Chapter 12
Utilization of Germplasm for the Genetic Improvement of Mung bean [*Vigna radiata* (L.) Wilczek]: The Constraints and the Opportunities

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Abstract Pulses are rich in proteins and serve as a main source of this essential component of nutrition particularly for the predominantly vegetarian population of India and adjacent countries. Mung bean [*Vigna radiata* (L.) Wilczek] also known as green gram is an important pulse crop due to its widespread consumption throughout the Indian subcontinent. It is increasingly becoming popular in other parts of the world in recent years due to its value added products that are rich in several nutrients. However, unlike cereal crops, mung bean yields have not been able to meet the demands of the consumers leading to its import from other countries thereby resulting in steep rise in prices. Low productivity in mung bean is pushing it to the marginal lands and further decreasing its competitiveness in comparison to other crops. Despite developing several cultivars suitable for specific agro-climatic zones, mung bean crop is affected by a wide range of biotic and abiotic stresses. Further, some quality traits of mung bean also need to be improved for enhancing its nutritional value. Large germplasm collections are available in national and international gene banks; however, their vast potential is yet to be exploited. Effective utilization of these genetic resources requires their trait-based evaluations for identification of the elite genotypes and core sets. Conventional breeding approaches will get strong impetus by the identification of primary, secondary and tertiary genepools in order to select the donor and design the judicious approaches for the transfer of useful genes. Detailed molecular characterization of genetic diversity of the available germplasm and assessment of phylogenetic relationships among the related taxa done so far can provide useful leads in this regard.

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Recent developments in the large-scale genomic tools have resulted in the availability of whole genome sequence of mung bean which is a significant boost for the exploitation of biotechnological advancements for its improvement. However, lack of efficient protocols for transformation and regeneration of mung bean pose the important challenges that need to be addressed at the earliest. Appropriate combination of conventional and molecular approaches aimed at exploitation of the available germplasm is the need of the hour for successful development of high yielding cultivars in this crop.

Keywords Pulses · *Vigna radiata* · Green gram · Mung bean · Wild relatives · Germplasm · Genomic resources

12.1 Introduction

Pulses or food legumes including beans, peas and lentils are the most versatile and nutritious food available. They are the major source of protein for the predominantly vegetarian population of Indian subcontinent and other adjacent countries. Pulses provide significant nutritional and health benefits as they are low in fats contain no cholesterol and are high in proteins, folate, potassium, iron and magnesium. They also have phytochemicals; a group of compounds that are known for preventing chronic ailments such as cardiovascular diseases and cancers. In addition, pulses are a good source of fibre that can reduce the risk of developing diabetes and help in lowering of blood cholesterol levels, thereby also help in reducing the risk of heart diseases. Due to their ability to fix atmospheric nitrogen, they play a key role in maintaining soil fertility and ensuring sustainability of production system, particularly in low input, small-scale agriculture.

India is the largest producer, importer and consumer of the pulses in the world, accounting for 25 % of the global production, 15 % of the trade and 27 % consumption, as sizeable population of the country still depends on the pulses to meet its protein requirement. Cereals are generally deficient in the lysine which is an essential amino acid and can be provided by legumes. On the other hand, legumes are low in sulphur containing amino acids, which cereals can provide (Thirumaran and Seralathan 1988). Therefore, a combination of cereals and pulses is the most prevalent meal in common households in many countries including India.

The country produces variety of pulses including chickpea, pigeon pea, urdbean, mung bean, lentil, field pea and others to the tune of 13–15 million tonnes from an area of 22–33 million hectares with an average yield of 600–650 kg/h. In India, Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka and Rajasthan are the major states which grow pulses and contribute to the 80 % of the total production of pulses.

Domestic production of pulses after its peak of 14.94 million tonnes in 2003–04 had declined to 13.38 million tonnes in 2004–05 and to 13.11 in 2005–06, due to adverse climatic conditions prevalent in the major production zones. In the year 2007–08, the production has increased marginally to 14.9 million tones. This still fell short of the domestic requirement, which is increasing consistently with growing population, rising income, value addition and market opportunities. The widening gap in demand and supply has continued and led to soaring prices of pulses during past 2 years. Also, inclusion of pulses in future trading and limited availability in the international market has further fueled the prices.

12.2 Constrains that Results in Low Productivity of Pulses

The stagnant production of pulses in the past decade is raising a concern among the stakeholders and thus demands technological breakthrough immediately for improving productivity to balance the demand and supply of pulses. The major factor responsible for poor growth in the production of food legumes is their low productivity that in turn makes pulses economically less competitive in comparison to other crops. In fact, there has been only a marginal annual increase (less than 1 %) over the past more than five decades in case of pulse crops. They are therefore increasingly getting pushed to less endowed and marginal lands (areas), particularly in developing countries. The greatest challenge for legume researchers is to enhance the economic competitiveness of these legumes by improving their niches available in various cropping systems, enhancing their end use quality for diversified uses, and reducing their susceptibility to a host of biotic stresses (diseases, insect pests, parasitic and other weeds, etc.) and abiotic stresses (drought, extremes of temperature, salinity, nutrient deficiencies and toxicities, etc.). Therefore, conventional as well as biotechnological interventions that could either enhance their productivity or improve their resistance against biotic stresses and abiotic stresses are required.

Further in India, the national agriculture policies stress on promotion of cereal crops as pulses are generally considered neither as staple food nor as cash crops. They occupy low priority in the farming system because of lack of stability and high risk involved in their production. Also, pulses are prone to high losses during storage. Further, they have so far proved to be less responsive to inputs including irrigation (Singh and Kochhar 2005). To add to it, there are extremely fluctuating markets for the pulses. As productivity of pulses is low, they are generally grown in rain-fed area with poor soil fertility and low moisture retention capacity. Thus, these crop face moisture stress at various growth stages. Nonavailability of quality seed is another important constrains that limits pulse production. Seeds of newly developed varieties are either not available to the farmers or are not available in required quantities. Thus, varieties with better yield advantages and desired characteristics to suit various agro- climatic conditions need to be developed.

