

Morphophysiological and Molecular
Diversity in Mung Bean (*Vigna radiata* L.) **5**

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Abstract

Mung bean [Vigna radiata (L.) Wilczek] is one of the upsurging, highly economical, nutritive Asiatic leguminous crops. The crop is getting higher attention in terms of the consumption and production worldwide being an important source of amino acids, proteins, dietary fibre and unsaturated fatty acids. It possesses folate and iron in significant amount along with several phytochemicals. The short life cycle and nitrogen-fixing ability make it more suitable for sowing along with other crops. In spite of several advantages, it has got less attention in terms of development of morphophysiological and molecularly diverse varieties. Mung bean has a small genome, and fortunately it has been sequenced; therefore, it may be utilized as an exemplary plant to understand other legumes. Development of wild mung bean pool from diverse origins and environmental conditions would help to conserve the genetic wealth of the crop. Higher yields, shorter maturity period, higher harvest index, photoperiod insensitivity, resistance to major insect pests/diseases, compact canopy and synchronous maturity are some of the important objectives for crop improvement in mung bean. This chapter reviews the morphophysiological and molecular diversity of mung bean and also gives an insight about mutagenesis, plant protection and abiotic stresses associated with the crop.

Keywords

Mung bean · Diversity · Biometric · Physiological · Molecular level

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P. Muthu Arjuna Samy et al. (eds.), Legumes: Physiology and Molecular Biology of Abiotic Stress Tolerance, [https://doi.org/10.1007/978-981-19-5817-5_5](https://doi.org/10.1007/978-981-19-5817-5_5#DOI)

5.1 Introduction

Mung bean [Vigna radiata (L.) Wilczek], also known as green gram, golden gram, green bean or mash bean, is an important fast-growing, highly economical, nutritive, multipurpose leguminous crop cultivated in tropical and subtropical regions of Asia (Tah [2006](#page-30-0); Yang et al. [2008\)](#page-32-0). It is a self-pollinating species belonging to the genus Vigna of Fabaceae family. The crop is mainly grown in frost-free regions from Asia to Africa, South America and Australia (Nair et al. [2012\)](#page-27-0). India is one of the largest producers of mung bean and shares about 50% of global annual production (Nair et al. [2012](#page-27-0)).

Being a leguminous crop, mung bean is an important source of amino acids, proteins, dietary fibre and unsaturated fatty acids (Hou et al. [2019](#page-24-0)). It is easily digestible, produces low flatulence as compared to other legumes and contains higher folate and iron (Keatinge et al. [2011](#page-25-0)). The crop makes the soil fertile and improves its texture (Graham and Vance [2003\)](#page-24-0). Similarly, it has also been observed that the cereals intercropped with mung bean have a lesser incidence of pest infestation and have a higher yield due to the availability of nitrogen fertilizer (Yaqub et al. [2010\)](#page-32-0).

Owing to higher vitamin, calcium, iron and phosphorus content as compared to other leguminous crops, mung bean is a preferred nutritive food. The presence of amino acids, proteins, polyphenols and oligosaccharides in the crop has been exploited for antioxidant, antitumor, anti-inflammatory and antimicrobial activities (Anjum et al. [2011](#page-21-0); Randhir et al. [2004\)](#page-28-0). Mung bean has also been reported to contain several phytochemicals, viz., steroids, triterpenoids, glycosides, flavonoids, alkaloids, polyphenols, tannins, saponins, daidzin, daidzein, ononin, formononetin, isoformononetin, quercetin, kaempferol, myricetin, rhamnetin, etc. (Priya et al. [2012;](#page-28-0) Ramesh et al. [2011](#page-28-0); Tang et al. [2014](#page-31-0)).

Mung bean has also been reported to contain a good amount of antifungal proteins (Solanki et al. [2018\)](#page-30-0) that can be used against human and plant pathogens. Mung bean seeds possess alkaloids, coumarin and phytosterol that support the physiological metabolism in human beings. The seeds are also free from antinutritional factors, viz., trypsin inhibitors, phytohemagglutinins and tannins (Xin et al. [2003](#page-32-0)).

Mung bean has been used as a model crop for physiological studies (Musgrave et al. [1988\)](#page-27-0) and for understanding the beginning and expansion of adventitious roots (Norcini et al. [1985](#page-27-0); Tripepi et al. [1983\)](#page-31-0). The rooting bioassay of this plant has also been used to assess the root-promoting potential of growth regulators (Kling et al. [1988\)](#page-25-0). Mung bean is used globally for human consumption, cattle feed and medicinal purposes (Jo et al. [2006\)](#page-24-0). Its sprouts and splits are very nutritious, and as a component of soups, noodles, cake or ice cream fillings, it is commonly used in human foods. Its haulm, green and dry fodder are used as nutritious animal feed (Garg et al. [2004](#page-23-0)). Studies have revealed its importance in the treatment of hepatitis, gastritis, etc., and it has antihypertensive, antidiabetic and anticancer properties (Kumar and Singhal [2009](#page-25-0)). Keeping in view the importance of the crop, the consumption of mung bean has increased considerably along with its production

(Shanmugasundaram et al. [2009\)](#page-29-0). Therefore, mung bean is considered among cash crops and has attracted the interest of researchers.

5.2 Origin

Mung bean has diploid ($2n = 2x = 22$) chromosome numbers. Vavilov [\(1951](#page-31-0)) proposed Central Asian regions as the basic genetic centre of mung bean and India as the centre of its domestication (Singh et al. [1970;](#page-30-0) Smartt [1985](#page-30-0)). The diversity data and archaeological confirmations also suggested India to be the origin place of mung bean (Fuller and Harvey [2006;](#page-23-0) Jain and Mehra [1980](#page-24-0)), although the wild relatives of mung bean have been reported from the subtropical and tropical provinces of northern and eastern Australia (Lawn and Cottrell [1988](#page-26-0)). Studies carried out based on protein and enzyme variability suggest that modern mung bean has several series of domestication (Lambrides and Godwin [2007](#page-26-0); Viña and Tomooka [1994\)](#page-31-0).

5.3 Genetic Resources

Availability of germplasms having superior alleles and wide genetic diversity is one of the prerequisites for a sustainable breeding programme. Therefore, numerous organizations have collected mung bean germplasm to sustain the genetic resources. To facilitate the effective utilization and easier access to genetic resources, germplasms have been conserved in China, India, Korea and the USA. Asian Vegetable Research and Development Center has established a core collection of about 1700 mung bean accessions. These accessions have been morphologically and molecularly characterized (Shanmugasundaram et al. [2009](#page-29-0)). Germplasms having variable characteristics are the most important resource for crop improvement and play an important role in widening the genetic background of cultivars.

