themselves is of utmost importance in starting a breeding program aimed at higher levels of micronutrients (biofortification). To start such a mapping study good mapping populations and a good DNA marker system are needed. Recombinant Inbred Lines are in principle homozygous and make it possible to harvest as many genetical identical seeds as needed. In other words they are a permanent resource for testing and repeating experiments. This is particularly useful in studying the effects of different environments (i.e. soil conditions) on the level of Fe and Zn in leaves and seeds. We created two different RIL populations with parents differing in a number of characteristics especially Fe and Zn concentration. The results showed a 2-3 fold difference in concentrations between individual lines and also some transgressive segregation was seen (values higher than the values of the parent with the highest value). In general the results showed that also in mungbean it is possible to breed for new cultivars with high micronutrient content. Studies in other pulses have shown similar results (Blair *et al.* 2009; Cichy *et al.* 2009; Khoshgoftarmanesh *et al.* 2010). Our results showed a positive or no correlation in mungbean between Fe and Zn content. This makes it possible to breed for new varieties high in both; similar observations have been reported in literature (Beebe *et al.* 2000; Guzman-Maldonado *et al.* 2003). The uptake of both might be under control of similar mechanisms (Blair *et al.* 2009).

To allow efficient breeding for good mungbean cultivars with high levels of Fe and Zn it is important that the tools are available that allow this. Since over 20 years Marker Assisted Selection has been such a tool in many crops (Babu *et al.* 2004; Sean May *et al.* 2010). Two steps are of importance: identification of marker trait associations and later using this information in molecular breeding programs. The molecular marker technology has been changing rapidly the last few years; due to rapid changes in efficiency of sequence technology it is possible to find enough polymorphisms. Regrettably, when we had to analyse our RIL populations we only had access to the AFLP technology, a marker system that can be used in mapping populations without prior knowledge. It turned out that only few scorable markers could be generated by this technology in our populations and we were forced to stop before we had gathered enough marker data to make high density linkage maps that would make it possible to look for marker trait associations. With our limited data set we didn't find any marker trait associations. However, in genotyping the 30 cultivars two markers were identified linked to genes underlying the difference in Fe and/or Zn. Genetic studies are needed to show that these markers are linked or not.

We hope that in the near future our mapping populations will be of use in combination with modern sequence based marker technology such as described in Viquez Zamora *et al.* (2013). Highly likely genotyping by sequencing will also be an affordable option in the near future (Elshire *et al.* 2011). These methods will highly probable lead to markers linked to the genes playing a role in micronutrient levels in mungbean. This will give mungbean a role in solving the problem of the hidden hunger.

References

Anjum T, Gupta KS, Datta S (2010) Mapping of *Mungbean Yellow Mosaic India Virus* (MYMIV) and powdery mildew resistant gene in black gram [*Vigna mungo*(L.) Hepper]. Electronic Journal of Plant Breeding 1: 1148-1152.

Babu R, Sudha K. Nair, Prasanna BM, Gupta HS (2004) Integrating marker-assisted selection in crop breeding – Prospects and challenges. Current Science 87 (5): 607-619.

Beebe S, Gonzalez A, Rengifo J (2000) Research on trace minerals in the common bean. Food and Nutrition Bulletin 21:387–391.

Blair MW, Giraldo MC, Buendía HF, Tovar E, Duque MC, Beebe SE (2009) Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.) Theoretical and Applied Genetics 113: 100-109.

Cichy KA, Caldas GV, Snapp SS, Blair MW (2009) QTL analysis of seed iron, zinc and phosphorus levels in an Andean bean population. Crop Science 49 (5): 1742-1750.

Ding Guangda,Mei Yang, Yifan Hu, Yuan Liao, Lei Shi, Fangsen Xu, Jinling Meng (2010) Quantitative trait loci affecting seed mineral concentrations in *Brassica napus* grown with contrasting phosphorus supplies. Annals of Botany 105(7): 1221–1234.

Dixit GP (2005) Project Coordinators Report. Annual Group Meet (*kharif*, 2005). Indian Inst. Pulses Res., Kanpur.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K. (2011) A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. PLoS ONE 6 (5): e19379. doi:10.1371/journal.pone.0019379.

Grusak MA, Cakmak I (2005) Methods to improve the crop delivery of minerals to humans and livestock. In: MR Broadley, PJ White (eds) Plant Nutritional Genomics. CRC Press, Boca Raton, 265–286.

Haq Zia Ul-M, Iqbal S, Ahmad S, Imran M, Niaz A, Bhanger MI (2007) Nutritional and compositional study of Desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. Food Chemistry 105: 1357-1363.

Humphry M, Lambrides C, Chapman S, Aitken E, Imrie B, Lawn R, McIntyre C, Liu C (2005) Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. Plant Breeding 124: 292-298.

Islam FMA, Basford KE, Jara C, Redden RJ, Beebe SE (2002) Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genetic Resource and Crop Evolution 49: 285–293.

Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbaksh B, Afyuni M (2010) Micronutrient efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agronomy Sustainable Development 30: 83-107.

Lambrides CJ, Godwin I D (2007) School of Land and Food Sciences, Plant Improvement Group, The University of Queensland, St Lucia, QLD 4072, Australia (chapter mungbeans).

Maldonado-Salvador H Guzmán, Octavio Martínez, Jorge A. Acosta-Gallegos, Fidel Guevara-Lara, Octavio Paredes-López (2003) Putative Quantitative Trait Loci for Physical and Chemical Components of Common Bean. Crop Science 43: 1029-1035.

Sadeghipour O, Monem R, Tajali AA (2010) Production of Mungbean (*Vigna radiata* L.) as affected by nitrogen and phosphorus fertilizer application. Journal of Applied Science 10: 843-847.

Sean Mayes, Florian Stadler, Gracia Ribas-Vargas, Shraani Basu, Festo Massawe, Aurelie Bonin, Chiew Foan Chin, Neil Graham, Martin bRoadley, Andrzejkilian, Jeremy Roberts, John Fouikes, Sayed Azam Ali (2010) Marker-assisted breeding in crops: wheat and bambara groundnut as two contrasting examples of progress. Academia.edu (http://www.academia.edu/2124771/Markerassisted_breeding_in_crops_wheat_and_bambara_groundnut_as_two_contrasting_examples_of_progress).

Sholihin, Hautea DM (2002) Molecular mapping of drought resistance in mungbean (*Vigna radiata*): 2: QTL linked to drought resistance. Journal of Biotechnology Pertanian 7: 55-61

Somta Prakit, Srinives Peerasak (2007) Genome Research in Mungbean [V*igna radiata* (L.) Wilczek] and Blackgram [*V. mungo* (L.) Hepper]. Science Asia 33: 69-74 Supplement 1.

Thavarajah D, Thavarajah P, See Chai-Thiam, Vandenberg A (2010) Phytic acid and Fe and Zn concentration in lentil (Lens culinaris L.) seeds is influenced by temperature during seed filling period. Food Chemistry 122: 254-259.

Van Ooijen (2006) JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands.

Víquez-Zamora, Ben Vosman, Henri van de Geest, Arnaud Bovy, Richard G.F. Visser, Richard Finkers, Adriaan W. van Heusden (2013) Tomato breeding in the genomics era: Insights from a SNP array. Marcela BMC Genomics 14:354.

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany 55 (396): 353-364.

CHAPTER 8

General Discussion and Perspectives

In the given framework the step to reconnect food production with food consumption and nutrition was taken into account. In this thesis an effort was made to strengthen the local mungbean food networks by exploring and studying mungbean production in the north Indian region and also studying the associated mungbean production constraints along with its germplasm diversity and micronutrient content. The specific objectives of this thesis were:

i. Determining the major constraints, limitations and preferences of producers (Chapter 3).

ii. Characterising the eating habits of consumers especially in the context of mungbean and its iron, zinc and protein content (Chapter 4).

iii. Assessing the diversity in the available germplasm with emphasis on yield and related traits along with micronutrient iron and zinc concentrations (Chapter 5).

iv. Assessing the effects of different environments on selected cultivars for their mineral micronutrients (Chapter 6).

v. Mungbean recombinant inbred lines were developed and assessed for their iron and zinc content. A start was made to develop tools for marker assisted breeding (Chapter 7).

The main objective of this general discussion is to describe the major outcome of this thesis and to reflect on it. In addition, the effect and contribution of this study to the goals of the "Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition" (TELFUN) project along with overall impact of this project is discussed. In the end perspectives and recommendations for future research are given.

Study area and sampling methods

The interdisciplinary Indian TELFUN team carried out a survey on the production and consumption of mungbean in Haryana, India in local food networks. First of all a coordinated network survey was performed in the Hisar district of Haryana state. These regions are important mungbean producing areas in Haryana (Chapter 3, Table 1). In the Hisar district two villages (Mangali and Dhiktana) were selected on basis of the importance of mungbean production, the presence of school feeding programmes and the accessibility of the area from the university to the villages. A majority of the farmers of this selected area produces mungbean, in a large or small scale and thus all the actors of the mungbean food networks could be found, namely farmers, processors, consumers and traders. Very often the actors played interchangeable roles for example, simultaneously acting as a farmer, processor and consumer. The presence of a school with children of 6 to 8 years was essential for the study of the human nutritionist. The school feeding programme allowed the investigation of part of the TELFUN programme in a controlled environment, i.e. the effect of mungbean foods on the health of school going children. Twenty farmers (ten in each village) actively involved in mungbean cultivation were selected after identification of the major constraints and limitations in mungbean production during the focus group discussions. Details about the farmers' visits are given in Chapter 3 and 4. The main objective of this survey was to make the team aware about the production, processing, consumption and trade of mungbean at a small scale level.