12.3 Mung Bean

12.3.1 Taxonomy and Distribution

The genus *Vigna* named after Domenico Vigna, Professor of Botany in Pisa (Savi 1826; Baudoin and Maréchal 1988) of the family Fabaceae includes many pulse-yielding species. It is an important legume taxon widely distributed in tropical and subtropical regions of both hemispheres and contains about 150 species, divided into seven subgenera. Of which, subgenus *Ceratotropis* mainly comprising species found in Asia, has been differentiated into morphologically homogenous group with specialized and complex floral morphology (Maréchal et al. 1978). *Vigna mungo* (L.) Hepper (urdbean) and *V. radiata* (L.) Wilczek (mung bean) are the two most important pulse-yielding taxa belonging to subgenus *Ceratotropis* of genus *Vigna*. Indian centre is extremely rich and possesses considerable landrace diversity of these species and several allied ancestral wild taxa. *V. radiata* and *V. mungo* are morphologically similar; however, minor differences exist in morphology and few other important plant characters. Variations have also been reported in karyotype, DNA content, proteins, amino acid composition and species hybridity.

12.3.2 Cultivation and Economic Importance of Mung bean

In general, pulses are grown mostly in two seasons; warmer, rainy season or kharif (June-Oct) and cool, dry season or rabi (Oct-April). Mung bean is grown on approximately six million hectares, mainly in Asia; however, cultivation has been extended to parts of Australia, USA, Canada and Ethiopia (Schafleitner et al. 2015). However, 90 and 54 % of world production of mung bean comes from Asia and India, respectively. Thus, 65 % of the world acreage under mung bean is accounted by India alone. In India, mung bean is grown on 34.4 lakh hectares that accounts for 18.07 % of the land under pulse production, whereas the production is approximately 14 lakh tonnes accounting for 11.48 % of the total pulse production. With a productivity of 406.98 kg/ha, mung bean is primarily grown in seven states in India, namely Rajasthan, Maharashtra, Andhra Pradesh, Karnataka, Orissa, Tamil Nadu and Uttar Pradesh. However, yield of mung bean has not shown any dramatic increase during the past several years (Fig. 12.1).

12.3.2.1 Export–Import Status and World Trade

India imports substantial amount of pulses from the world market for its domestic consumption. Import of mung bean and urdbean has been increased to 624.12 thousand tonnes in the year 2013–2014. These pulses are mainly imported from Myanmar (82.83 %), Tanzania (4.23 %), Kenya (3.55 %), Australia (3.05 %) and

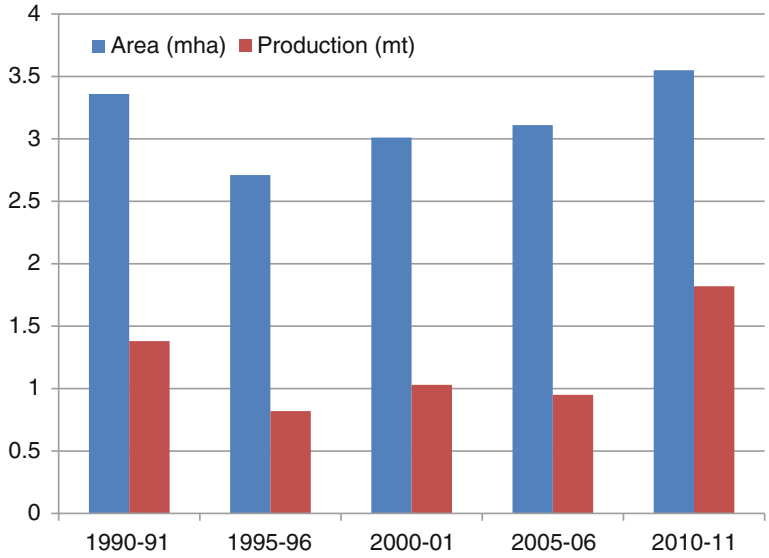

Fig. 12.1 Graphical representation showing area and production of *Vigna* sp. across India during 1990–2010

Mozambique (1.61 %). Interestingly, India exports also a small quantity of urad and mung bean (approximately 1.16 % of the total pulses export) to USA, Sri Lanka and Kenya.

12.3.3 Nutrients

Mung bean along with other pulses constitutes the major source of protein in Asia and constitutes an important supplement to the predominantly cereal-based diet. Seed of mung bean contain 26 % protein, 62.5 % carbohydrates, 1–2 % fats and 4.2 % fibres. Mung bean is also used for bean sprouts, starch noodles, green pods as peas in cooking and mung bean soup. Seeds and sprouts of mung bean with high levels of total phenols and flavonoids, DPPH (2,2-diphenyl-1-picrylhydrazyl), tyrosinase inhibition, anti-proliferative and ADH (Alcohol Dehydrogenase) activities could be recommended as preventative or/and therapeutic agents for human diseases in addition to normal prescription drugs. Yao et al. (2008) discussed the antidiabetic properties of extracts from mung bean sprouts and mung bean seed coat in their study of type 2 diabetic mice. Thus, selection of varieties with high levels of polyphenol content during sprouting was recommended as a very good option for addressing diabetes problem.

The oil content in mung bean seeds is relatively low (2.1–2.7 %). Oil of mung bean contains linoleic acid, palmitic acid and oleic acid as the dominant fatty acids.

Mung bean oil also contains vitamin E, including tocopherols and tocotrienol. The vitamin A content of 70 and 100 μg RAE (Retinol Activity Equivalent) has been reported in mung bean grains and sprouts, respectively.

Mung bean has low content of phytic acid as compared to other leguminous crops and cereals. Phytic acid is commonly found in cereals and other legume crops which have a negative impact on Fe and Zn availability in plant–based diets. Therefore, due to its palatable taste, other nutritional qualities and low cost, mung bean has been used as good source of iron especially in baby foods. Additionally, Vitamin C the most potent enhancer of iron absorption though low in mung bean grains (0.05 g kg−1 dry weight basis) is significantly high in its sprouts (1.38 g kg −1 dry weight basis).