5.4 Cultivation

Mung bean is a short-day crop and is generally grown during the rainy seasons. It takes about 90–120 days to mature. It is the third most important leguminous crop after chickpea and pigeon pea cultivated in India (Ahmad and Belwal [2019](#page-20-0)). Mung bean is globally cultivated on nearly seven million hectares and is mostly limited to Asian countries (Nair et al. [2019\)](#page-27-0). The total production of the crop in India from 2018 to 2019 was 2455.37 thousand tonnes with an average productivity of 516 kg per hectare (Anonymous [2020](#page-21-0)), suggesting that India is one of the largest producers of mung bean.

The production and partitioning of dry matter potential in mung bean are an outcome of several growth stages of the plant. The changes in the growth stage mainly depend upon the temperature and photoperiod. Manipulation in the process of the growth stage in context to the environmental conditions may lead to grain yield improvement. The time taken for mung bean crop to mature is an important yield factor. The duration may change with the environmental conditions, sowing time and cropping season. It helps to determine the suitability of crops under various cropping systems. Mung bean is sensitive to photoperiod, and flowering in the crop is influenced by the duration of light (Aggarwal and Poehlman [1977](#page-20-0)). It has been reported that short days lead to early flowering, while long days result in delayed flowering (Aggarwal and Poehlman [1977](#page-20-0)). A higher yield can be realized from the crops grown under proper drainage conditions in sandy loam soil, while higher humidity and excessive rainfall may lead to several diseases and lower yields in mung bean (Oelke et al. [1990](#page-27-0)). The determinants influencing the crop duration in mung bean have been discussed by several workers (Robertson et al. [2002;](#page-28-0) Summerfield and Lawn [1987](#page-30-0)).

Mung bean has broad and trifoliate leaves that overlap horizontally bounding the light into the canopy. It has been noticed that the mung bean plants having narrow leaves capture maximum light and give comparatively higher yields (Lee et al. [2004\)](#page-26-0). Mung bean has epigeal germination, and cotyledons have to arise from the soil for the growth of the seedling. However, low-moisture conditions and crusting of soil under higher temperatures may limit this type of germination (Cook et al. [1995\)](#page-23-0), resulting in poor germination and simultaneously poor establishment (Harris et al. [2005\)](#page-24-0). Seedling vigour may be important under such conditions, but no relation could be noticed between seedling vigour and crop yield in mung bean (TeKrony and Egli [1991](#page-31-0)). A plant stand of about 30 plants under each square meter is considered significant to provide higher yields in mung bean (Rachaputi et al. [2015\)](#page-28-0).

The flowering and pod maturity in mung bean do not take place evenly, and differences between these two incidences are higher (Tah and Saxena [2009](#page-30-0)), leading to non-synchronous maturity and yield losses (Alam Mondal et al. [2011](#page-21-0)). Early and uniform maturity of a crop has a positive effect on the grain yield; however, this important characteristic is not known in the case of mung bean (Chen et al. [2008\)](#page-22-0). High-yielding, uniform-maturity and disease-resistant varieties are of choice for the successful cultivation of mung bean (Tomooka et al. [2005\)](#page-31-0), while low-yielding potential, poor harvest index and vulnerability to diseases and biotic and abiotic stresses (Srinives et al. [2007](#page-30-0)) are some of the major challenges in its cultivation. Wild species of mung bean may serve as a better genetic material as the cultivated germplasm may have lost many alleles during the process of domestication and/or breeding programmes (Hyten et al. [2006\)](#page-24-0). Therefore, beneficial alleles from uncultivated species have been accustomed to the crop improvement in mung bean (Nair et al. [2012](#page-27-0)).

5.5 Genetic Variability

Self-pollinated crops generally have composite floral structures and low natural variability. Therefore, the selection of such plants for crop improvement becomes difficult; nevertheless, estimation of the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability estimates and genetic advance

(GA) provides immense opportunity to choose better genotypes. Estimation of these variabilities reveals the influence of several gene effects operating towards total variability for the desired traits. Several studies reported the importance of GCV, PCV, heritability and GA in the improvement of traits in different crops (Denton and Nwangburuka [2011](#page-23-0); Johnson et al. [1955;](#page-24-0) Kim et al. [2015a\)](#page-25-0). Evaluation of phenotypic or genotypic variability offers better insight into the utilization of available germplasm resources (Bisht et al. [1998;](#page-22-0) Schafleitner et al. [2015](#page-29-0)). Wide variation in morphological traits, viz., number of pods per plant, yield per plant, 100-seed weight, fruit-setting capacity, flowering period, maturity, number of pod-bearing peduncles, plant height, primary branches, length of branches, nodule and leaf pattern, has been observed in mung bean (Bisht et al. [1998](#page-22-0)).

The study on the inheritance of narrow trifoliate leaves in mung bean revealed the inheritance of larger leaflets over smaller leaflets (Dwivedi and Singh [1985\)](#page-23-0). Lobed leaf shape was found dominant over the entire leaf shape, while pentafoliate leaf was reported to be an inherited characteristic in mung bean (Chhabra [1990\)](#page-22-0). The inheritance of dullness and shininess of leaf surface suggest that it is governed by a digenic interaction (Bhadra et al. [1991\)](#page-22-0) with the dominance of dullness over shininess. Inheritance of plant and flower bud colour in mung bean suggested that the dark purple colour of the plant was dominant over the green plant colour; similarly, purple flower buds showed dominance over the green flower buds (Khattak et al. [2000](#page-25-0)). It was found that black and green seed colour is governed by similar genes; however, black seed is dominant over green seeds (Chen et al. [2001](#page-22-0)). The occurrence of anthocyanin is a dominant character so is the black-colour seed coat over the green colour (Chen et al. [2001\)](#page-22-0). The study also revealed that the genes responsible for purple petiole and black seed colour have higher lineages. Single recessive gene was observed to control mung bean yellow mosaic virus resistance in the crop with susceptible behaviour being dominant over the resistant behaviour (Win et al. [2021\)](#page-32-0).

PCV and GCV along with heritability estimates provide an insight into the improvement of requisite characters (Burton and de Devane [1953\)](#page-22-0). Mung bean has been reported with higher PCV and GCV for seed yield and pod numbers (Makeen et al. [2007\)](#page-26-0), plant height, pod numbers and grain yield, while it was low with respect to days to 50% flowering (Anand and Anandhi [2016\)](#page-21-0). Primary branches, pod numbers, seed yield and clusters showed higher GCV and PCV in mung bean (Asari et al. [2019\)](#page-21-0). Higher PCV and GCV were reported for 100-seed weight, flowering period, seed length and seed breadth (Tripathi et al. [2020\)](#page-31-0). The number of pods, seed yield and number of clusters have been recorded with high PCV and GCV in mung bean (Salman et al. [2021\)](#page-29-0), suggesting the presence of higher variabilities for these traits, and therefore, there are more opportunities for further improvement using several genetic influences.