The survey showed that farmers prefer growing mungbean mostly in marginal areas as it is not a staple crop in this region. Also, mungbean was associated with a severe disease problem, namely mungbean yellow mosaic virus (MYMV) infection which results in 30-100% loss of the crop (Nene 1972). Farmers had in general poor knowledge about the health benefits of eating mungbean. When the farmers were invited for selection of desirable cultivars in the field, a majority of them selected only the resistant cultivars with high yield potential. These farmer selected cultivars were later on included in further experiments (diversity and chemical analysis). Selected advanced cultivars were provided to the food science researcher and the nutritionist of TELFUN team for their experiments and one best genotype for mid-day school feeding programme. This thesis on mungbean production doesn't include the entire North-Indian region such as Uttar Pradesh, one of the biggest mungbean producers in the region. Nevertheless, we believe that our findings apply to the entire mungbean growing region because of the fundamental nature of our investigations.

Role of mungbean in alleviation of the nutritional deficiencies

According to World hunger facts 2011, two billion people are anaemic, suffering from protein and energy malnutrition. These health problems are increasing at an alarming rate and appropriate action is required. Micronutrient malnutrition has been addressed through food fortification, dietary supplementation and biofortification of staple crops, but to date such programs have had limited success. The major limitations involved in such programmes are; the costs involved in research (lack of public funds), limited or poor regulatory compliance, rural populations with limited access to processed foods, low intakes (small serve sizes) and low bioavailability of micronutrients. Mungbean has always been considered as a good supplier of protein and energy amongst the vegetarian population of India. Though mungbean is a well-known food legume particularly in South East Asia, and its nutritional value has long been recognised, its popularity has not improved with many farmers because of the relatively low yields that have been obtained, partly due to its susceptibility to pests and diseases. The mineral micronutrient of several legume crops along with staple food grains were studied by a number of researchers. Cultivars of common beans show variability for iron concentrations ranging from 3.0 to 12.0 mg/100 g and zinc concentrations ranging from 2.0 to 6.0 mg/100 g (Blair *et al.* 2009). Micronutrient analysis was also carried out in lentils which showed iron concentrations from 7.3 to 9.0 mg/100 g and zinc concentrations ranging from 4.4 to 5.4 mg/100 g (Thavarajah *et al.* 2011). Similar studies in mungbean germplasm show a range for iron content varying from 1.6 to 9.3 mg/100g with a mean of 4.0 mg/100g and for zinc from 1.5 to 3.9 mg/100g with a mean of 2.6 mg/100g. However, recombinant inbred lines in the present studies showed a range of iron from 1.7 – 8.2 mg/100g and zinc ranges from 1.0 to 8.4 mg/100g in individuals of both crosses. For the RIL analysis the parents should have a maximum diversity. However, utmost care has to be taken with respect to other characters like susceptibility to prevalent diseases, days to maturity and yield of the parents for the success of the experiments in the field.

Different aspects of mungbean study and research

One of the benefits of studying mungbean was that unlike rice which is polished before eating (resulting in a significant loss of nutrients), mungbean is usually consumed without such treatments, thus conserving all its nutritional content. For any programme aimed at supplying extra nutrients a few things must be kept in mind. Firstly, what is the amount of food that is consumed; secondly what is the level of micronutrient content present in the food and thirdly what is the bioavailability of the micronutrients. In case of iron deficiency, three possible ways at different levels can be used to solve the problem, supplementation by adding available iron supplements to the diet, biofortification by breeding for higher levels of micronutrients and finally enhancing bioavailability by reducing the presence of chelating agents in the food. Biofortification is one of the cheapest methods for increasing the micronutrient availability to the human population through crop breeding programmes. Success of a breeding programme mainly depends on the genetic variation for the trait of interest and a good method to incorporate the trait in a breeding program. These approaches can improve the nutritional level and as a consequence also the health of the people who consume it. Therefore, in the framework of TELFUN, mungbean germplasm was evaluated for two main attributes i.e. yield and level of mineral micronutrients (iron and zinc). We found appreciable variation for the micronutrient and protein levels (Chapter 5). But in order to use mungbean in solving the problem relating to mineral malnutrition this variation must be explored further to increase mineral content in eatable parts (seed) of the plant. No genotype showed stability for a given trait across different soil conditions. But a significant positive correlation between the levels of iron and zinc was found which showed a ray of hope to further improve mungbean for micronutrient content. A negative correlation was found between yield and protein content however this was non-significant. On the other hand a positive correlation was present between micronutrients and yield. Thus there is a possibility to explore mungbean in elevating the problem of mineral micronutrient deficiency. Similar results were also reported in other crops such as wheat, rice, maize, beans, cassava etc. (Graham *et al.* 2001). A logical next step was to study the inheritance underlying the differences in levels of iron and zinc. In this way genes with an active role in iron absorption, translocation and accumulation are targeted and their chromosomal locations should be as far as possible be determined. Also genes with an effect on bioavailability should be further studied. Overall the interests of different actors in the chain i.e. from producer to processor to consumer should be considered in order to identify applicable solutions. And a more sustainable production of nutritious and healthy food must be the goal, but breeding for higher trace mineral density in seeds should not have a yield penalty. That this can be complex is exemplified by a study in which it was shown that high levels of minerals in seeds resulted in a lower yield but in more resistance to disease and abiotic stresses (Bouis 2003). Here the potential yield (without biotic and abiotic stress) is lower but there is a positive effect on the realized yield and thus no economic loss to poor farmers.

It was already known that variation in the content of micronutrients can be different due to climatic conditions. Therefore a stability study was an important aspect to appreciate the real value of the variation of iron and zinc content in mungbean. The accumulation of micronutrients is a complex process and till date it was not very well understood. Nutrient absorption, translocation and redistribution are tightly controlled and many barriers do not allow plants to accumulate nutrients beyond the limit which is toxic to plant tissues. The first barrier is the rhizosphere which allows the root to absorb micronutrients from the soil. The presence of micronutrients in seeds thus also depends on environmental conditions (soil and spraying) and therefore we studied this aspect in TELFUN. A differential response of genotypes to different environments/soils (genotype environment interactions; G X E) makes it more problematic to identify those genotypes capable of accumulating the highest levels of nutrients. Significant differences in grain Fe & Zn levels along with significant G X E were observed in grains of various crops (Gregorio 2002; Oikeh *et al.* 2004; Jiang Li-Na *et al.* 2010). The development of genotypes with a wide adaptability has long been a universal goal for breeders. One of the objectives of the present study was to understand the nature and magnitude of the GxE interaction (Chapter 6) for micronutrients. Different conditions were created and the condition named environment 6 was supplemented with single super phosphate along with iron and zinc which favours yield and other agronomic traits was found. For iron, environment 4 was found to be favourable as this environment was supplemented with foliar spray of $FeSO_4$ and with no supply of $ZnSO_4$ which otherwise hinders iron absorption while for zinc, environment 5 was the most favourable. This environment was supplemented with $ZnSO_4$ along with SSP, this helps in better zinc absorption from the soil. The farmers should therefore be advised to apply these nutrients through fertilizer application on the basis of soil tests.

In GxE experiments, only few cultivars show a predictable response for the seed iron content while for zinc prediction this was not possible. The most promising genotypes can be potentially utilized in breeding programs in which the micronutrient and protein levels are selectable traits. Negative correlations could be broken for economic benefits through conventional or novel breeding techniques. Besides, nutritional advantages such cultivars can have other benefits such as a root system with better capacity to tap subsoil water. Increase in the micronutrient content in the soil or by spraying, results in higher levels in the mungbean plants and subsequently in plant foods (seed). These results are in accordance with experiments carried out in related crops like soybean, cowpea etc. (Soheil Kobraee *et al.* 2013). The survey showed that mungbean is one of the favourite legumes consumed in the community and therefore this was a promising crop to investigate. The micronutrient levels in the plant are important but even more important are the way of processing of the mungbeans and subsequently the bio-availability in different recipes. Research in genetic modification of plants to increase the uptake of nutrients holds promising possibilities. For long term solutions to micronutrient deficiencies, it is really beneficial to improve the nutritional quality of grains through genetic modification and improved agricultural practices. Transgenic approaches were used in biofortifying staple crops like high β-carotene 'golden rice' grain, high ferritin-Fe rice grain. Efforts were also carried out in developing transgenic improved beans, maize, wheat and cassava. But many such transgenic crops were no success as masses were not aware about the benefits and even if they knew there was a low public acceptance. Developing micronutrient enriched plant foods with better yield and protein using traditional plant breeding methods in combination with Marker Assisted Breeding will be more acceptable to the masses. For farmer acceptance productivity must be maintained or increased. Thus in our opinion molecular assisted breeding must take the lead in solving the problem of mineral malnutrition and along with this, improved agronomic practices appropriate for improved yield and quality must be followed by farmers. For this, fertilizer application based on soil tests need to be popularised.