12.4 Major Areas that Need Improvement in Vigna

Mung bean and urdbean are planted in kharif season and have to cope up with the problems of water management, nonsynchronous late maturity, diseases and pest. Therefore, attempts should be made to develop varieties with early and synchronous maturity, resistant to major diseases and pest which could be successfully cultivated in spring/summer and/or in kharif season (Khanal et al. 2005). There is an urgent need to developed varieties that are widely adaptable, tolerant to both low and high moisture stress, resistant to biotic stress such as cercospora leaf spot, web blight, thrips sucking bugs, etc. Also there is need to explore and decipher possibilities of growing mung bean and urdbean as inter or mixed crop with vegetables, fruits and maize. In addition to this there is requirement of developing suitable weed management technologies. Finally, the need to explore wild genepool for identification of desirable traits cannot be overemphasized.

12.5 Major Diseases in Mung bean

Mung bean is susceptible to diseases caused by fungi, bacteria and viruses. Yellow Mosaic is one of the most devastating diseases of mung bean. It is caused by bipartite begomo viruses namely mung bean yellow mosaic virus (MYMV), mung bean yellow mosaic India virus (MYMIV) and HgYMV in different mung bean growing areas of the world. The virus is transmitted by whitefly (*Bemisia tabaci*). The diseased plants have characteristic yellow coloration on the leaves and results in poorly developed pods thus affecting the yield significantly. First reported by Capoor and Verma (1948) in case of lima bean (*Phaseolus lunatus*) and dolichos bean, it was soon reported to affect mung bean with its to its first detail description

from the farms of IARI (Nariani 1960). In the first two decades after its detection in mung bean, the efforts were made to screen germplasm for the resistant lines. These attempts have been successful in identifying some resistant lines in the cultivated genepool aand some wild species. Genetic studies have led to the identification of genetic loci controlling the resistance.

Tobacco streak virus (TSV) causes a minor to severe impact on yields. Thrips are the only known vector of TSV. In Australia, several common broadleaf weed species are hosts of TSV with parthenium weed being a widespread and key host of the virus in central Queensland. The physical damage caused by thrips feeding on the plant allows infected pollen to enter the mung bean plant where the virus multiplies. TSV-infected mung bean plants are usually stunted and wilted with dead (necrotic) stems and tips.

Various leaf and stem pathogens, such as powdery mildew and bacterial blight, are frequently seen, especially in growing crops. Powdery mildew caused by the fungus, *Podosphaera fusca*, is favoured by cooler growing conditions and is often widespread in late crops. Charcoal rot caused by *Macrophomina phaseolina* results in infected plants to die prematurely and may reduce yield with its biggest impact upon the marketability of sprouting grade beans. Seed borne infection creates a soft, wet rot of the sprouts during the germination process. Legume little leaf affected plants often fail to produce pods, and if they do, they are generally distorted with the beans either failing to develop inside the pod or turning brown in colour.

Other diseases affecting mung bean include Sclerotinia white mould, Cercospora leaf spot, bacterial blight, etc.

12.6 Abiotic Stresses Affecting Mung bean

Abiotic stresses affect plant metabolism, disrupt cellular homeostasis and uncouple major physiological and biochemical processes (Arora et al. 2002; Srivalli et al. 2003). There are various abiotic stress factors that act as inhibitors to reduce vegetative growth, pod set, flower initiation in mung bean (Morton et al. 1982). The major environmental factors which affect *Vigna* production are water and salt stress. Mung bean is highly sensitive to these stresses. Initially, it is the root system which is affected more than the shoot and subsequently these stresses act on the growth rate by affecting the major physical and biochemical parameters and changes the growth pattern. Abiotic stresses can also lead to number of insect pests and several disease conditions which ultimately result in further loss of productivity and quality of mung bean (Singh and Singh 2011).

12.7 Drought Stress

Basic metabolism of the plant is mainly affected by water stress which controls the plant growth, plant yield and quality of a crop (Hsiao and Acevedo 1974; Begg and Tuener 1976). Mung bean is reported to be more susceptible to drought than many other grain legumes (Pandey et al. 1984). This affects the productivity of mung bean mainly during spring and summer season. Thomas et al. (2004) reported that mung bean plants under water stress attained maturity earlier than the well-watered treatment. Mung bean crop is more sensitive to water deficit during the flowering period. Severe drought reduces vegetative growth, flower initiation and pod set (Morton et al. 1982). Drought induces early flowering in mung bean which results in poor pod setting. Increased drought during the development of reproductive organs also has a pronounced effect on fruit development and yield mainly due to an increased rate of floral and pod abortion (Liu et al. 2003). Water stress also decreases the number and shape of root hairs (Worral and Roughley 1976) and when nodules are formed, drought also alters the nodule structure and weight (Ramos et al. 2003).

12.7.1 Water Logging Stress

Excess of water can also affect the growth of mung bean. Heavy rain coupled with strong winds damages the mature crop resulting in severe yield losses. Death of mung bean plants occurs due to waterlogging and surviving plants, if any, become very sensitive to various fungal diseases and insect pests. During early stages of growth, mung bean cannot withstand waterlogging (Tickoo et al. 2006). Main effect of waterlogging occurs on the dry weight of roots. Oxygen concentration reduces around the roots of submerged plants and restricts nodule activity and nitrogen fixation during the waterlogging. Infact, it is because of this reason that mung bean is not suited to the wet tropics where the annual precipitation is >1000 mm (Fernandez and Shanmugasundaram 1988). However, water logging has no effect on flower colour parameters (Musgrave and Vanhoy 1989). Some other effects of water logging are decrease in chlorophyll content, flower partitioning, pod setting, photosynthesis rate, number of nodules, membrane stability index, crop growth rate, root dry weight, quality and crop yield. Varma and Rao (1975) observed that the number of pods and seed yield/pod in mung bean reduces sharply with excess moisture level. Duong et al. (1988) reported that 48 h of waterlogging reduced plant height up to 76 %, leaf area up to 46 % and dry matter production up to 57 %. Interrelationship between root and shoot carbon budgets was shown by Musgrave and Vanhoy (1989) in mung bean as a response to waterlogging stress and damage. Mung bean genotypes exhibiting differential sensitivity during recovery from water logging have been identified and characterized. This selection is required to identify the stress tolerance in germinated seed for the advancement of the crop and to

increase the yield of the legume grain. (Haqqani and Pandey 1994; Tickoo et al. 2006; Kumar et al. 2012a, b). At AVRDC also efforts have been made to identify waterlogging tolerant cultivars and several tolerant selections such as 'V 1968', 'V 2984', 'V 3092' and 'V 3372' have been made (Tickoo et al. 2006).