Heritability is the amount of phenotypic variance among different genotypes due to the effect of inherited genes. The estimation of heritability is done to find the similarity between the genotypes (Falconer and Mackay [2005\)](#page-23-0). It also explores the association between phenotypic and genotypic variance (Lourenço et al. [2017\)](#page-26-0). Heritability in combination with genetic advance gives better insight into the desired genotype (Nwangburuka and Denton [2012](#page-27-0)). The traits presenting higher heritability

along with higher genetic advance in mung bean may be enhanced by the selection method (Degefa et al. [2014](#page-23-0)) because these characteristics are under the influence of additive gene action. The influence of both additive and non-additive gene effects has been reported for several traits in mung bean (Khattak et al. [2002\)](#page-25-0). Days to first pod maturity (Khattak et al. [2001](#page-25-0)) and seed yield (Sharma [1999\)](#page-29-0) exhibited higher heritability. It has also been reported that the additive gene effect governs the seed yield in mung bean (Joseph and Santhoshkumar [2000](#page-24-0)).

The number of pods, plant height and test weight had a high value of heritability coupled with a higher genetic advance in mung bean, suggesting the influence of additive gene effect in their manifestation (Makeen et al. [2007\)](#page-26-0). Higher variability was recorded during the assessment of genetic diversity among yield-attributing traits comprised of 9 qualitative and 21 quantitative characters among 340 cultivated mung bean collections (Yimram et al. [2009](#page-32-0)). Several yield-attributing traits showed higher genetic variability and heritability (Yimram et al. [2009\)](#page-32-0). The number of seeds, seed yield and biomass yield exhibited higher heritability coupled with higher genetic advance, suggesting their importance in the selection of mung bean for better yield potentials (Degefa et al. [2014](#page-23-0)).

Genetic architecture of synchronous pod maturation and yield-related traits in mung bean were studied, and domination of additive and environmental components for days to flowering, pod maturation, synchrony in pod maturation and yield-related characters were recorded (Iqbal et al. [2014](#page-24-0)). The study suggested that inter-crossing of F2-generation plants having earliness and synchronized pod maturation along with high-yielding potential and their subsequent selection may be useful for manipulation of complex inherited characters in the development of mung bean lines for plant improvement (Iqbal et al. [2014](#page-24-0)). Seed yield, plant height and number of pods exhibited high values for heritability (Anand and Anandhi [2016](#page-21-0)).

High heritability coupled with higher genetic advance was reported for plant height, number of primary branches, number of clusters, number of pods and seed yield, signifying the dominance of additive gene action (Asari et al. [2019](#page-21-0)). Higher heritability was reported for seed dimension-related traits, days to 80% maturity, 100-seed weight, days to 50% flowering, pod length and days to initial maturity, suggesting that these traits are appropriate for mung bean breeding (Tripathi et al. [2020\)](#page-31-0). Pod numbers, seed yield, clusters, number of branches, seeds and height had high heritability coupled with high genetic advance, suggesting the influence of additive genes in the inheritance of these morphological characters (Salman et al. [2021\)](#page-29-0).

5.6 Mutation

Mutation is an unexpected genetic modification caused by variation in the gene sequences, leading to alteration in several plant characteristics including height, branches, flowers, pods, etc. It may occur naturally or may be induced artificially. The natural mutation is sudden, and its frequency is very low; therefore, it cannot be considered realistic. Hence, artificial methods of mutation were discovered to create

variability in the crops. The introduction of mutations has played an important role in the field of genetic studies and plant breeding (Raina et al. [2016](#page-28-0)). The mutation is considered a promising tool for evolution, and induced mutagenesis is an ideal methodology for the creation of required genetic variability in crops (Auti [2012;](#page-21-0) Dubinin [1962](#page-23-0)). It may be induced using physical and chemical mutagens either individually or in combination. Various physical and chemical mutagens have been recognized in various crops (Pathak [2015](#page-27-0); Shah et al. [2008](#page-29-0)). X-rays and gamma rays

are generally applied as physical mutagens, while ethyl methane sulphonate (EMS), diethyl sulphonate (DES), sodium azide (SA), methyl methane sulphonate (MMS), nitrosoguanidine (NG), nitroso-methyl urea (NMU), etc. are the chemical mutagens used for creating variability. The genetic material generated through mutagenesis and the mutants with better desired characteristics may be included in the breeding programmes. Several attempts have been undertaken to improve the genetic variability in mung bean using different mutation techniques. The variability lost during the adaptation or evolution of a crop can be refurbished or renewed with the help of induced mutations. Selection of morphologically varied mutants, viz., plant type, chlorophyll, leaf, flower and seed-type mutants, has enhanced genetic variability and showed higher level of resistance towards abiotic and biotic stresses (Mounika [2020\)](#page-27-0).

5.6.1 Mutations Induced Through Physical Factors

Physical mutagenesis is an effective method for creating variability for crop improvement in self-pollinated crops including mung bean (Sarkar and Kundagrami [2018;](#page-29-0) Shah et al. [2008\)](#page-29-0). Irradiation with ionizing or non-ionizing rays is used to induce physical mutation. It was started with X-rays, but at the later stage, gamma rays got more popular (Auerbach and Robson [1946\)](#page-21-0) due to better effects over plant growth and development by stimulating cytological, genetical, biochemical, physiological as well as morphological variabilities (Gunckel and Sparrow [1967](#page-24-0)). The influence of gamma irradiation on morphological and cytological changes in mung bean was recorded, wherein decreased seed germination, seedling survivability and growth rate were observed with increased doses of gamma rays (Subramanian [1980\)](#page-30-0). Dosage of 10–30 kR gamma rays was reported to be appropriate to obtain earliness, synchrony in the maturity and resistance towards yellow mosaic disease in mung bean (Singh and Chaturvedi [1982\)](#page-30-0). Substantial variability for the number of clusters was recorded with 10, 30 and 40 kR gamma radiation in different mung bean genotypes (Tah [2006](#page-30-0)), and a 16–20% increase was observed over the control. Mung bean varieties treated with 10–40 Gy gamma rays resulted in mutants having synchronous maturity (Tah and Saxena [2009\)](#page-30-0).

Gamma rays were applied to create synchrony in the pod maturity, and the obtained mutants exhibited synchronous pod maturity along with variegated leaves (Sangsiri et al. [2007](#page-29-0)). The shallow rooting system of high-yielding and MYMVresistant mung bean variety (Samrat) was improved using 450 Gy gamma rays, and a long-root mutant possessing a root length of 71 cm was identified in the M_2 generation (Dhole and Reddy [2010\)](#page-23-0). The mutant showed better performance in terms of water uptake as compared to 'Samrat' and survived better under drought conditions. Gamma rays (300, 400 and 500 Gy) and EMS (10, 20 and 30 mM) were applied to screen the yellow vein mosaic virus disease-resistant mutants in mung bean, and several disease-resistant mutants were identified in M3 generation (Vairam et al. [2016\)](#page-31-0). Gamma radiation was applied to advance the genetic constitution of mung bean, and 20 mutants from M5 progeny having an early maturing period and high yield potential were identified (Sarkar and Kundagrami [2018](#page-29-0)). Four doses of gamma rays (100, 200, 300 and 400 Gy) were applied to improve genetic variation in mung bean varieties, and mutants showing higher harvest index were isolated in M7 generation from 200 and 400 Gy dosages of gamma rays (Dewanjee and Sarkar [2018\)](#page-23-0). The mutants having potential characteristics may be released as a variety, or the potential character may be transformed in other varieties to get better yields in mung bean (Pratap et al. [2020](#page-28-0)).