Molecular marker technology

Mungbean is a self-pollinating diploid plant with $2n = 2x = 22$ chromosomes and a genome size of 515 Mb/1C (Parida *et al.* 1990). Genomic studies in this crop are lagging far behind other legume crops and not many markers were specifically developed for mungbean. RFLPs from soybean and common bean were used extensively in mungbean mapping studies (Somta and Srinives 2007). A limited number of SSRs were developed for mungbean (Miyagi *et al.* 2004; Gwag *et al.* 2006). Sithichoke *et al.* (2009) used high throughput genome shotgun sequencing techniques and identified 1,493 SSR motifs that could be used as potential molecular markers. These markers could prove useful in germplasm analysis, assessing genetic diversity and linkage mapping of mungbean. Further knowledge of the extent of genetic diversity is useful for the utilization and preservation of genetic resources. In case of mungbean low to moderate genetic diversity was observed (Lavanya *et al*. 2008; Datta *et al.* 2012). Fast and cost-effective assessment of genetic diversity between genotypes can be determined using phenotypic variation and/or molecular markers. Morphological characteristics alone are inadequate because interactions between the genotype and environmental factors might influence the expression of the morphological differences. In contrast to morphological characters, molecular markers based on differences in DNA sequences are more reliable, independent of environmental conditions and virtually unlimited in number (Agarwal *et al.* 2008). To perform a genetic study in mungbean with respect to iron and zinc two Recombinant Inbred Line (RIL) populations were made by repeated selfing and single seed descent. An inventory of the level of amplified fragment length polymorphisms (AFLP) was made and this turned out to be too low to obtain within the framework of the project a medium- or even high density genetic linkage map. Recent advances in marker technology (such as sequence based Single Nucleotide Polymorphism identification) will very likely make it possible to use these two Recombinant Inbred Line populations in the near future for mapping studies in order to identify marker trait associations. For future studies efforts should also be made to make crosses in between more diverse parents with respect to mineral micronutrients. In the present study the variation between the parents selected was considerable for iron i.e. 2.4 - 6.0 mg/100g but for Zn the value was 2.0 - 3.0 mg/100g which was not diverse. This was because agronomic traits were also considered in the present studies. Therefore it was suggested that if working for mineral micronutrients more diverse and contrasting parents have to be selected in order to find marker trait associations.

The interdisciplinary approach to improve local mungbean food networks

TELFUN was an interdisciplinary and comparative research programme designed to investigate how production of local food crops is linked to the nutritional status of the resource poor in order to stimulate food sovereignty. The overall objective of TELFUN was to investigate whether food sovereignty can be achieved by taking into account the preferences of farmers, processors and consumers and the best ways to produce crops and process them in food products.

Food sovereignty is supposed to grow from household to community to regional and finally to the national level. Food sovereignty (*anna swaraj*) is the right and freedom to grow diverse (production) and nutritious food (consumers) and the right to have access to save healthy adequate and affordable food (market).

TELFUN used collaborative and combined approaches involving four disciplines with an overall objective of producing a nutritionally rich crop which upgrades the nutritional status of the masses. In order to fulfil this objective an effort was made to study and access production, processing, consumption pattern of the locally grown and acceptable crop. The role of plant breeding research was to contribute to selection and evaluation of the mungbean germplasm for the most preferred agronomic traits, micronutrient levels and protein concentration. In this thesis, the research focussed on mungbean based production and factors influencing micronutrient levels. Participatory varietal selection (PVS) gives awareness of the views and expectations of farmers and also reflects the knowledge of farmers. Farmer's knowledge was defined as the capability of a farmer to co-ordinate and moulds a wide range of social-technical growth factors within the specific local networks towards desired outcomes. The farmer's knowledge involves the art of developing agriculture within local conditions and to rebalance growth factors towards the local conditions. A related term used for this is 'indigenous knowledge' (Scoones and Thompson 1994). Therefore the agronomic evaluation was carried out according to the constraints and demands of the farmers as told in the initial stage survey. The germplasm was assessed for variation in micronutrient content, but in conventional breeding it is difficult to incorporate iron and zinc content as a selectable trait. Therefore an attempt was made to find molecular markers associated to high levels of the micronutrients. Regrettably this was not achieved in the timeframe of the project. The cultivars with the highest concentrations were used by the food science and nutrition researchers to develop affordable processing techniques in order to improve the bioavailability of the micronutrients. GxE showed that mungbean cultivars somewhat adapted to various ecological zones which can be further used in designing future experiments and programmes. The interdisciplinary approach of this project allowed us a better appraisal of the needs of local farmers and challenges related to the local networks of mungbean. The inter-discipline together provides more holistic evaluation of the case to be studied. Thus inter-discipline cohort ensures in overall improvement of nutritional and social status of the people of selected area.

Our study objective was to improve the nutritional status of the resource poor-people (farmer) using a local crop (mungbean). Our interdisciplinary project had some strong points but also some weaknesses. One of the major strong points of this project was to have the same goal and objectives. This keeps the whole team focused but time limitations and limited interdisciplinary activities restricted the researchers. Time to time all the four researchers tried to share their research experiments and information but less inter-experimental activities didn't allow us to plan more interdisciplinary work together. Besides that an independent PhD project also demands a specific depth often reducing the available time for interdisciplinary activities. Further interactions with other disciplines were also limited because of personal workloads and time constraints. Even then efforts were made to maximize possible interdisciplinary interactions and exchange of knowledge. For micronutrient deficiency studies food scientists and nutritional researchers used the selected cultivars provided by the breeder. The main achievement of TELFUN is that it tried to identify the problem related to mineral micronutrient deficiencies in the selected research area of the study and also making a step to solve this problem by targeting different actors (producers, consumers and processors) in the mungbean chain.

Future recommendations and perspectives

Yield and micronutrient stable cultivars will work

According to the view point of farmers the major trait is yield for any crop. Therefore new traits such as higher micronutrient content should be combined with high yield to even have a chance that farmers choose the improved cultivars. It would be optimal that these cultivars behave optimal in different environments and climatic conditions (trait stability across different environments). For this, cultivars should be tested over many generations and in as many as possible environments.

Bioavailability of micronutrients

Enriching micronutrient content alone will not solve the problem of micronutrient deficiency completely. Another important aspect must be considered and research should also be focussed on bioavailability of these micronutrients inside the body. Application of fertilizers in the deficient soil is also an important aspect. Micronutrient rich varieties should be resistant to prevalent diseases especially

MYMV besides and be higher in yield. There are many other factors (enhancers, promoters, inhibitors etc.) involved in the bioavailability of these micronutrients. Therefore the nutritionist and processor can work along with the breeder to develop a scientific chain of research and also make aware the masses about the basic techniques to enhance the bioavailability of these micronutrients.

Interdisciplinary projects

TELFUN was designed to deliver interdisciplinary research yielding PhD theses in different fields. But such projects need to be further examined to achieve better productivity. There was only some level of interaction among the scientists and disciplines. There was an active participation with the local supervisor in conducting the field researches but there were only limited interactions between the different disciplines of the same research group. These interactions should have been more scheduled and more regular. Furthermore certain disciplines like breeding and food processing were supposed to give some deliverables to the team and this interdependence of researches limited the whole interdisciplinary approach. Moreover this puts extra pressure on certain disciplines. Therefore interdisciplinary projects must be designed in a manner that every discipline involved in the project should equally contribute. If this is not the case then there is no added value of the other disciplines and independent disciplinary researches might be more beneficial. This might for instance already be achieved by conducting the whole programme over a longer time period and let the different subprojects start at the most favourable moments relative to each other.

Concluding remarks

The micronutrient content in many accessions of the mungbean germplasm was determined for the first time. Besides this genetic diversity and GxE interaction study with relation to the mineral micronutrients uncovered options for improvement in mungbean. Along with these descriptive analyses an effort was made to study molecular trait association with regard to iron and zinc in mungbean. For the first time in mungbean any initiative in this area was taken up and thus this study may prove helpful in designing future research related to legumes especially mungbean and micronutrients. Also the variation with respect to micronutrients in mungbean cultivars can be further used in future experiments and crop improvement programme.

Farmers were well adapted to this crop and they prefer to grow mungbean as it is a short duration crop and very well fits in the rain fed as well as in rice-wheat based system. However, a poor yield due to MYMV disease warrants the development of resistant cultivars. Moreover it offers a good market price because people prefer to have mungbean in their diet in different forms. Mungbean can act as one of the target crops to alleviate the problem of malnutrition in the Indian **Haryana** region, since wheat, rice and millets are the staple food crops and food legumes are the major protein source for the vegetarian population. Mungbean is one of the food legumes which are part of the diet of the population and therefore enriching mungbean with mineral micronutrients will partly help in solving the problem. In the target area, mungbean is an important food legume crop as it increases soil fertility and it is a short duration crop. The short growing season makes it possible to grow mungbean as rain fed crop in *kharif* season and between wheat and rice growing seasons during the summer in irrigated areas.

References

Agarwal Milee, Shrivastava Neeta, Padh Harish (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports 27:617–631.

Blair MW, Giraldo MC, Buendía HF, Tovar E, Duque MC, Beebe SE (2009) Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics 113: 100-109.