12.7.2 Salinity Stress

Salinity stress is one of the most important abiotic factors as it leads to significant loss in mung bean production (Abd Alla et al. 1998; Saha et al. 2010). It is estimated that 6.5 % of the total land of the world is affected by salinity stress which is around 831 M ha of land (Hasanuzzaman et al. 2013). Salt stress along with other pests (stem and pod borer) and yellow mosaic disease was found to cause 80–100 % yield loss in mung bean particularly during rainy season. Paliwal and Maliwal (1980) had reported that germinating mung bean can resist 6 m mhos/cm of salinity, whereas, a mung bean plant can tolerate 9–18 m mhos/cm salinity. To enhance the yield and growth of mung bean under saline conditions, seeds are inoculated with L-TRP and *Rhizobium* and used as complement (Zahir et al. 2010). With increasing salt concentration, delayed and decreased germination is observed in mung bean. However, no effect was observed in some of the concurrences of *Vigna radiata* when they are cultivated on extremely alkaline (pH > 8.5) calcareous soils (Lawn et al. 1988).

On treating the germinating seeds of mung bean it has been found that high salt concentration has a drastic effect on roots as compared to shoots. Length of the roots, branches and the number of root hairs decreases during salinity. Salt stress causes chlorosis, necrosis and reduced the chlorophyll content (Wahid 2004). Polyamine synthesis is also affected by an increased amount of salt concentration in mung bean grain (Friedman et al. 2006). Increase in proline oxidases (which convert proline to glutamate) leading to decrease in proline content has been reported.

In case of mung bean, salinity also induces desiccation which results in more flower shedding and pod shattering during the summer season. Therefore, reduced yield in mung bean under salt stress may be due to more flowers shedding, reduced photosynthetic efficiency per day of plant to fill the developing seeds and shattering of the pods (Wahid et al. 2004; Ahmed 2009). In another study, different doses of salt (NaCl) concentration given to three varieties of *V. radiata* (T 44, SML 66, Sarif) showed a reduction in seedling growth, germination percentage, relative growth rate and photosynthetic pigments after 15 days only (Arulbalanchandran et al. 2009).

Gulati and Jaiwal (1994b) examined accumulation of ions in cellular and whole plant response against different salt concentration and demonstrated that salt stress is regulated at cellular level. NaCl salt stress was overwhelmed by pretreatment with salt concentration which modified antioxidant enzyme activities; reduced malondialdehyde and H_2O_2 content so that accumulation of osmolytes (proline)

increases (Saha et al. 2010). Thus, in this way, plants of mung bean could adapt to survive even in salt stress.

12.7.3 Temperature Stress

Like other crops, mung bean is also sensitive to change in temperature and photoperiods. Most of the genotype of mung bean has a photoperiod of 12–13 h and beyond this photoperiod a progressive decrease in flowering is observed. According to Poehlman (1978) if the photoperiod is extended up to 16 h then in some short seasons early strain flowering was delayed for only a few days, however, in photosensitive strains this delay was of 30–40 days and some strains even fail to flower. Limited information is available about flower shedding in mung bean and scanty work has been carried out in this area. Kumari and Verma (1983) reported that high temperature stress has a negative impact on the retention of flower and consequently on the pod formation also. They also observed that due to high temperature extent of flower shedding has been extended up to 79 %. A Genotype with maximum flower retention and productive pods have been screened and selected during temperature >40 °C.

12.8 Resources Available and Their Utilization

12.8.1 Status of Global Collections and National Germplasm

A total of over 10,550 accessions of various *Vigna* species comprised of mung bean (3704), urdbean (3131), mothbean (1486), ricebean (2045) and azuki bean (185) have been stored at −18 °C in a long-term repository of National Gene Bank at NBPGR, New Delhi. Globally, mung bean germplasm accessions are maintained by more than 35 institutions, which hold more than 25,000 accessions. AVRDC at Taiwan maintains 5510 accessions of mung bean, and over 12000 accessions belonging to various *Vigna* species are held in the conservation unit in Georgia, USA.

12.8.2 Mung bean Varieties—the Cultivated Genepool

Though, a large number of varieties have been released and recommended for cultivation in different agro-climatic zones of India, a satisfactory increase in the yield has not been achieved so far. In India, out of a total of 50 varieties released

during 1985–2010, 37 were cross-bred, seven were selections from local landraces and five varieties were developed by mutation breeding. In addition, one variety was a selection from an exotic accession. A critical analysis of the pedigree data of the varieties released reveals limited use of vast germplasm and frequent use of previously released varieties as the background material for developing novel varieties. Lakhanpaul et al. (2000) had pointed out that source material for 13 distinct varieties released by different agricultural institutes/universities could be traced back to only four local collections originating from a single state of India.

Thus, a narrow genetic base owing to high degree of commonness in the pedigrees prevails in the cultivated germplasm of mung bean though large germplasm having significant variation in important morpho-agronomic traits is available.