5.6.2 Mutations Induced Through Chemical Factors

Mutations carried out by irradiation of ionizing rays may lead to chromosomal aberrations; therefore, chemical mutagens were taken as a substitute to create variabilities. Chemical mutagens have become more popular as no specific equipment is involved during their applications, and it is comparatively easy to induce. Compared to physical mutagens, it induced point mutations causing single base pair changes (Sikora et al. [2011](#page-30-0)). Two important groups of chemical mutagens, viz., alkylating agents and base analogues, are usually applied for creating mutations. However, out of these chemical mutagens, alkylating agents such as EMS and NMU are generally used to induce mutation in crops. Various chemical mutagens, viz., ethyl methane sulphonate (EMS), sodium azide (SA) and hydrazine hydrate (HZ), have been used in mung bean (Auti and Apparao [2009;](#page-21-0) Khan and Goyal [2009](#page-25-0); Wani [2006\)](#page-31-0). Variation in seed size of mung bean was observed when it was treated with EMS and nitroso-methyl carbamide (Singh and Chaturvedi [1982\)](#page-30-0). Higher seed yield, fertile branches and pods were reported in mung bean mutant lines acquired after the application of EMS and HZ (Wani [2006\)](#page-31-0). EMS induces mutations more efficiently in mung bean as compared to gamma rays (Singh and Rao [2007\)](#page-30-0). The crop duration in the M2 generation of mung bean was reduced with the help of SA mutagen (Lavanya et al. [2011](#page-26-0)).

5.6.3 Mutations Induced Through Physical and Chemical Factors

Physical and chemical mutagens individually have several advantages and induce random changes in the genome. However, the genetic variability induced by the combination of physical and chemical mutagens is comparatively more efficient, and the possibility of obtaining the required characteristics is significantly higher (Raina et al. [2017](#page-28-0)). A combination of lower doses of physical and chemical mutagen is more acceptable for artificial mutation (Medina et al. [2004\)](#page-26-0). Both effectiveness and efficacy are important parameters for mutagens. The effectiveness and efficacy give information regarding the rate of point mutations concerning dosage and other biological effects, respectively, induced by the mutagen (Konzak et al. [1965\)](#page-25-0). It relies upon the genotype and the mutagen. Varied effectiveness and efficiency of mutagens have been reported in several crops including mung bean (Wani et al. [2017](#page-31-0)). EMS and gamma rays were applied to create variability in mung bean and subsequently for the development of novel cultivars having higher yields and resistance towards insect pests (Khan and Goyal [2009;](#page-25-0) Wani [2006](#page-31-0)). Mung bean seeds treated with different concentrations of SA and EMS and different doses of gamma radiation were grown to study mutagenesis in mung bean (Auti and Apparao [2009\)](#page-21-0), and several viable morphological and physiological mutants were obtained.

Seeds of mung bean were treated with gamma rays (10–60 KR) and EMS $(0.1–0.4%)$ alone and in various combinations, and several chlorophyll and morphological mutants were identified in the M2 generation (Kumar et al. [2009\)](#page-26-0). Chlorophyll-deficient mutants are considered genetic markers and are used to study the photosynthesis process (Rungnoi et al. [2010](#page-29-0)). Maximum mutations were recorded with EMS followed by gamma rays and their combinations. Higher numbers of albina-, chlorina- and viridis-type chlorophyll mutants were observed with the treatment of EMS, MMS and SA in mung bean (Khan and Siddiqui [1993\)](#page-25-0). Similarly, albina, xantha, viridish, sectorial and chlorina mutants have also been recognized by Singh and Rao ([2007\)](#page-30-0) in mung bean. Chlorophyll mutation in mung bean has also been observed with gamma radiations and EMS alone and in its combinations (Kumar et al. [2009](#page-26-0)), wherein maximum frequency was recorded with EMS followed by gamma rays and their combinations. A higher number of chlorophyll mutation was observed when 300 Gy gamma rays were used in combination with 10 mM EMS in M2 generation (Vairam et al. [2016](#page-31-0)). Bifoliate, tetrafoliate and pentafoliate leaves have been reported in mung bean with the treatment of EMS (Auti and Apparao [2009](#page-21-0)). Mutation in flower colour has also been reported by various workers. Comb-like flowers having pollen sterility have been reported in mung bean upon mutation (Sangsiri et al. [2005](#page-29-0)). Variations in seed shape, seed size and seed colour were observed in mung bean mutants developed through treatment with gamma rays, EMS and SA (Auti and Apparao [2009\)](#page-21-0).

5.7 Genotype × Environment Interaction and Stability

Improvement in the quality and quantity of crops coupled with enhanced stability over the varied environmental conditions is the most important requirement in the breeding programme. The best varieties always have higher yields along with better stability (Eberhart and Russell [1966\)](#page-23-0). Genotype \times environment (G \times E) interaction suggests the variable responses of a trait of genotypes evaluated under different environments. It also reveals the comparative suitability of a genotype within a particular environmental situation (Allard [1960\)](#page-21-0). The genotype may acquire stability alone or may be due to the buffering effect of the population; however, the yield is

validated due to the effect of $G \times E$ interactions (Allard and Bradshaw [1964\)](#page-21-0). Nevertheless, the comparison of varieties in a chain of environments provides relatively different positions resulting in difficulties to identify superior varieties (Eberhart and Russell [1966\)](#page-23-0). The comparative performance of genotypes differs from one environment to another, and it can be articulated as a linear function of an environmental variable (Pathak [2015;](#page-27-0) Tan et al. [1979](#page-31-0)). Therefore, to assess the stability of a variety for the desired trait, an understanding of $G \times E$ interactions is essential. Stable varieties have great significance in several crops including mung bean for cultivation in variable environmental conditions (Verma et al. [2008\)](#page-31-0). Variable performance of a variety towards different environmental conditions compels to search novel breeding materials under multi-environmental trials for years to evaluate their stability for desired traits (Fehr [1987;](#page-23-0) Kang [1993](#page-25-0)). A decrease in the interactions between genotype and environment is necessary to find a stable genotype that has less interrelation with the environment wherein it is cultivated. Significance of genotypes upon environment and adaptation of varieties towards yield and yield-attributing traits with respect to stability has been thoroughly underlined by several workers in mung bean (Abbas et al. [2008](#page-20-0); Dwivedi [2006;](#page-23-0) Mahalingam et al. [2018](#page-26-0)). While highlighting the importance, it was suggested that the environment and $G \times E$ interactions must be considered during the designing and selection of materials for breeding in mung bean (Singh et al. [2009\)](#page-30-0).