Bouis Howarth E, Bruce M Chassy, James O. Ochanda (2003) Genetically Modified Foods for Human Health and Nutrition. Trends in Food Science & Technology 14 (5-8): 191-209.

Datta S, Gangwar S, Kumar Shiv, Gupta S, Rai R, Kaashyap M, Singh P, Chaturvedi SK, Singh BB, Nadarajan N (2012) *Genetic Diversity in Selected Indian Mungbean [Vigna radiata (L.) Wilczek] Cultivars Using RAPD Markers.* American Journal of Plant Sciences 3 (8): 1085-1091.

Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Advances in Agronomy 70: 77–142.

Gregorio GB (2002) Symposium "Plant Breeding: A New Tool for Fighting Micronutrient Malnutrition" given at the Experimental Biology 2001 meeting, Orlando, Florida, on April 1, 2002. International Food Policy Research Institute, Washington, DC.

Gwag JG, Chung WK, Chung HK, Lee JH, Ma KH, Dixit A, Park YJ, Cho EG, Kim TS, Lee SH (2006) Characterization of new microsatellite markers in mung bean, *Vigna radiata* (L.). Molecular Ecology Notes 6:1132-1134.

Jiang Li-Na, Hao Bao-Zhen, Zhanng Dai-Jing, Shao Yun, Li Chun-Xi (2010) Genotypic and environmental differences in grain contents of Zn, Fe, Mn and Cu and how they relate to wheat yield. Chinese Journal of Eco-Agriculture 18(5): 982−987.

Lavanya GR, Srivastava J, Ranade SA (2008) Molecular assessment of genetic diversity in mung bean germplasm. Journal of Genetics 87: 65–74.

Miyagi M, Humphry M, Ma ZY, Lambrides CJ, Bateson M, Liu CJ (2004) Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). Theoretical and Applied Genetics 110: 151–156.

Nene YL (1972) A survey of viral diseases of pulse crops in Uttar Pradesh, G.B. Pant University Agriculture and Technology, Pantnagar, Research Bulletin No. 4.

Oikeh S, Menkir A, Maziya-Dixon B (2004) Assessment of iron bioavailability from twenty elite late-maturing tropical maize varieties using an *in vitro* digestion Caco-2 cell model. Journal of the Science of Food and Agriculture 84: 1202-1206.

Parida A, Raina SN, Narayan RKJ (1990) Quantitative DNA variation between and within chromosome complements of *Vigna* species (Fabaceae). Genetica 82:125-133.

Scoones I, Thompson J (1994) Beyond Farmer First - Rural People's Knowledge, Agricultural Research and Extension Practice. Intermediate Technology Publications Ltd (ITP), London.

Sithichoke Tangphatsornruang, **Prakit Somta**, **Pichahpuk Uthaipaisanwong**, **Juntima Chanprasert**, **Duangjai Sangsrakru**, **Worapa Seehalak**, **Warunee Sommanas**, **Somvong Tragoonrung**, **Peerasak Srinives (2009)** Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). BMC Plant Biology 9:137-147.

Soheil Kobraee, Ghorban NoorMohamadi, Hosein HeidariSharifAbad, Farokh DarvishKajori, Babak Delkhosh (2013) Micronutrients Distribution in Soybean Plant with Zn, Fe, and Mn Application. Annual Review & Research in Biology 3(2): 83-91.

Somta Prakit and Srinives Peerasak (2007) Genome Research in Mungbean [V*igna radiata* (L.) Wilczek] and Blackgram [*V. mungo* (L.) Hepper]. ScienceAsia 33 (1): 69-74.

Sylvester O Oikeh, Abebe Menkir, Bussie Maziya-Dixon, Ross M Welch, Raymond P Glahn (2004) Assessment of iron bioavailability from twenty elite late maturing tropical maize varieties using an *in vitro* digestion/Caco-2 cell model. Journal of the Science of Food and Agriculture 84:1202–1206.

Thavarajah D, Thavarajah P, Wejesuriya A, Rutzke M, Glahn R, Combs Jr GF, Vandenberg (2011) The potential of lentil (*Lens culinaris* L.) as a whole food for increased selenium, iron, and zinc intake: Preliminary results from a three year study. Euphytica 180 (1): 123-128.

World Hunger Facts (2011) Why Are People Hungry? World Hunger Education. http://www.worldhunger.org/articles/Learn/world%20hunger%20facts%202002.ht

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SUMMARY

Malnutrition in India, particularly among women, children and adolescents is an emergency that needs immediate attention in this fast growing and developing country. Micronutrient deficiencies are threatening public health in India more and more. Deficiencies of micronutrients drastically affect growth, metabolism and reproductive phase in humans as it does in plants and animals. Cereal and pulse based Indian diets are qualitatively deficient in micronutrients such as iron, calcium, vitamin A and zinc. This is due to a low intake of income-elastic protective foods such as pulses, vegetables, fruits, and foods of animal origin. It is presumed that if we restore the geographical connection between food production and consumption in local food networks it will help in solving this nutritional problem in India. This offers new opportunities to tailor science & technology to location specific patterns of food production and consumption, which may lead to environmentally and socially sustainable agriculture. Despite global pressure (including from science and technology) to focus agricultural cultivation on a limited number of food crops, still many so-called orphan crops like mungbean do exist and are cultivated in location-specific crop rotation systems. Particularly, the seed legumes are of major nutritional importance, especially in developing countries, because they have high protein contents of good biological value. Out of the total sales of mungbean, about half of the sales are within the village which clearly establishes the need for development of infrastructure and facilities at the village level to serve the interests of the farm households. Moreover, it is also necessary to shift the focus of development from the urban market centres (largely developed) to the rural market centres. Linking breeding, nutrition, processing and standardisation of food products, may be designed within the experimental framework of empowering poor farmers. Hence, tailoring plant, food and social sciences to empower local mungbean production and consumption patterns has been designed as an interdisciplinary program of plant breeding, food technology, human nutrition and sociology of science and technology. Thus the 'Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition' (TELFUN) project aimed to help people in selecting their own way of local food production, processing and consumption of the best suited local food. The main objective is to strengthen "The Science in Society" approach by remodeling participatory research and development and the general aim of TELFUN was to attune disciplinary research objectives within an interdisciplinary framework to enhance food sovereignty and to improve mungbean based production and consumption patter in selected research area (Haryana, India). As an example our research focused on further improving one of the potential nutritional crops, namely mungbean. The present work emphasizes on mungbean in general and especially on the available micronutrient variation in the mungbean germplasm. The mungbean (green gram), *Vigna radiata* (L.) Wilczek is native to the Indian subcontinent. They are warm season annuals, highly branched and having trifoliate leaves like the other legumes. Seeds of mungbean are small, ovoid in shape, and green in color. Mungbean seeds are high in protein (21%–28%), calcium, phosphorus and certain vitamins. Moreover they are easily digested and they replace scarce animal protein in human diets in vegetarian populations of the world. The selected area of research centres contain a high level of local biodiversity and are the locations for domestication of mungbean. This legume has co-evolved with their natural ecosystems and is well-adapted to withstand the local biotic and abiotic stresses. This will help in enabling the reconnection of the cultivation of the mungbean with their natural environments. Moreover, as domestication has taken place by local farmers during many centuries, they have accumulated local endogenous knowledge, which is very relevant for local food networks (www.telfun.info). Thus to explore the potential mungbean network, the present thesis set its objectives. They were: i) identification of the major constraints, limitations and preferences of producer's with regard to mungbean, ii) assessing the diversity in the available germplasm and assessing the effects of different environments on selected cultivars for their mineral micronutrients and iii) to make a start to develop tools for marker assisted breeding with regard to iron and zinc.

Initially a literature study was carried out to know as much as possible about the selected crop (mungbean). Thus in **Chapter 2**, an effort was made to study the necessity of genetic improvement of mungbean emphasizing on increasing the levels of iron and zinc through a multi-disciplinary team approach including genetic improvement, bioavailability and social awareness. In the review paper information was gathered about the role of iron, zinc and protein content at different stages of life in humans. Their role in plant and animal development was mentioned. Besides this, the bioavailability aspect was also touched upon and thus it was found that combining breeding with good processing methods and making people aware about improved varieties available on the market further helps in improving their health status.

In **Chapter 3**, genetic diversity among selected cultivars of mungbean was estimated using ISSR and analysis shows moderate genetic diversity ranging from 65 to 87 percent among the selected cultivars. From the interdisciplinary point (**Chapter 4**) of view a joint venture involving a survey of the selected research area was carried out. From the breeding aspect major constraints and limitations in the mungbean production were investigated. Field surveys revealed that disease resistance and low yield of the mungbean cultivars were the major hindrance in its production. From the nutritionist survey it was revealed that school going children in the selected locale were anaemic in varying degrees. A combined effort was made to distribute the high yielding, disease resistance and nutritionally superior cultivars to local farmers.