12.8.3 Wild Germplasm and Its Utilization

Crop Wild Relatives (CWR)—the putative progenitors including wild plant species that are related to crop plants are potential gene donors for the desirable traits (Ford-Lloyd et al. 2011). Thus, wild relatives, the important constituent of the total genetic diversity of the cultivated species are extremely valuable for the improvement of a crop. Genetic resources thus are important source of useful genes for resistance to diseases or for adaptability to changing climatic conditions. Likewise, some of the wild *Vigna* species have many useful genes (Table 12.1), which are transferable to cultivated crops by direct crosses (Tomooka et al. 2008; Pandiyan et al. 2008). Fuzi and Miyazaki (1987) reported an accession (TC1966) of *V. radiata* var. *sublobata* that showed perfect resistance against azukibean weevil (*Collasobruchus chinensis*). The resistance was found to be controlled by a single dominant gene (Kitamura et al. 1988). Fuzii et al. (1989) further found that TC1966 is completely resistant against *C. maculatus, C. phaseoli* and *Zabrotes subfasciatus*. Tomooka et al. (1992) developed a bruchid resistant mung bean line in Thailand by using TC1966 as a gene source. In addition to bruchid resistance, high resistance to yellow mosaic virus, high methionine content in seeds (Babu et al. 1988), higher photosynthetic activity and tolerance to drought (Ignacimuthu and Babu 1987),

Table 12.1 Wild/related species of *Vigna* as sources of useful genes

Species	Trait of interest
V. radiata var. *sublobata*	-Resistance to Mung bean Yellow Mosaic Virus (MYMV) -High number of seeds per pod and pods per plant -High methionine content
V. trilobata	Resistance to Mung bean Yellow Mosaic virus (YMV)
V. mungo var. *sylvestris*	Resistance to YMV
V. mungo var. *mungo*	Synchronous maturity and tolerance to Cercospora leaf spot
V. umbellata	High yield, tolerance to bruchid, responsive to inputs

higher tolerance to saline and alkaline soils (Lawn et al. 1988) have been reported for *V. radiata* var. *sublobata*. In comparison to *V. radiata* var. *sublobata,* there are very few studies on evaluation of *V. mungo* var. *silvestris* as genetic resource. However, this variety is cross-compatible with *V. mungo* (Miyazaki 1982).

Nevertheless, the varying extent of crossability barriers of the crop taxa with the wild and weedy relatives are the important determinants of the success of such conventional breeding programs. Thus, an insight into the results obtained so far while making interspecific crosses involving mung bean is important.

12.8.4 Hybridization

Interspecific crosses within the genus *Vigna* have been attempted by various workers. Barriers while crossing mung bean with other species range from failure of pollen tube to penetrate the style or stigma (Chowdhary and chowdhary 1977) to embryo abortion after fertilization and poor pod or seed set. Bharathi et al. (2006) while crossing *V. radiata* as female parent with four other species obtained highest crossability with *V. umbellata* (29.63) followed by *V. trilobata* (8.48 %) and *V. aconitifolia* (7.69 %). Interestingly, a reciprocal cross using *V. umbellata* as the female parent showed least number of pods (0.005 %) with shrunken pods and shrivelled seeds.

Sehrawat et al. (2015) while evaluating mung bean germplasm in saline environment identified the most salt tolerant wild relative of mung bean. Two genotypes namely EC528960 and TCR86 belonging to *V. luteola* and *V. trilobata,* respectively, were identified and are the potential source of genes to be introgressed in the salt sensitive mung bean genotypes through breeding.

12.8.5 Phylogenetic Relationships: Mungo-radiata *and Their Allies*

The taxonomic and nomenclatural uncertainty in *Vigna* occurred due to wrong nomenclature by Linneaeus, who named urdbean as *Phaseolus mungo* and mung bean as *P. radiatus*. Verdcourt (1970) reviewed the genus and on the basis of morphological and biochemical evidences transferred Asian species of *Phaseolus* to genus *Vigna.* Later, *Phaseolus mungo* was named as *V. mungo* and *P. radiatus* as *V. radiata.* Also, it was considered that both these species evolved from a common ancestor *P. sublobatus*, which afterwards was named as *V. sublobata*. Further misunderstanding occurred due to wrong cytogenetical relationships based on cytological study and scanning electron microscopy of seed coat pattern (Jain and Mehra 1980). The authors contended that *V. sublobata* was not likely to be the progenitor of mung bean (*V. radiata*) but instead the progenitor of urdbean (*V. mungo*). Morphological and biochemical studies by Arora et al. (1973) on wild populations of *Vigna* provide the first constructive evidence about evolutionary

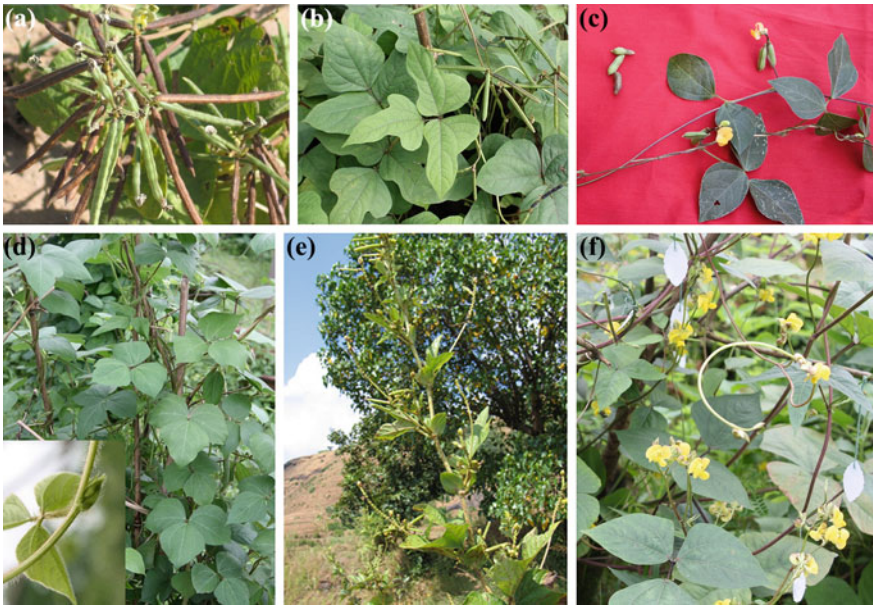

Fig. 12.2 Mung bean and some of its wild relatives occurring in India: **a**. *V. radiata*; **b**. *V. radiata* var. *sublobata*; **c**. *V. hosei*; **d**. *V. trinervia var. trinervia*; **e**. *V. khandalensis*; **f**. *V. trinervia var. bourneae*

relationships among *V. radiata-mungo-sublobata-silvestris* group. They identified two botanical varieties, which, respectively, gave rise to *V. radiata* and *V. mungo.*

Of the seven subgenera under the genus *Vigna*, only subgenus *Ceratotropis* has its centre of species diversity in Asia. In the subgenus *Ceratotropis*, there are crop complexes associated with each of the domesticated species. The Asian *Vigna* genepool includes 21 species out of which eight are used for human and animal food. The subgenus includes wild relatives and their wild forms.