Stable varieties of mung bean have been identified over the years under varied environmental conditions by several researchers (Abbas et al. [2008;](#page-20-0) Baraki et al. [2020;](#page-22-0) Raturi et al. [2012b](#page-28-0)), and the prominence of some genotypes over the environment was also observed (Mahalingam et al. [2018](#page-26-0)). The environment imposes a higher impact on several characteristics of mung bean including flowering time, pod formation as well as yields. Kamannavar and Vijaykumar ([2011\)](#page-25-0) assessed $G \times E$ interactions in mung bean cultivars grown in different agro-climatic zones and reported that genotype, environment and $G \times E$ interaction were significant for all the characters signifying the existence of variabilities for genotype and environment along with non-linear influence of genotypes over the environment. However, the partitioning of interaction into linear and non-linear components suggests the involvement of both predictable and unpredictable sources of variables. Non-significant $G \times E$ interaction was recorded for 100-seed weight, suggesting the variable response of genotypes towards variable environmental conditions (Revanappa and Kajjidoni [2004\)](#page-28-0). On the basis of stability analysis and their influences, Henry and Mathur ([2007\)](#page-24-0) categorized the genotypes for favourable, adverse and variable environmental conditions.

Raturi et al. ([2012a,](#page-28-0) [b\)](#page-28-0) reported significant $G \times E$ interactions for 1000-seed weight, days to 50% flowering, number of seeds per pod and number of primary branches revealing varied responses of genotypes to varied environments. Significant $G \times E$ interactions have been recorded for seed yield among genotypes of mung bean grown under varied environmental conditions (Baraki et al. [2020](#page-22-0)). A crossover $G \times E$ interaction is usually observed if genotypes are evaluated under multi-location trials. Studies suggest that the variation in the seed yield of mung bean due to $G \times E$ interactions is inherited, and the genotypes perform differently to the varied

environmental situations of the site of sowing (Baraki et al. [2020](#page-22-0); Waniale et al. [2014\)](#page-31-0). Therefore, mung bean genotypes may essentially be tested at multi-locations.

5.8 Correlation and Path Analysis

The morphophysiological characteristics of a genotype depend on several factors, and therefore, several aspects are taken into account during the selection of a genotype including the fact related to the association of characters and the influence of direct and indirect effects of each trait. Correlation provides the information with respect to the association between the traits, but it does not reveal the cause and/or consequence of association (Roy [2000\)](#page-29-0), while the path coefficient analysis gives a better insight into the influence of one trait on another during identification of a predictor variable (Akanda and Mundt [1996\)](#page-21-0). Thus, path analysis informs about the cause and reveals the comparative influence of the traits, while correlation analysis just provides reciprocal relation of traits (Dewey and Lu [1959](#page-23-0)).

The findings on correlation coefficient in mung bean recommend that a plant with more number of branches, clusters, pods and higher number of seeds in a pod is anticipated to provide higher seed yields. Thus, an increase in the number of branches and pods may be culminated into higher seed yield as branches bear pods and pods bear seeds. The association between seed yields was significantly positive with the number of branches, number of pods and total biomass in mung bean (Nawab et al. [2001](#page-25-0)), indicating the influence of these traits on the seed yield. The number of pods and plant height had a significantly positive association with seed yield (Makeen et al. [2007](#page-26-0); Upadhaya et al. [1980\)](#page-31-0); similarly, these traits along with test weight showed a maximum direct effect on the seed yield (Makeen et al. [2007\)](#page-26-0). A significant positive association was observed between seed yield and days to 50% flowering, primary branches, secondary branches, clusters, pods, pod length, seeds, pod mass, pod wall mass, seed mass, shelling percentage, seed and harvest index (Singh and Kumar [2014](#page-30-0)), suggesting that these traits may be useful for selecting genotypes for yield improvement in mung bean. Seed yield had highly significant and positive correlations with pods, clusters and seed numbers (Singh and Kumar [2014](#page-30-0)), whereas days to maturity had a negative association with seed yield. The study also showed that seed yield had no significant association with protein content.

Number of clusters and number of pods showed a significantly positive association with seed yield, suggesting that these are the most important components for crop improvement in mung bean (Anand and Anandhi [2016](#page-21-0); Asari et al. [2019\)](#page-21-0). Similarly, the study also revealed a positive and direct impact of days to 50% flowering, test weight, number of clusters, number of pods and number of primary branches on seed yield (Asari et al. [2019](#page-21-0)), suggesting that emphasis may be given on these traits during the crop improvement in mung bean. Seed weight was reported to be negatively associated with seed roundedness, days to first flowering, days to 50% flowering, flowering period and days to maturity (Tripathi et al. [2020](#page-31-0)), while pod length showed a positive correlation with seed weight, seed area and seed dimensions.

5.9 Genetic Divergence

Quantification of divergence within the characters required to be improved gives the understanding to find suitable parents for breeding programmes (Mahalanobis [1936\)](#page-26-0). It was suggested that the measurement of the metric distance between population centroids may help in the consideration of high-yielding parents having wider genetic divergence that are found beneficial in the development of highyielding hybrids (Murty and Arunachalam [1966](#page-27-0)). The analysis also measures the magnitude of divergence and simultaneously provides an understanding of the evolutionary patterns in terms of the comparative influence of various traits on the entire divergence functioning at intra- and inter-cluster levels. Genetic divergence studies help in the identification of suitable parents for hybridization during crop improvement (Mohammadi and Prasanna [2003](#page-27-0)) as the involvement of genetically different parents brings gene constellation in the progressive generations.

Several studies have been carried out to find the nature and extent of genetic divergence in mung bean using Mahalanobis D^2 statistics (Goyal et al. [2021;](#page-23-0) Rahim et al. [2010;](#page-28-0) Ramana and Singh [1987;](#page-28-0) Ramanujam et al. [1974](#page-28-0); Sen and De [2017](#page-29-0)), and it was concluded that the genotypes grouped in different clusters with higher statistical distances may be utilized in the hybridization programmes for crop improvement in mung bean. The comparative influence of each character on the total genetic divergence, the clusters having the highest statistical distance and the collection of at least one genotype from such clusters are some of the most significant points for the identification of parents using D^2 statistics. It has been observed that there is no relation between geographic and genetic diversity in mung bean (Naidu and Satyanarayana [1991;](#page-27-0) Raje and Rao [2000;](#page-28-0) Tripathi et al. [2020](#page-31-0)).