In **Chapter 5** ninety two mungbean cultivars were selected and screened for their agronomic performance in the research fields along with the chemical estimation of iron and zinc content. On basis of a biodiversity analysis, the cultivars were grouped into 5 clusters. The highest numbers of cultivars (thirty four) grouped in cluster II, this group comprises cultivars which show early maturity and resistance to the virus disease Mungbean Yellow Mosaic Virus. Chemical analysis showed a fair range of micronutrient and protein variation (range for Fe = 1.6 to 9.3 mg/100g; for Zn = 1.5 to 3.9 mg/100g and Protein = 21.1 to 30 per cent). Fe and Zn content showed a positive correlation (r= 0.469). Heritabilities for individual traits were calculated and for iron and zinc the values came out to be $h^2 = 0.259$ and 0.727 respectively. The values show that further selection for higher contents is possible as both show fair heritability values and selection of one trait indirectly helps in selection for higher values of the other trait. On the basis of the screening thirty cultivars were selected and the performance and stability of iron and zinc concentrations in different environmental conditions were estimated. As mungbean shows in general high genotype x environment interactions, this was investigated for Fe and Zn and described in **Chapter 6**. For iron content, GxE interaction was linear for eleven cultivars and the Fe content of these cultivars could be more or less predicted across different environments; this was for Zn content hardly the case. A soil environment supplemented with all the nutrients i.e. single superphosphate, iron and zinc along with the recommended dosage of fertilizers was found to have a positive effect on the seed yield per plant. This shows that iron and zinc play a role in increasing the seed yield of mungbeans.

Chapter 7 focuses on finding DNA markers to be used in marker assisted breeding in mungbean a start was made. Contrasting parents were chosen and crosses were made (BG39 X 2KM138 and SMH99-1 X BDYR1), after five generations of single seed descent the RIL's were assessed chemically for iron and zinc content. The level of amplified fragment polymorphisms (AFLP) was determined but this level was too low to make it feasible to generate within the framework of the TELFUN program enough markers to make a high density linkage map. A chemical analysis of the RILs shows as expected a positive correlation between iron and zinc content (r = 0.474). The content of iron and zinc varied between individuals of the populations: Cross 1: 1.7 to 5.9 mg/100g (Fe), 2.0 to 3.8 mg/100g (Zn); Cross 2: 2.1 to 8.2 mg/100g (Fe), 1.0 to 8.4 mg/100g (Zn), and these values show transgressive segregation. For QTL analysis, marker trait association was done with the limited number of markers but no significant association between the markers and the phenotypic traits was found.

Finally, in **Chapter 8**, all the findings were integrated and an effort was made to see the applicability of our results and to study the possibility in alleviating the micronutrient deficiencies prevailing in the selected area. The breeding contribution to the interdisciplinary approach of the TELFUN programme was discussed together with future research possibilities.

SAMENVATTING

Ondervoeding is in India, vooral bij vrouwen, kinderen en adolescenten een groot probleem. Deze ondervoeding vraagt om onmiddellijk aandacht in dit snel groeiende en zich ontwikkelende land. Het tekort aan micronutriënten bedreigt de volksgezondheid in India meer en meer. Deze tekorten bedreigen bij zowel mensen als planten groei, metabolisme en het vermogen zich voort te planten. De op granen en peulvruchten gebaseerde Indiase diëten hebben vaak te lage concentraties aan micronutriënten zoals ijzer, calcium, vitamine A en zink. Dit komt doordat deze diëten te weinig onderdelen, zoals peulvruchten, groenten, fruit en vlees bevatten met hogere concentraties micronutriënten. Het idee is dat als in lokale voedselnetwerken de geografische banden tussen voedselproductie en voedselconsumptie hersteld worden bovengenoemd voedselprobleem opgelost zou kunnen worden. Deze aanname creëert ook nieuwe mogelijkheden om wetenschap en technologie af te stemmen op lokale specifieke patronen van voedselproductie en consumptie, wat kan leiden tot een milieuvriendelijke en sociale, duurzame landbouw. Ondanks een wereldwijde druk (mede door wetenschap en technologie) om te focussen op een landbouw met maar een paar grote gewassen, zijn er ook nog steeds kleine gewassen zoals mungbeans die verbouwd worden in locatie-specifieke rotatie systemen. Vooral peulvruchten hebben een hoge voedingswaarde (o.a. eiwit) en zijn daarom belangrijk in ontwikkelingslanden. De helft van de geproduceerde mungbeans wordt lokaal, binnen het dorp verkocht. Dit geeft duidelijk aan dat er, in het belang van de boerengezinnen, een behoefte is aan infrastructuur in de dorpen. Daarom is het nodig de focus te verschuiven van stedelijke markten (met een hoog organisatieniveau) naar meer landelijke, lokale markten. De koppeling van plantenveredeling, voeding, voedselbereiding en het standaardiseren van voedselproducten kan een rol spelen binnen een experimentele raamwerk dat bedoeld is om arme boeren meer mogelijkheden te bieden. Daarom is een interdisciplinair project geformuleerd waarin plantenveredeling, voedseltechnologie, humane voeding en sociale wetenschappen samen werken om meer kansen te bieden aan lokale mungbean productie en consumptie. De 'Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition' (TELFUN) project was bedoeld om mensen te helpen de keus te maken hoe ze lokaal het meest geschikte eten kunnen produceren, bereiden en consumeren. Het belangrijkste doel is de aanpak "Wetenschap in de Samenleving" te versterken door een andere benadering van participatief onderzoek en ontwikkeling. Het algemene doel van TELFUN was om de disciplinaire onderzoeksdoelen in overeenstemming te brengen met het interdisciplinaire netwerk om op deze manier zowel voedselsoevereiniteit als mungbean productie en consumptie te bevorderen in een geselecteerd gebied (Haryana, India). Ons onderzoek was gericht op het veredelen van mungbean in het algemeen maar speciaal gericht op hogere concentraties van micronutriënten.

De mungbean (ook wel *green gram*), Vigna radiata (L.) Wilczek heeft als centrum van herkomst het Indiase subcontinent. Het zijn eenjarige planten die goed kunnen groeien tijdens het warme seizoen. Mungbean is sterk vertakt en heeft kleine zaden die eivormig en groen zijn. De zaden hebben een hoog gehalte aan eiwitten (21%–28%), calcium, fosfor en bepaalde vitaminen. Ze worden gemakkelijk verteerd en kunnen een vervanging zijn voor dierlijke eiwitten in het dieet van vegetariërs. Het door ons geselecteerde onderzoeksgebied heeft veel mungbean diversiteit en ook veel van de domesticatie van mungbean heeft hier plaats gevonden. Mungbean is geëvolueerd met de plaatselijke ecosystemen en is goed aangepast om de lokale biotische en abiotische stressfactoren te kunnen weerstaan. Dit maakt het mogelijk om mungbean te verbouwen in zijn natuurlijke omgeving. Bovendien hebben lokale boeren gedurende het eeuwenlange proces van domesticatie veel kennis over mungbean gekregen wat erg belangrijk is voor het opzetten van lokale voedsel netwerken (www.telfun.info). Om het potentiele mungbean netwerk verder te onderzoeken, heeft dit proefschrift de volgende doelen gesteld. Deze waren: i) identificeren van de belangrijkste beperkingen en voorkeuren van de producenten met betrekking tot mungbean, ii) bepalen van de diversiteit in de aanwezige genenpool en het bestuderen van het effect van verschillende omgevingen op geselecteerde rassen met betrekking tot de concentratie van micronutriënten en iii) het in gang zetten van het ontwikkelingen van methodologiën voor marker gestuurde selectie op ijzer en zink gehalte.

Om te beginnen is een literatuuronderzoek uitgevoerd om de aanwezige kennis over mungbean in kaart te brengen. In **Hoofdstuk 2**, is de noodzaak bestudeerd om mungbean te veredelen op een hoger ijzer- en zinkgehalte. Een multidisciplinaire aanpak, waar naast een genetisch verbeterde mungbean ook gekeken werd naar biologische beschikbaarheid en de sociale aspecten, moet laten zien of dit kan leiden tot hogere ijzer en zinkconcentraties in mensen. In het overzichtsartikel wordt ook de rol beschreven van ijzer, zink en eiwitten in de verschillende levensfases van mensen.

Hun rol tijdens de ontwikkeling van dieren en planten wordt behandeld. Hoge concentraties in mungbean in combinatie met verbeterde verwerkingsmethodes en een goede biologische beschikbaarheid kunnen, als er een besef is bij de consument dat er betere variëteiten op de markt aanwezig zijn, helpen in het verbeteren van de gezondheid.

In **Hoofdstuk 3**, wordt de genetische diversiteit geschat in een selectie van variëteitens. Dit werd bepaald op basis van de gevonden verschillen in de amplificatiepatronen van ISSR markers, de analyse laat een genetische diversiteit zien tussen de 65 en 87%. Vanuit het interdisciplinaire gezichtspunt (**Hoofdstuk 4**) is gemeenschappelijk een enquête uitgevoerd in de gekozen regio. Voor het veredelingsaspect werden de voornaamste verplichtingen en beperkingen onderzocht. Dit veldonderzoek liet zien dat ziekteresistentie en lage opbrengst van mungbeans de grootste problemen veroorzaken in de productie. De voeding studie liet zien dat onder schoolgaande kinderen in het gebied verschillende mate van bloedarmoede voorkwam. Gezamenlijk werden een aantal mungbean variëteiten met hoge opbrengsten, resistentie tegen ziekte en qua voedingswaarde superieur gedistribueerd onder boeren.