The species of *mungo-radiata* group has wide distribution throughout Indian subcontinent. The group includes two cultivated species *V. mungo* and *V. radiata* and their respective wild forms namely, *V. mungo* var. *silvestris* and *V. radiata* var. *sublobata* and *V. radiata* var. *silvestris*. Group also includes another wild species, i.e. *V. hainiana* which can be regarded as most ancestral type. All the species included in the group are variable in morphological characters (Fig. 12.2).

Lukoki et al. (1980) accepted specific distinction between two forms and their relation as wild ancestor to the cultivated species. They noted that Plx 416 was similar to *V. mungo* biochemically in possessing glutamyl-methionine and its sulphoxide; and morphologically possesses narrower stipules, brighter yellow flowers, 6–8 ovules per ovary, erect pods with long white hairs (or glabrescent) and seeds with a raised arillate hilum. Therefore, they recognized these plants as the wild ancestor of black gram and described it as a new taxon, namely *V. mungo* var. *silvestris* Lukoki, Maréchal and Otoul. Lukoki et al. (1980) also described Plx 274 similar to *V. radiata*

var. *radiata* in possessing α-glutamyl-s-methylcysteine and its sulphoxide in its seeds and have broader stipules, paler yellow flowers, 10–14 ovules per ovary, spreading pods with short brown hairs and seeds with a flat non-arillate hilum. Chandel et al. (1984) and Miyazaki (1982) also supported the view that mung bean and urdbean were domesticated from *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris*, respectively, on the basis of their biochemical and morphological studies.

Babu et al. (1988) assessed the seed protein and amino acid composition of *V. radiata* var. *sublobata* and two cultivated forms *V. mungo* and *V. radiata*. They reported high range of variation for all the amino acids indicating broad genetic base and suggested the usefulness of wild populations of *V. radiata* var. *sublobata* in the nutritional upgrading of mung- and urdbean. The amino acid profiles were population-specific and wild populations of *V. radiata* var. *sublobata* were shown to differ contrastingly in cysteine content with *V. radiata* as well as *V. mungo*. *V. mungo* contained high methionine content, which could be due to Y-glutamylmethonine and its sulphoxide while *V. radiata* possessed cysteine and its sulphoxide. These results were similar to those reported by Lukoki et al. (1980). However, Babu et al. (1985) continued to stress upon that *V. mungo* and *V. radiata* var. *sublobata* are closely related. Some indirect evidences from nodulation studies also indicate the origin of *V. mungo* and *V. radiata* from *V. mungo* var. *silvestris* and *V. radiata* var. *sublobata,* respectively (Kavimandan and Chandel 1988).

Various workers have analysed phylogenetic relations between mung bean and closely related taxa using molecular markers as well as sequence level variation in the phylogenetically useful loci. Ajibade et al. (2000) used ISSR amplification to study genetic relationships among 18 species of the genus *Vigna*. Tomooka et al. (2002) performed AFLP analysis of the diploid species in the genus *Vigna* subgenus *Ceratotropis*. The phenograms generated revealed that the species belonging to sections *Ceratotropis* and *Aconitifoliae* are well separated. However, most of the species in section *Angulares* showed a high level of similarity suggesting a low level of genetic variation.

Goel et al. (2002) reconstructed molecular phylogeny based on internal transcribed spacer (ITS) sequences to resolve the taxonomic contradictions in *Vigna* and its relation to the genus *Phaseolus*. ITS phylogeny was congruent with classification based on morphological, biochemical and cytogenetical and palynological features. However, subgenus *Plectotropis* of Neotropical origin was revealed to be very closely related to subgenus *Vigna* instead of forming a link between African and Asiatic *Vigna*. Sequence-based analysis of ITS and atpB-rbcL intergenic spacer split the subgenus *Ceratotropis* into three distinct sections namely Aconitifoliae, Angulares and *Ceratotropis* (Doi et al. 2002). Earlier, Vaillancourt and Weeden (1993) reconstructed chloroplast DNA phylogeny of old world *Vigna* which supported monophyly of old world *Vigna* with the only exception *V. frutscens* of subgenus *Vigna* that fell within the new world. Kaga et al. (1996) studied species relationships in subgenus *Ceratotropis* of genus *Vigna* through RAPD analysis and observed that the wild forms were always grouped with their most closely related cultivated forms. The largest intraspecific variation was found in mung bean and least variation was found in adzuki bean.