5.10 Plant Protection

Mung bean is susceptible to several viral, bacterial and fungal diseases leading to major economic losses to the crop (Mbeyagala et al. [2017;](#page-26-0) Pandey et al. [2018](#page-27-0); Singh et al. [2000](#page-30-0)). Cercospora leaf spot, powdery mildew, anthracnose, dry root rot, web blight, fusarium wilt and Alternaria leaf spot are major fungal diseases (Pandey et al. [2018\)](#page-27-0); halo blight, bacterial leaf spot and tan spot are the important bacterial diseases; while mung bean yellow mosaic disease (MYMD) is a major viral disease (Nair et al. [2017\)](#page-27-0) found in mung bean. Maximum yield losses in mung bean have been reported due to MYMD (Karthikeyan et al. [2014](#page-25-0)) followed by several fungal diseases (Bhat et al. [2014](#page-22-0); Maheshwari and Krishna [2013;](#page-26-0) Shukla et al. [2014\)](#page-30-0). Effect of several bactericides and fungicides in the seed treatment and foliar spray along with the influence of good agronomic practices have been reported to combat these infections (Pandey et al. [2018\)](#page-27-0). The use of disease-resistant varieties and the

employment of integrated disease management are the best cost-effective ways to control the incidence of diseases in mung bean.

5.10.1 Viral Diseases

Mung bean yellow mosaic virus (MYMV) is a major threat to mung bean cultivation. The reference genome of this virus is available (Morinaga et al. [1993\)](#page-27-0). The virus is comprised of two DNAs of about 2.7 kb. There are several views concerning genetic resistance associated with MYMV. It was suggested that it is controlled by a solo recessive gene (Reddy [2009\)](#page-28-0), a dominant gene (Sandhu et al. [1985](#page-29-0)), while others reported that it is controlled by two recessive genes and a complementary recessive gene (Ammavasai et al. [2004](#page-21-0); Dhole and Reddy [2012](#page-23-0); Pal et al. [1991\)](#page-27-0). The infected plant shows yellow-coloured spots on the young leaves that become yellow mosaic shape in the later stage, and simultaneously drooping of leaves takes place after the entire yellowing and drying of the leaves. Presently, fully resistant varieties to MYMV are unavailable. However, resistant varieties exhibit high variability and depend on climatic conditions (Nair et al. 2017) as the virus is transmitted through whitefly. The occurrence, distribution and transmission of this vector are well known that may help to cope with the spread of the virus. The variation in pathogen because of several other factors makes its control more cumbersome (Alam et al. [2014](#page-21-0)).

5.10.2 Fungal Diseases

Cercospora leaf spot (CLS) disease caused by fungus Cercospora canescens is one of the important foliar diseases in mung bean. The disease may reduce the yield up to 40%. There is chaos on the genetic basis of CLS-resistant gene, whether it is monogenic or multigenic. It has been reported that CLS resistance is governed by a single dominant gene (Lee [1980](#page-26-0)); besides this, studies also suggest the presence of quantitative genetic control (Chankaew et al. [2011\)](#page-22-0) and a single recessive gene influence (Mishra et al. [1988](#page-26-0)) in respect to CLS resistance in mung bean. Variability among *C. canescens* strains is a major problem in crop breeding as it varies in the same region and within the same host including mung bean. Variable mycelial characteristics have also been reported with CLS (Joshi et al. [2006](#page-24-0)).

5.10.3 Bacterial Diseases

Blight caused by Xanthomonas axonopodis is a distressing bacterial disease in mung bean. Seeds are the primary source of bacteria, and therefore proper treatment of seeds before sowing is the best practice to control the disease (Baker and Smith [1966\)](#page-21-0). A bacterial disease showing symptoms of marginal and veinal necrosis of leaves caused by *Curtobacterium flaccumfaciens* subsp. *flaccumfaciens* has been reported (Wood and Easdown [1990\)](#page-32-0). The pathogen does not cause any wilting. The disease can be generally seen in rainfed crops suffering with water stress. Another bacterial disease showing the symptoms of necrotic spots on the leaves and collapsing of the upper part of the stem was observed in mung bean, and it was reported that the disease is caused by Pseudomonas syringae pv. syringae (George and Tripepi [1990\)](#page-23-0). Besides this, irregular necrotic spots encircled with slender chlorotic and water-soaked radiance are seen on the leaves of mung bean that may result in blight. The disease is caused by X. *axonopodis* pv. *phaseoli* and may lead to severe loss to the crop (Osdaghi [2014](#page-27-0)). Necrotic spots surrounded with yellow halo caused by P. syringae pv. phaseolicola have been observed in China (Sun et al. [2017\)](#page-30-0). A foliar disease caused by P. syringae pv. tabaci showing resemblance to wildfire has also been reported in mung bean (Sun et al. [2017\)](#page-30-0). The disease initially appears in the form of small rounded light green patches that becomes brown from the centre during later stage due to necrosis of parenchymatic tissues. The necrosis proceeds quickly, and the brown spot encircled with watery lesion increases in length and width. The severity of infection may lead to deformation and drooping of leaves.

5.10.4 Nematodes

Nematodes have destructive effect on agriculture. Several nematodes, viz., Rotylenchulus reniformis, Meloidogyne incognita, Bitylenchus vulgaris, Basirolaimus indicus, B. seinhorsti, Helicotylenchus indicus, H. retusus, Tylenchorhynchus mashhoodi and Tylenchus sp., have been reported to infest mung bean (Ali [1995\)](#page-21-0). Heterodera vigni is also known to infect mung bean crops, resulting in higher yield loss and dry matter content. Population-monitoring system (Saxena and Reddy [1987](#page-29-0)) and oil extracted from herbs (Sangwan et al. [1990;](#page-29-0) Siddiqui and Mahmood [1996\)](#page-30-0) are considered better approaches to getting rid of nematodes in mung bean.

5.10.5 Insect Pests

Several insect pests are known to infest mung bean from its sowing to storage and lead to severe yield losses. Some of the insect pests found on mung bean are stem fly, thrips, aphids, whitefly, pod borer complex, pod bugs and bruchids (Swaminathan et al. [2012\)](#page-30-0). They may directly attack the crop or work as vectors of diseases. Bean fly (Ophiomyia phaseoli) is the important pest found on mung bean. Besides O. phaseoli, other species of bean flies such as Melanagromyza sojae and O. centrosematis also infest mung bean crops (Talekar [1990\)](#page-30-0). The flies attack the crop within a week after the germination, and under severe conditions, it may lead to complete loss of the crop (Chiang and Talekar [1980\)](#page-22-0). Whitefly (Bemisia tabaci) is another pest that affects the crop directly and indirectly. It feeds on phloem and excretes honeydew on the plant that becomes black sooty moulds; besides this, it is the well-known vector of MYMV. Thrips also infest the crop at different stages. Several thrips, i.e., seedling thrips (Thrips palmi and Thrips tabaci) and flowering

thrips (Caliothrips indicus or Megalurothrips spp.), are found on the crop. Spotted pod borer (Maruca vitrata) is also an important pest found on mung bean crops grown in tropical and subtropical regions. The larvae of this pod borer attack the flower, stem, peduncle and pod of mung bean (Sharma [1999\)](#page-29-0). Azuki bean weevil (Callosobruchus chinensis) and cowpea weevil (Callosobruchus maculatus) are some of the most serious pests of mung bean in the field, while bruchids are the serious pests found in storage conditions (Somta et al. [2007;](#page-30-0) Tomooka et al. [1992\)](#page-31-0).