In **Hoofdstuk 5** zijn 92 mungbean variëteiten geselecteerd en gescreend in de onderzoeksvelden op hun landbouwkundige eigenschappen en tevens is hun ijzer en zink gehalte gemeten. De biodiversiteitsanalyse verdeelde de variëteiten in vijf groepen. De meeste variëteiten waren aanwezig in groep II, deze groep bestaat uit vroege variëteiten met een resistentie tegen *Mungbean Yellow Mosaic Virus*. De chemische analyse toonde een redelijke hoeveelheid variatie aan voor micronutriënt- en eiwitconcentratie (Fe 1.6 – 9.3 mg/100 g; Zn 1.5 – 3.9 mg/100g en eiwit 21.1 tot 30 procent). Fe en Zn gehalte zijn positief gecorreleerd (r=0.469). De erfelijkheidsgraden (h^2) voor de individuele eigenschappen waren respectievelijk 0.259 en 0.727. De waarden laten zien dat selectie op de hoogst mogelijke waarden mogelijk is en dat door te selecteren op een eigenschap indirect ook een selectie is op de andere eigenschap. Gebaseerd op de screening werden 30 variëteiten geselecteerd voor verdere studies. In **Hoofdstuk 6** werden ijzer en zink concentraties en de stabiliteit ervan bepaald onder verschillende condities. Met betrekking tot het ijzergehalte was het bij elf variëteiten mogelijk het ijzergehalte in verschillende omgevingen te voorspellen, voor het zinkgehalte was dit voor bijna geen variëteit het geval. Een bodem met aanbevolen bemesting maar ook gesupplementeerd met bestanddelen zoals superfosfaat, ijzer en zink had een positief effect op de zaadopbrengst. Dit toont aan dat ijzer en zink een rol spelen in het verhogen van de zaadopbrengst in mungbeans. In **Hoofdstuk 7** wordt beschreven hoe de eerste stappen gezet zijn om DNA merkers te vinden die gebruikt kunnen worden in merker gestuurde veredeling. Ouders met contrasterende eigenschappen werden gekozen en onderling gekruist (BG39 X 2KM138 en SMH99-1 X BDYR1), na vijf generaties *single seed descent* zijn van de Recombinant Inteelt Lijnen (RIL) ijzer- en zinkgehalte bepaald. Er is ook bepaald hoe gemakkelijk het is voldoende moleculaire merkers (*amplified fragment length polymorphisms,* AFLPs) te vinden. Helaas bleek het binnen het raamwerk van TELFUN onmogelijk voldoende merkerdata te genereren. Een chemische analyse van de RILs liet de verwachte positieve correlatie tussen ijzer- en zinkconcentratie zien (r=0.474). Het gehalte ijzer en zink varieerde tussen de individuele lijnen van de populaties. Kruising 1: 1.7 tot 5.9 mg/100g (Fe), 2.0 tot 3.8 mg/100g (Zn); Kruising 2: 2.1 tot 8.2 mg/100g (Fe), 1.0 tot 8.4 mg/100g (Zn), transgressieve segregatie werd gevonden. Een QTL analyse, uitgevoerd om associaties tussen eigenschappen en merkers te vinden, leverde helaas geen resultaat op.

Tenslotte zijn in **Hoofdstuk 8** alle resultaten geïntegreerd en is de toepasbaarheid van de resultaten besproken. Wat zijn de mogelijkheden de bestaande micronutriëntentekorten in ons onderzoeksgebied te verminderen. De bijdrage van de veredeling aan de multidisciplinaire aanpak van het TELFUN programma wordt bediscussieerd samen met mogelijk toekomstig onderzoek.

सारांश

भारत में कुपोषण विशेष रूप से महिलाओं, बच्चों, किशोरों के बीच आपातकालीन स्थिति है। जिसे विकासशील देशों में तत्कालीन ध्यान देने की जरूरत है। भारत में सूक्ष्म पोषक तत्वों की कमी अधिक से अधिक सार्वजनिक स्वास्थ्य को धमकी दे रही है। सूक्ष्म तत्वों की कमी मनुष्य की वृद्धि, चयापचय और प्रजनन को प्रभावित करते हैं वैसा ही प्रभाव पशुओं और पेड़ों पर होता है। अनाज और नाडी आधारित भारतीय आहार में लोहा, कैल्शियम, विटामिन ए और जिंक जैसे सूक्ष्म पोषक तत्वों की गुणात्मक कमी है। यह दालें, सब्जियां, फल और जानवर मूल के खाद्य पदार्थ के रूप में आय लोचदार सूरक्षात्मक खाद्य पदार्थों की कम मात्रा खाने के कारण है। यह माना गया है कि यदि हम स्थानीय खाद्य नैटवर्क में खाद्य उत्पादन और खपत के बीच भौगोलिक सम्बन्ध बहाल कर दें तो इससे भारत में पोषण की समस्या को हल करने में मदद मिलेगी। यह खाद्य उत्पादन और खपत के स्थान विशिष्ट के लिए खाद्य विज्ञान और प्राद्योगिकी के लिए नए अवसर प्रदान करता है। जोकि पर्यावरण व सामाजिक रूप से स्थायी कृषि को जन्म दे सकती है। खाद्य फसलों की सीमित संख्या पर कृषि खेती ध्यान केन्द्रित करने के वैश्विक दबाव (विज्ञान और प्राद्योगिक सहित) के बावजूद मूंग की तरह अभी भी कई तथाकथित अनाथ फसलें मौजूद हैं और स्थान विशेष फसल चक्र व्यवस्था में खेती की जाती है। विशेष रूप से बीज, फलियां विकासशील देशों में प्रमुख पोषक महत्व के हैं क्योंकि वे अच्छा जैविक मूल्य के उच्च प्रोटीन युक्त हैं। मूंग की कुल बिक्री में से आधी बिक्री गांव के भीतर ही होती है जोकि स्पष्ट रूप से खेत परिवारों के हितों की सेवा करने के लिए गांव स्तर पर बुनियादी ढांचे और सुविधाओं के विकास की जरूरत को स्थापित करता है। इसके अलावा शहरी बाजार केन्द्रों (मौटे तौर पर विकसित) से ग्रामीण बाजार केन्द्रों के विकास पर ध्यान केन्द्रित करने की आवश्यकता है। खाद्य उत्पादों की प्रजनन, पोषण, प्रसंस्करण और मानकीकरण जोड़ने, गरीब किसानों को सशक्त बनाने की प्रयोगात्मक ढांचे के भीतर तैयार किया जा सकता है। अतः स्थानीय मूंग उत्पादन व खपत के तरीकों के सशक्तिकरण के लिए पौधा प्रजनन, खाद्य प्राद्योगिकी, मानव पोषण और विज्ञान और प्राद्योगिकी के समाजशास्त्र के एक अन्तःविषय कार्यक्रम के रूप में डिजाईन किया गया है। खाद्य विज्ञान मार्गदर्शन–भविष्य पोषण के लिए स्थानीय खाद्यापूर्ति के स्थानीय तरीके परियोजना का लक्ष्य लोगों के लिए स्वयं खाद्य उत्पादन, प्रसंस्करण और उपभोग के लिए सर्वोत्तम स्थानीय खाद्य फसलों का चयन करना है।

इसका मुख्य उद्देश्य अन्तःविषय ढांचे के भीतर अनुशासनात्मक अनुसंधान उद्देश्यों के अनूकूल भागीदारी, अनुसंधान और विकास और telfun के सामान्य उद्देश्य remodeling द्वारा समाज में विज्ञान दृष्टिकोण को मजबूत करने के लिए, खाद्य संप्रभुता को बढ़ाने के लिए चयनित अनुसंधान क्षेत्र (हरियाणा, भारत) मूंग आधारित उत्पादन और खपत तरीकों में सुधार करना।

एक उदाहरण के रूप में हमारे अनुसंधान मूंग फसल में संभावित पोषण सुधार लाने पर जोर दिया गया । वर्तमान कार्य में सामान्य रूप से मूंग पर और विशेष रूप से मूंग जर्मप्लाजम में उपलब्ध सूक्ष्म पोषक तत्वों की भिन्नता पर जोर देती है। मूंग बीन (हरा चना), विग्ना रेडियाटा (एल) विलजैक भारतीय उपमहाद्वीप के मूल निवासी हैं। यह अत्यधिक टहनीदार और अन्य फलियां तरह तिपत्तियां पतियों वाली गर्म मौसम की वार्षिक फसल है। मूंग के बीज छोटे आकार में अण्डाकार, हरे रंग के हैं। मूंग बीज में उच्च प्रोटीन की मात्रा है **(21–28 प्रतिशत),** कैल्शियम, फास्फोरस और कुछ विटामिन आदि भी हैं। इसके अलावा मूंग आसानी से पचता है और दुनिया की शाकाहारी आबादी में मानव आहार में दुर्लभ पशु प्रोटीन की जगह ले रहा है। अनुसंधान केन्द्रों में से चयनित क्षेत्र के स्थानीय जैव विविधता का एक उच्च स्तर होता है। और मूंग को घरेलूकरण के स्थान रहे यह दलहनी फसल प्राकृतिक पारिस्थितिक अन्तर के साथ मिलकर विकसित की गई है और स्थानीय जैविक और अजैविक तनाव को झेलने के लिए अच्छी तरह से अनुकूल है। इससे प्राकृतिक वातावरण के साथ मूंग की खेती के पुर्नसम्बन्ध को सक्षम करने में मदद मिलेगी। इसके अलावा सदियों से मूंग का घरेलूकरण होने से स्थानीय किसानों ने मूंग का स्थानीय ज्ञान संचित कर लिया है। जोकि स्थानीय खाद्य नैटवर्क (www.telfun.info) के लिए बहुत प्रासंगिक है। इस प्रकार संभावित मूंग नैटवर्क का पता लगाने के लिए वर्तमान थिसीस अपने निम्नलिखित उद्देश्यों के लिए निर्धारित किया गया है वे हैं –