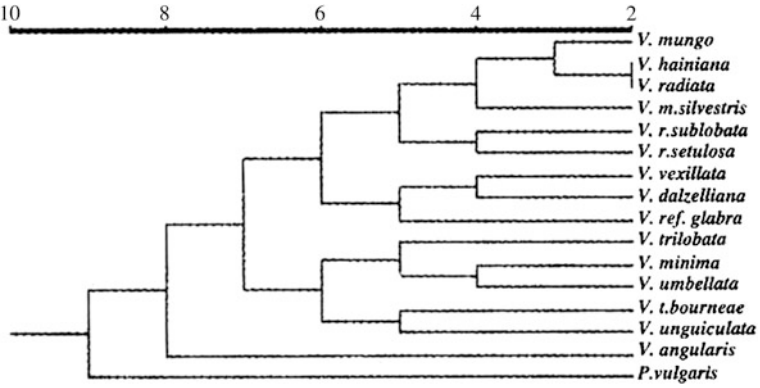

Fig. 12.3 Neighbour joining tree showing the relationship among 15 *Vigna* species using *Phaseolus vulgaris* as the outgroup (adapted from Vir et al. 2008)

Later, Vir et al. (2009) analysed population substructure, genetic differentiation and phylogenetic relationships among selected Asiatic *Vigna* species. Phylogenetic tree indicated that *V. mungo, V. radiata, V.mungo* var *silvestries, V. radiata* var *sublobata* and *V. radiata* var *setulosa* are distinct taxonomic groups. Also wild relatives were placed along with their cultivated forms reflecting their origin from a common ancestors (Fig. 12.3). Vir et al. (2010) also employed ISSR markers for diversity analysis, genotyping and assessment of species relationships in Asiatic *Vigna* from Indian subcontinent. The wild forms were grouped with their cultivated forms as expected whereas another wild forms *V. hainiana* emerged as a distinct taxon and seemed to be more primitive in comparison to the other wild relatives of green gram and black gram. Javadi et al. (2011) investigated phylogeny and biogeography of the subgenus *Ceratotropis* using chloroplast DNA sequence data. The study revealed three eco-geographical groups and late Pilocene-Pleistocene diversification.

12.8.6 Mung bean Germplasm and Core Collections

The effective utilization of huge germplasm collections maintained in the genebanks world over has necessitated the development of core set for each crop plant that has minimum similarities between the entries and yet represents the total collection in terms of genetic diversity (Frankel and Brown 1984; Van Hintum et al. 2000) With this realization, Bisht et al. (1998) developed a representative core set for Indian mung bean collections based upon their morpho-agronomic characteristics along with the passport data. The accessions from six major mung bean growing zones and one group of exotic collections subjected to principle component score strategy resulted in 152 accessions that could represent as a core of Indian mung bean germplasm collections. A comparison of the genetic diversity of

this core collection with the total collection was also made to test the validity of the representative collection. Additionally, the variation pattern of the core set was also used to discriminate among accessions in order to enhance the accessibility and utilization of the germplasm. Recently, Schafleitner et al. (2015) established AVRDC—the world vegetable centre mung bean core collection by geographical stratification of the whole collection comprising more than 5000 accessions and subsequent diversity analysis based on eight phenotype descriptors. This core along and mini core collection together with the evaluation and genotypic data, is available for distribution to breeders. The core collection of 1481 entries has been under continuous evaluation for traits of interest for breeding biotic and abiotic stress tolerance and thus will add information of significant value to these collections.

12.8.7 Diversity Analysis

Analysis of genetic diversity in mung bean is important since diversity present within the species is paramount for maintaining its generic potential. Assessment of genetic diversity provides an opportunity to identify desirable traits such as higher yield, pest and disease resistant that are useful in the development of improved varieties. Also, genetic diversity within the species reflects its ability to adapt in changing environment. Genetic diversity within mung bean has been assessed by using different molecular markers such as RAPD (Lakhanpaul et al. 2000; Saini et al. 2008; Lavanya et al. 2008; Sony et al. 2012; Datta et al. 2012; Bhuyan et al. 2014) ISSR, AFLP (Chattopadhyay et al. 2005), SSR (Gwag et al. 2010; Chen et al. 2015) suggesting their utility in the analysis.

In addition, Vir et al. (2009) demonstrated the transferability of mung bean STMS primer to urdbean, wild relatives and other pulse taxa. Narsimhan et al. (2010) evaluated six mung bean Yellow Mosaic Virus resistant and susceptible genotypes of mung bean and urdbean using RGA primers from cowpea to assess the molecular diversity to develop suitable mapping population that can be used to identify and validate markers related to resistant loci.

Das and Singh (2014) conducted comparative analysis of genetic diversity across certain mung bean and urdbean cultivars of west Bengal using ISSR. Information generated was helpful in identification of the germplasm and diversity assessment. ISSR markers proved useful in assessment of genetic diversity, through detection of duplicate samples in germplasm collection and the selection of a core collection to enhance the efficacy of germplasm management for use in breeding and conservation programs. Recently, Chen et al. (2015) assessed genetic diversity and population structure of mung bean germplasm employing EST-based genomic SSR markers for germplasm evaluation.

12.9 Genomic Resources

12.9.1 Molecular Maps and Gene Tagging

In the pioneering study by Young et al. (1992), a major bruchid resistant gene was mapped in mung bean using RFLP markers. Bruchids are the most destructive pests of mung beans and other members of genus *Vigna*. Earlier bruchid resistance had been identified in wild mung bean accession TC1966. Fifty-eight F_2 progeny from a cross between TC1966 and a susceptible mung bean cultivar were analysed for 153 markers. Resistance mapped to a single locus on linkage group VIII, approximately 3.6 centimorgans from the nearest RFLP marker.

Menancio-Hautea et al. (1992) investigated genome relationships between mung bean and cowpea based on the linkage arrangement of random genomic restriction fragment length polymorphism (RFLP) markers. A common set of probes derived from cowpea, common bean, mung bean and soybean PstI genomic libraries were used to construct the genetic linkage maps. The mung bean and cowpea genomes were compared on the basis of the copy number and linkage arrangement of the 53 markers mapped in common between the two species. Results also suggested that the nucleotide sequences are conserved; however, there is variation in copy number and rearrangements in linkage order which happened after the divergence of species. Also, entire linkage groups were not conserved, but several large linkage blocks were maintained in genomes.