5.11 Physiology and Abiotic Stresses

Abiotic stresses have an adverse effect on plant growth and productivity, leading to major economic losses (Ye et al. [2017\)](#page-32-0). These stresses may include several atmospheric issues along with drought, flooding, radiation, salinity, temperature, etc. The effect of climatic aberrations over the periods also reduced crop yields (Boyer et al. [2013;](#page-22-0) Rosenzweig et al. [2014\)](#page-28-0). Mung bean is highly sensitive to salinity, drought and fluctuating temperatures during the flowering and pod formation stages, leading to severe yield losses. Understanding of physiological limits influencing the seed yield in mung bean is critical, and it should be properly identified before devising solutions.

5.11.1 Water Stress and Drought

Mung bean is generally grown under limited soil moisture conditions and does not require any additional input. Nevertheless, its growth is highly influenced by the availability of moisture in the field. However, it is highly susceptible to waterlogging conditions (Singh and Singh [2011\)](#page-30-0). It was observed that water stress during the flowering stage resulted in 50–60% yield reduction (El Nakhlawy et al. [2018\)](#page-23-0) in mung bean, and the study also revealed that seed formation was the most sensitive stage to water stress. Further, studies also suggest that the extreme drought conditions may lead to a reduction of plant biomass, pod numbers and consequently great toll on seed yield (Kumar and Sharma [2009\)](#page-25-0). A decline in the pace of pod initiation, its development (Begg [1980](#page-22-0)) and flower shedding (Moradi et al. [2009](#page-27-0)) are the significant impacts of water stress during the reproductive growth of the crop. Drought condition during the reproductive stage has a negative effect on flowering and simultaneously leads to a reduction in the yield (Raza et al. [2012](#page-28-0)).

Drought conditions during flowering and podding stages may lead to 31–57% and 26% yield reduction, respectively (Nadeem et al. [2019\)](#page-27-0). Drought condition leads to the production of destructive superoxide molecules that damages cells, and this oxidative stress depends mainly upon the level of ascorbic acid and glutathione pools (Anjum et al. [2015](#page-21-0)). Heat and cold stress are highly dangerous to different growth stages and may result in higher yield losses. The optimum temperature for plant growth is 28–30 °C. Higher temperatures (>45 °C) during the flowering stage may lead to flower shedding. Several developmental stages of mung bean including germination, seed emergence, vegetative phases, flowering stage and pod/seed setting stage are highly sensitive to temperature extremity (HanumanthaRao et al. [2016\)](#page-24-0). Crops grown during February or March months face major problems of water stress due to insufficient or no rainfall; hence, sowing of short-duration varieties may be preferred to avoid the stress (Pratap et al. [2013](#page-28-0)). Mung bean varieties/lines having tolerance against several abiotic stresses, viz., drought, heat and salt, have been identified over the period (Bindumadhava et al. [2018](#page-22-0); Dutta et al. [2016](#page-23-0); Dutta and Bera [2008](#page-23-0); Manasa et al. [2017](#page-26-0); Sharma et al. [2016](#page-29-0)).

5.11.2 Salt Stress

Salt stress adversely affects seed germination, biomass and shoot and root growth along with several yield-attributing traits (Ahmed [2009](#page-21-0); Promila and Kumar [2000\)](#page-28-0). Lesser seed germination was observed in mung bean with the increasing salinity levels (Kandil et al. [2012;](#page-25-0) Maliwal and Paliwal [1982](#page-26-0)). It may be due to the fact that salinity evades water uptake or causes toxic effects, resulting in a reduction of seed germination (Murillo-Amador et al. [2002\)](#page-27-0). Salt stress is usually exhibited as a general stunning of plant growth. Symptoms of salt injury such as chlorosis and necrosis have also been reported in mung bean due to increased levels of salinity (Reddy [1982](#page-28-0); Wahid et al. [2004](#page-31-0)). Significant variability was observed for growth, yield, yield components and chemical composition in mung bean seeds under different salinity levels (Mohamed and El-Kramany [2005](#page-27-0)). Mung bean plants have been reported to have higher proline content in the root and shoot due to increased salinity and or salinity stress (Misra and Gupta [2006](#page-26-0)). It was suggested that salt stress may affect the filling of seeds in the pods of mung bean, leading to a reduction in the number of seeds in the pods and simultaneously a reduction in the yield potential (Ahmed [2009\)](#page-21-0). Yield variability in mung bean upon salt stress has also been noticed by various workers (Hossain et al. [2008;](#page-24-0) Jahan et al. [2020](#page-24-0)). It has been observed that salt-stressed plants of mung bean had a higher concentration of sodium and chloride ions in their leaves, roots and shoots and a lower concentration of potassium and calcium ions as compared to the non-stressed plants (Mohammed [2007](#page-27-0)). Owing to this condition, the electrolyte leakage in mung bean was comparatively higher (Alharby et al. [2019\)](#page-21-0). A decrease in seed germination, plant height, shoot and root length, dry matter, biomass, and root, stem and leaf weights has been reported in mung bean due to an increase in salt stress (Mohamed and El-Kramany [2005;](#page-27-0) Mohammed [2007\)](#page-27-0). It has been observed that 50 mM NaCl significantly affected the yield of mung bean (Saha et al. [2010](#page-29-0)). Accumulation of a higher quantity of salt leads to a reduction of the osmotic ability of soil sap, resulting in water stress in plants and consequently nutritive deficiency and oxidative stresses (Tavakkoli et al. [2011\)](#page-31-0) along with reduction in photosynthesis rate. This may also stimulate physiological and metabolic pathways (Misra and Dwivedi [2004\)](#page-26-0) of the cells. Reduction in root length due to salt stress impedes the uptake and supply of nutrients. Number of nodules also reduced with the increase in salinity; however, their size increased due to salinity (Naher and Alam [2010](#page-27-0)). Pre-treatment of mung bean with sub-lethal

dosage of sodium chloride may help in adaptation of the crop to the lethal levels of salinity (Saha et al. [2010\)](#page-29-0). There is comparatively little work available on the development of salt-tolerant varieties of mung bean. Decline in relative water content, cellular dehydration and osmotic stress have been observed in mung bean due to salt stress (Singh et al. [2021\)](#page-30-0). The biometric, morphophysiological, biochemical and biophysical characters in mung bean were highly affected due to salt stress (Kumar et al. [2012\)](#page-26-0). It suggests that salt stress imposes water insufficiency in plants and may cause physiological drought. It has been reported that salinity tolerance depends on the genotype and different growth stages; hence, salt tolerance at seedling stage may not suggest that it may show tolerance at maturity stage (Sehrawat et al. [2013\)](#page-29-0). Salinity has different responses in the plant, which can be manifested at tissue, canopy, physiological or molecular level (HanumanthaRao et al. [2016](#page-24-0)).