1. मूंग फसल को लेकर उत्पादक प्रमुख बाधाओं, सीमाओं और प्राथमिकताओं की पहचान करना।
2. उपलब्ध जर्मप्लाज्म में विविधता का आकलन करने और उनके खनिज पोषक और चयनित किस्मों पर अलग–अलग वातावरण के प्रभाव का आकलन

3. मूंग में जिंक व लौह सम्बन्धित मार्कर सहायता प्राप्त प्रजनन उपकरण विकसित करने के लिए

प्रारम्भ में एक साहित्य अध्ययन चयनित फसल मूंग के बारे में जितना सम्भव हो सका पता किया गया। इस प्रकार **अध्याय 2** में एक प्रयास किया गया जिसमें अनुवांशिक सुधार, जैव उपलब्धता और सामाजिक जागरूकता सहित बहु अनुशास्नात्मक टीम के दृष्टिकोण के माध्यम से लौह और जिंक के स्तर को बढ़ाने पर बल के अनुवांशिक सुधार की आवश्यकता का अध्ययन किया गया। समीक्षा पेपर जानकारी में मानव के जीवन के विभिन्न चरणों में लौह, जिंक और प्रोटीन की भूमिका के बारे में जानकारी इकट्ठा की गई। पौधों और जानवरों के विकास में उनकी भूमिका का उल्लेख किया गया। इसके अलावा शरीर में उपलब्धता (बायोअवेलेबिलिटी) के पहलू को भी छुआ और यह पाया कि प्रसंस्करण विधियों के साथ प्रजनन के संयोजन और बाजार में उपलब्ध उन्नत किस्मों के बारे में लोगों को जागरूक बनाने के आगे उनके स्वास्थ्य की स्थिति में सुधार लाने में मदद करता है।

अध्याय 3 में मूंग की चयनित किस्मों के बीज आनुवांशिक विविधता ISSR (आइएसएसआर) और विश्लेषण प्रयोग से अनुमानित की गई जोकि चयनित किस्मों के बीच **65–87 प्रतिशत** तक मध्यम आनुवांशिक विविधता का पता चलता है। अन्तःविषय बिन्दू **(अध्याय 4)** से चयनित अनुसंधान क्षेत्र के एक सर्वेक्षण का संयुक्त उपक्रम किया गया। प्रजनन के पहलू से मूंग उत्पादन में प्रमुख बाधाओं और सीमाओं की जांच की गई। क्षेत्र सर्वेक्षण से पता चला कि रोग प्रतिरोध और कम उपज मूंग के उत्पादन में प्रमुख बाधा थे। पोषण सर्वेक्षण से यह पता चला कि एक चयनित स्थानीय क्षेत्र के स्कूली बच्चों में खून की भिन्न भिन्न कमी पाई गई। स्थानीय किसानों को अधिक उपज, रोग प्रतिरोधी, बेहतर पोषण देने वाली मूंग की कल्टीवार बांटने का एक संयुक्त प्रयास किया गया।

अध्याय 5 में मूंग की **92 कल्टीवार** का खेती के लिए चयन किया गया और लौह तथा जिंक सामग्री की रासायनिक आकलन के साथ अनुसंधान के क्षेत्र में उनके कृषि प्रदर्शन के लिए जांच की। एक जैव विविधता के विश्लेषण के आधार पर, खेती को पांच समूहों में बांटा गया। खेती में सबसे अधिक संख्या (चौंतीस) कलस्टर द्वितीय में वर्गीकृत किया है, इस समूह में जल्दी परिपक्वता और पीला मोजेक विषाणु (YMV) के लिए

प्रतिरोध दिखा। रासायनिक विश्लेषण में सूक्ष्म पोषक तत्व और प्रोटीन भिन्नता की एक उचित सीमा का पता चला **(लौह 1.6–9.3 मिलीग्राम/100 ग्राम के लिए सीमा, जिंक के लिए 1.5–3.9 मिलीग्राम/100 ग्राम और प्रोटीन 21.1–30 प्रतिशत)।** लौह और जिंक सामग्री ने एक सकारात्मक सहसम्बन्ध **(नि 0.469)** दर्शाया। व्यक्तिगत गुण के लिए हैरिटेबिलिटी की गणना की और लौह तथा जिंक के लिए **h^2 मान = 0.259 और 0.727** क्रमशः पाया गया। हैरिटेबिलिटी के मान में दर्शाया कि निष्पक्ष अनुवांशिकता मूल्यों और एक विशेषता का चयन दोनों परोक्ष रूप से अन्य विशेषता के उच्च मूल्यों के चयन में मदद करता है। और उच्च सामग्री के लिए आगे चयन सम्भव है। स्क्रीनिंग के आधार पर 30 किस्मों का चयन किया गया और विभिन्न प्र्यावरणीय स्थितियों में लौह व जिंक की सांद्रता का प्रदर्शन और स्थिरता का अनुमान लगाया गया।

सामान्य उच्च जिनोटाईप एवं पर्यावरण इन्टरेक्सन में पता चला, लौह और जिंक के लिए जांच की गई जोकि **अध्याय 6** में वर्णित है। लौह सामग्री के लिए जिनोटाईप एवं प्र्यावरण इन्टरेक्सान 11 कल्टीवार के लिए रेखीय था और इन किस्मों की लौह सामग्री कम या ज्यादा अलग वातावरण में भविष्यवाणी हो सकता है। यह शायद ही जिंक सामग्री के लिए कहा जा सकता था। एक मिट्टी प्र्यावरण एकल अधिभास्वीय यानि सभी पोषक तत्वों के साथ पूरक, उर्वरकों की सिफारिश की खुराक के साथ लौह और जिंक संयत्र प्रति उपज पर एक समारात्मक प्रभाव पाया गया था। यह लौह और जिंक मूंग के बीज की उपज बढ़ाने में भूमिका निभाते हैं।

अध्याय 7 मूंग में मार्कर सहायता प्रजनन इस्तेमाल करने के लिए डीएनए मार्कर खोजने पर केन्द्रित है। विषम माता पिता को चुना गया और एक बीज वंश की पांच पीढ़ियों आरआईएल **(RILs)** के लौह और जिंक सामग्री के लिए रासायनिक मूल्यांकन किया गया। व **BG39 x 2KM138 और SMH99-1 x BDYR1** का क्रॉस बनाया गया। प्रवर्धित टुकड़ा बहुरूपताओं **(AFLP)** का स्तर निर्धारित किया गया लेकिन इस स्तर पर एक उच्च घनत्व लिंकेज नक्शा बनाने के लिए TELFUN कार्यक्रम काफी मार्करों के ढांचे के भीतर उत्पन्न करने के लिए यह संभव बनाने के लिए बहुत कम था। RILs का रासायनिक विश्यलेषण के रूप में लौह और जिंक सामग्री **(नि. = .474)** के बीच एक सकारात्मक सम्बन्ध पाया गया। आबादी के व्यक्तियों के बीच विभिन्न लौह और जिंक की सामग्री क्रॉस **1 लौह – 1.7–5.9 मिलीग्राम/100 ग्राम , 2.0–3.9**

मिलीग्राम / 100 ग्राम (जिंक), क्रॉस 2 लौह – 2.1–8.2 मिलीग्राम / 100 ग्राम, 8.4 मिलीग्राम / 100 ग्राम जिंक में उत्क्रामी अलगाव दिखा। **QTL** के विश्लेषण के लिए, मार्कर विशेषता संघ मार्करों की सीमित संख्या के साथ किया गया लेकिन मार्करों और प्रारूपी लक्षण के बीच कोई महत्वपूर्ण सम्बन्ध नहीं पाया गया।

अन्त में **अध्याय 8** में सभी निष्कर्षों को एकीकृत किया गया और हमारे परिणामों की परियोजिता को देखने के लिए और चयनित क्षेत्र में प्रचलित सूक्ष्म पोषक तत्वों की कमी को समाप्त करने की संभावना का अध्ययन करने के लिए एक प्रयास किया गया था। TELFUN कार्यक्रम के अन्तःविषय दृष्टिकोण में प्रजनन योगदान व भविष्य में अनुसंधान संभावनाओं की चर्चा की गई।

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ABOUT THE AUTHOR

Curriculum Vitae

Renu Singh was born in Hisar, Haryana, India on 12th December 1982. After completing secondary school at Campus School in Hisar, she started the bachelors programme from Kurukshetra University, India. During her graduation she also actively participated in the NCC services and honoured by the college colour and she was selected as the best cadet. After successfully completing her graduation she enrolled in the Master programme in the Department of Biotechnology and Molecular Biology at the CCS HAU, Hisar. She completed a Master's thesis in regeneration studies in female papaya plant (*Carica papaya* L.). During her masters research she standardized the protocol for sterilization and regeneration for papaya. She evaluated different growth regulators on the vegetative regeneration of female papaya plant using different explants. During her graduation and masters degrees she was awarded with the merit certificate and scholarship respectively. In March 2006, Renu obtained her master's degree afterwards she got a scholarship from Department of Biotechnology, India for industrial training. She joined Pepsi Co. Channo, Punjab as a trainee. Here, she learnt various techniques which were helpful to her in advancement of career. After successfully completing her training in April 2007 she started with a PhD project at Wageningen UR Plant Breeding, Wageningen University. The PhD project was funded partly by INREF and DBT, India. During her PhD she critically reviewed the micronutrient research status in pulse crops. She evaluated mungbean germplasm for its major agronomic traits like disease, yield and maturity. She estimated genetic diversity study in mungbean using AFLP and ISSR. Besides she carried out AFLP and chemical analysis of RIL's in order to identify markers for iron and zinc. During the course of her PhD she successfully cleared the national eligibility test and represented her work at national and international level. Since July 2013, she is employed as Assistant Professor (Biotechnology) at the JCDM College, JCDV, Sirsa, India.