Humphry et al. (2002) constructed a genetic linkage map of mung bean consisting of 255 RFLP loci using a recombinant inbred population of 80 individuals. The population derived from an inter-subspecific cross between the cultivated mung bean variety "Berken" and a wild mung bean genotype "ACC 41" (*V. radiata* subsp. *sublobata*). The total length of the map, which comprised 13 linkage groups, spanned 737.9 cM with an average distance between markers of 3.0 cM and a maximum distance between linked markers of 15.4 cM. This map was compared with the previous released map of lablab using a common set of 65 RFLP probes. The chromosomal arrangement between these two species was found to be highly conserved. The two genomes, however, accumulated large number of duplications/deletions after they have diverged.

Gene tagging involves identification of molecular markers tightly linked to the trait of interest, an essential requirement of marker aided selection (MAS) in plant breeding programs for screening large populations in an unambiguous manner. Such markers have been developed in nearly all important crops. However, limited efforts have been made in case of mung bean. Nevertheless, Souframanien and Gopalakrishna (2005) identified a molecular marker closely linked to YMV resistance gene in case of black gram, a closely related species of green gram. A SCAR marker namely, $ISSR81_{1357}$ was specifically present in the resistant genotypes and consistently absent in susceptible ones.

12.9.2 Whole Genome Sequence of Mung bean and Its Selected Allies

Genome size of mung bean is 579 Mb with 2n = 2x = 22 chromosomes. Kang et al. (2014) constructed a draft genome of cultivated mung bean (*V. radiata* var. *radiata* *v*c1973a) on a chromosomal scale. This is the first draft genome sequence within the genus *Vigna.* For understanding domestication, polyploidization and speciation in the genus *Vigna*, whole genome sequence of the wild mung bean relative (*V. radiata var. sublobata*) and a tetraploid relative of mung bean (*V. reflex-pilosa* var. *glabra*), as well as transcriptome sequences of 22 *Vigna* accessions of 18 species were produced. In the study, 80 % of the *V. radiata* var. *radiata* genome was constructed with identification of 22,427 high-confidence protein coding genes and 160 *Vigna* gene clusters. Genomic sequencing provided insights into the polyploidy in legumes. It has been suggested that the domestication and cultivation of mung bean was initiated in the northwest and far south in India 4000–6000 years ago, based on the geographical distribution of the wild mung bean and archaeological records from India (Fuller 2007). As only one accession of *V. radiata var. sublobata* was included in the study, they could not observe any population substructure in *V. radiata var. sublobata* and thus were not able to determine whether there are V. radiata var. sublobata lineages more closely related to cultivated mung bean or they obtained evidences of multiple origin of the crop variety.

12.10 Biotechnological Interventions

Though dramatic results have been obtained in cereal crops by conventional breeding, the success in improving the crop yield has remained largely elusive in case of pulse crops in general and mung bean in particular. The narrow genetic base of the germplasm and crossability barriers between cultivated genepool and its allies has often been cited as the primary causes of failure of traditional methods for improvement. Nevertheless, the biotechnological interventions have now enabled the utilization of secondary and even tertiary genepool as gene transfers can be made across the wide taxa using modern techniques of genetic transformation with or without the help of in vitro regeneration protocols. Consequently, transgenic plants have been produced in most of the major crops.

However, legumes are generally not amenable to such biotechnological interventions easily due to the recalcitrance exhibited by these taxa to efficient tissue culture as well as transformation protocols. Likewise, success in developing transgenic mung bean has been rather limited using both direct and indirect method of organogenesis. The efforts by various workers towards optimizing usable protocols has been extensively reviewed and summarized by Sahoo et al. (2003).

Mung bean though a recalcitrant grain legume, *Agrobacterium*-mediated transformation has been achieved by Jaiwal et al. (2001). Hypocotyl and primary leaves

excised from 2-day-old in vitro grown seedlings produced transgenic calli on B_5 basal medium supplemented with 5×10^{-6} M BAP, 2.5×10^{-6} M each of 2,4-D and NAA and 50 mg l^{-1} kanamycin after co-cultivation with *Agrobacterium tumefaciens* strains, LBA4404 (pTOK233), EHA105 (pBin9GusInt) and C58C1 (pIG121Hm), all containing β-glucuronidase (*gusA*) and neomycin phosphotransferase II (*npt*II) marker genes.

Sonia et al. (2007) developed fertile transgenic plants of mung bean with two transgenes namely bar and ά amylase inhibitors. Recently, Yadav et al. (2012) reported a reproducible and highly efficient protocol for *Agrobacterium*-mediated transformation of mung bean using double cotyledonary nodes (DCN). Transient and constitutive gene expression was observed in DCN explants and different tissues of T0 and T1 plants. Integration of annexin gene was further confirmed by southern blotting. However, undoubtedly, extensive and concerted research efforts are further required to develop efficient protocols for mung bean regeneration and transformation. Later, Mirza and Tazeen (2004) also optimized the *Agrobacterium tumefacians* mediated transformation protocol for mung bean by studying parameters like sensitivity of explants to kanamycin, pH of co-culture media, age of explants, types of explants, co-cultivation time and optical density of *Agrobacterium* culture medium. Transformed shoots were produced on shoot regeneration medium containing 50 mg/l kenamycin and 500 mg/l cefotaxine.

In conclusion, though germplasm holdings can be further enriched by conducting targeted explorations and through exchange programs, sufficient collections do exist in the national gene banks and international institutes such as AVRDC. Despite maintaining these large collections comprising of wild species, landraces, obsolete cultivars, etc., that are immensely endowed with a number of useful genes, efforts to utilize them in the ongoing improvement programs are less than satisfactory. Some of the major efforts for preventing the underutilization of mung bean germplasm include extensive screening, characterization and evaluation of the available holdings for identifying the elite genotypes and development of multiple core sets for the desirable traits. Intensive prebreeding efforts through strong interactions between germplasm curators and mung bean breeders are also required. On the other hand, developing novel varieties exploiting distant genepools necessitates the optimization of efficient protocols for regeneration and transformation for successful intervention of biotechnogical tools.

Efforts using a combination of traditional breeding and recent molecular approaches are the need of the hour to break the yield barriers in pulse crops such as mung bean that are the major source of nutrition for large populations and should be considered as the immediate and the urgent challenge for scientific community engaged in crop improvement programs.

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