List of publications

Renu Singh, Adriaan W. van Heusden, Ram Kumar, Richard G.F. Visser and Ram C Yadav. 2013. Genetic diversity of mungbean (*Vigna radiata* L.) in iron and zinc content as impacted by farmers varietal selection in northern India. *Ecology of Food & Nutrition*. 52: 148-162.

Renu Singh, Ram C. Yadav and Neelam R. Yadav. 2011. Effectiveness of different Sterilization Treatments in Combating Contamination Problem in Papaya (*Carica papaya* L.). *Indian Horticulture Journal*. 1(1):29-31.

Kanta Rani, **Renu Singh** and Pushpa Kharab. 2011. Effect of plant growth regulators on Periwinkle (*Catharanthus roseus* L.) G. Don using leaves, hypocotyls and epicotyl as explants. *Environment & Ecology*. 29 (3A):1291-1295.

Renu Singh, Pushpa Kharab and Kanta Rani. 2011. Rapid Micropropagation and Callus Induction of *Catharanthus roseus in Vitro* Using Different Explants. *World Journal of Agricultural Sciences*. 7 (6):699-704

Renu Singh, Ram C. Yadav and Neelam R. Yadav. 2010. Effect of growth regulators on *in vitro* plant regeneration of female papaya using shoot tip as an explant. *Indian Journal Horticulture* 67: 51-54.

Ram Kumar and **Renu Singh.** 2008. "Improvement in mungbean: present scenario and future challenges". *National. Journal of Plant Improvement.* 10 (2): 69-74.

Renu Singh, Ram C. Yadav and Neelam R. Yadav. 2009. Effect of growth regulators on *in vitro* plant regeneration of female papaya using axillary bud as an explant. *Journal of Applied Horticulture.* 11 (1): 50-53.

Renu Singh, Ram C. Yadav and Neelam R. Yadav. 2008. In vitro regeneration from mature female papaya plant cv. selection 1. *National Journal of Plant Improvement.* 10 (1): 1-4.

Renu Singh, Adriaan W. van Heusden & Ram C Yadav. A comparative genetic diversity analysis in mungbean (*Vigna radiata* L.) using ISSR & AFLP. ". Accepted in *African Journal of Biotechnology*

Renu Singh, Ram C. Yadav and Neelam R. Yadav. Effectiveness of different sterilization treatments in combating contamination problem in papaya (*Carica papaya* L.). Accepted in *Research Journal of Agricultural Sciences*

Abstracts

Renu Singh, Ram Kumar, Adriaan W. van Heusden, Richard G.F. Visser and Ram C Yadav. 2011. Breeding of mungbean for micronutrients: a solution to combat mineral malnutrition. 5th TELFUN workshop, Benin, 24th – 28th October' 2011 Cotonou, Benin

Renu Singh, Sjaak van Heusden, Ram Kumar and R.C.Yadav. 2010. Effect of micronutrients application on the uptake of iron and zinc in mungbean (*Vigna radiata* L.), paper presented at "Legumes for global health, legume crops and products for food, feed and environmental benefits" IFLRC V & AEP VII - April 26-30, 2010 - Antalya, TURKEY (Poster presentation)

Renu Singh, Sjaak van Heusden, R.C.Yadav and Ram Kumar. 2010. Molecular characterization of elite genotype of mungbean (*Vigna radiata* L.) using AFLP. Abstract in Indo-US bilateral workshop on plant genomics improvement with reference to biotic and abiotic stress, 25 -27, February, 2010. (oral presentation)

Renu Singh, Ram Kumar, Adriaan W. van Heusden, Richard G.F. Visser and Ram C Yadav. 2010. Development of high iron and zinc enriched mungbean variety with other traits in consideration. Fourth international workshop- TELFUN. 2-6, February, 2010. Accra, Ghana (oral presentation)

Renu Singh, Ram Kumar, R.C Yadav and Sjaak van Heusden. 2009 Genetic diversity for micronutrients (Fe & Zn) and protein content in mungbean (Vigna radiata L. Wilczek) cultivars. Accepted at International Conference on "Current trends in biotechnology & Implication in agriculture" 19-21, February, 2009. Modipur, Meerut - (Oral Presentation)

Renu Singh, Ram Kumar, R.C.Yadav and Sjaak van Heusden. 2009. Assessment of genetic variation for iron and zinc among mungbean (Vigna radiata L. Wilczek) germplasm lines. Accepted at International Conference on " Grain legumes: Quality Improvement , Value Addiction and Trade" 14-16, February, 2009. IIPR, Kanpur Feb. 14-16, (Poster presentation).

Renu Singh, R.C.Yadav, Ram Kumar and Sjaak van Heusden 2008 Assessment of genetic relatedness among mungbean (*Vigna radiata* L.) Cultivars using RAPD and Conventional Plant breeding methods. Abstract in NLS March 15-17. CPB, Hissar, India (Oral Presentation)

Renu Singh, Ram Kumar and Sjaak van Heusden. 2007. Evaluation of farmer's knowledge and the differences in the level of Zn and Fe content in mungbean (*Vigna radiata* L.). Abstract in NLS Nov.3-5. IIPR, Kanpur India (Poster Presentation).

Education Certificate

Education Statement of the Graduate School: Production Ecology and Resource Conservation
Issued to: Renu Singh
Date: 30 May 2013
Group: Plant Breeding, Wageningen University

Courses	Year
1. Start-up phase	
• **Writing of project proposal**	
○ Development of iron and zinc enriched mungbean (*Vigna radiata L.)* cultivar with agronomic traits in consideration	2007
• **Review of literature**	
○ Genetic improvement of mungbean (*Vigna radiata L*): necessity to increase the levels of the micronutrients iron and zinc: a review	2007
Subtotal Start-up Phase	*10.5 credits**
2. Scientific Exposure	
• **International symposia, workshops and conferences**	
○ National conference; oral presentation; Hisar, India	2008
○ International conference; Kanpur, India	2009
○ International conference; oral presentation; Meerut, India	2009
○ International conference; Hisar, India	2009
○ International conference; Antalya, Turkey	2010
▪ **Discussion groups / local seminars / other scientific meetings**	
○ Excursion to the Zeeuwse Vlegel in Zeeland: an example of sovereign wheat production; integrated and environmental friendly production of wheat processes locally to supply local bakeries that produce bread and pastries locally appreciated: history, activities and challenges; TEL, Zeeland, the Netherlands	2008
○ Lecture by Dr. Niel Koning and Wilhelmina Quaye (MSc): food sovereignty: origins, meaning and relation with other dicourses	2008
○ Lecture by Prof. Dr. Han Wiskerke: technologies for suitable food networks do locality matter?	2008
• **TELFUN Research Group: yearly meeting of one week**	2007-2010
• **Laboratory training and working visits**	
○ Selection of the mungbean cultivar against MYMV having high nutritional status (Zn and Fe); CCS HAU	2007
○ Molecular marker techniques on mungbean cultivar; CCS HAU	2008
Subtotal Scientific Exposure	*14.6 credits**
3. In-Depth Studies	
○ Multivariate analysis; PE&RC, WUR	2008
○ Statistics for life science; WUR	2007
• **Deficiency, refresh, brush-up courses**	
○ Basic of plant breeding; Department of Plant Breeding, COA, CCS HAU	2010

Subtotal In-Depth Studies	*6.0 credits **
4. Personal Development • **Skill training courses** ○ Introduction to EndNote; WUR ○ EndNote advance; WUR ○ Technique for writing and presenting scientific paper; WUR ○ Presentation skills; WUR • **Organisation of PhD students day/week** ○ PE&RC PhD Weekend, ○ PE&RC PhD Day	 2007 2007 2007 2008 2008 2011
Subtotal Personal Development	*5.2 credits**
TOTAL NUMBER OF CREDIT POINTS*	**36.3**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of PE&RC which comprises of a minimum total of 32 credits. **A credit represents a normative study load of 28 hours of study.*

Cover design and layout: by Renu Singh

Front and back cover picture represents disease resistant, early maturing, dwarf mungbean cultivar.