5.11.3 Other Abiotic Stresses

Rising application of synthetic fertilizers and higher human interference along with the mixing of contaminated industrial effluents have deteriorated the cultivated land, and indirectly the crops are grown on it. The water or air pollutants are significant threats to crop cultivation as they have a higher concentration of heavy metals (Lagerverff and Specht [1970](#page-26-0)). The metal accretion in the soil is increasing continuously due to uncontrolled usage of fertilizers, pesticides, industrial waste and sewage (Harland et al. [2000\)](#page-24-0). Soil pollution due to heavy metals is very hazardous because heavy metals cannot be despoiled naturally and may remain in the ecosystem for a longer time and simultaneously in the food chain (Igwe et al. [2005\)](#page-24-0). Lethal impacts of heavy metals have been observed on the soil microflora (Pawlowska and Charvat [2004\)](#page-27-0) along with amendment of the variability, quantity and entire activity of the microbial communities (Smejkalova et al. [2003\)](#page-30-0). Besides heavy metal contamination, air pollution has higher concentration of sulphur dioxide, nitrogen dioxide and ozone, which also have deleterious effects on biomass, seed quality and yield potential of crops including mung bean (Agrawal et al. [2003,](#page-20-0) [2006\)](#page-20-0). The toxic effect of heavy metals on mung bean seed germination was studied, and delayed germination was observed with a higher concentration of lead (Ashraf and Ali [2007](#page-21-0)). The study also suggests that silver was more toxic followed by lead and zinc. A decrease in the biomass and quality of seeds was reported due to air pollutants such as sulphur dioxide, nitrogen dioxide and ozone in mung bean (Agrawal et al. [2006](#page-20-0)). Heavy metal nickel adversely influences the photosynthetic pigments and yield in mung bean (Ahmad et al. [2007](#page-21-0)). It also supports the deposition of sodium, potassium and calcium ions.

An increased level of proline in the plant is suggestive of abiotic stress. The level of proline was tested in mung bean under cadmium, cobalt, lead and zinc stress (Saradhi [1991](#page-29-0)), and cadmium was found as the most poisonous metal triggering proline production. Cadmium increases glutathione reductase activity (Gill and Tuteja [2010\)](#page-23-0), inhibits photosynthetic activity (Wahid et al. [2008\)](#page-31-0) and affects the

activity and structure of chloroplast (Wahid et al. [2007\)](#page-31-0) in mung bean. Cadmium and lead induce changes in growth, biochemical attributes and mineral accumulation (Ashraf et al. [2016](#page-21-0)), while mercury induces changes in germination and biochemical attributes (Saminathan [2013\)](#page-29-0) in mung bean, suggesting that heavy metalcontaminated soil exhibits negative impacts on the development, production and protein content in the crop. Sharma et al. ([2021\)](#page-29-0) observed that cadmium had amended several morphological and biochemical characteristics of mung bean. It also affected the chlorophyll, carbohydrate, protein, polyphenol and antioxidant profile of the crop.

5.12 Tissue Culture and Genetic Transformation

The development of plants through the tissue culture technique permits the transfer of genes into plant cells (Chandra and Pental [2003\)](#page-22-0). The transgenic exploration in mung bean is sluggish owing to its recalcitrant behaviour towards tissue culture and lower frequency of regeneration after transformation (Eapen [2008;](#page-23-0) Varshney et al. [2015\)](#page-31-0). However, regeneration protocols for mung bean have been developed through embryogenesis (Sivakumar et al. [2010](#page-30-0)), organogenesis (Himabindu et al. [2014\)](#page-24-0) and axillary bud proliferation using cotyledonary node explants (Sagare and Mohanty [2015;](#page-29-0) Yadav et al. [2010\)](#page-32-0). Successful transformation in mung bean has also been reported in which transgenes were effectively inherited and conceded to the following generations (Baloda and Madanpotra [2017](#page-21-0)).

Genetic transformation in mung bean was initially carried out in hypocotyls and primary leaves (Jaiwal et al. [2001\)](#page-24-0), and a binary vector (selection marker: neomycin phosphotransferase and reporter gene: beta-glucuronidases) was successfully incorporated. Later, Saini et al. [\(2007](#page-29-0)) developed morphologically normal and fertile transgenic plants of mung bean comprising two transgenes, bialaphos resistance and alpha-amylase inhibitor, using cotyledonary node explants. A pathogenesis-related gene (bjnpr1) isolated from mustard was introduced into mung bean, and it was observed that the transgenic mung bean plants exhibited resistance against fungal diseases (Vijayan and Kirti [2012\)](#page-31-0). Similarly, annexin1bj gene was successfully incorporated into mung bean, and the consequently developed transgenic plants revealed better tolerance against drought stress (Yadav et al. [2012\)](#page-32-0). Transformation of mung bean plants for salt and drought tolerance was carried out by introducing a gene for an osmoprotectant glycine betaine (Saraswat et al. [2017\)](#page-29-0), and transformation and expression of the transgene (codA gene) were realized. Modification in the DNA structure of food crops is usually unacceptable; therefore, genetically engineered food crops have always been viewed with a question mark despite several advantages.

5.13 Genetic Markers and Biotechnology

Several molecular markers, viz., restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, have been employed to study the genetic diversity in mung bean. The molecular markers have also been used for the construction of linkage maps focusing on yield, nutritional aspects and disease resistance.

Mung bean has a smaller $(\sim 600 \text{ Mb})$ genome and takes a lesser period to complete its life cycle; therefore, it is comparatively more suitable to apply other approaches for crop improvement. It was observed that maximum genes found in mung bean showed synteny with the genes found in soybean (Kang et al. [2014\)](#page-25-0). Vigna radiata variety VC1973A was genetically sequenced, transcriptome sequences of 22 accessions were obtained (Kang et al. [2014\)](#page-25-0) and relatedness of two homologous genomes of V. reflex-pilosa (a wild species) was outlined. The study enhanced the understanding of the evolution of *Vigna* species that may enable crop improvement in mung bean. The molecular markers facilitate the identification of loci linked to the desirable characteristics, and their tracking is more accurate and effective as compared to traditional breeding (Collard and Mackill [2008\)](#page-23-0).

Bruchid resistance in mung bean was analysed using RFLP markers (Young et al. [1992\)](#page-32-0), and 153 RFLP markers were categorized into 14 linkage groups having an average interval of 9.3 cM. Further, RFLP markers were used to prepare a linkage map of mung bean comprising 11 linkage groups, and an interspecific hybrid population between V. radiata ssp. radiata and V. radiata ssp. sublobata was obtained (Menancio-Hautea et al. [1992](#page-26-0)). Humphry et al. ([2002\)](#page-24-0) exploited RFLP markers to construct a genetic map using recombinant inbred populations of 80 mung bean accessions derived from a cultivated variety and *V. radiata* subsp. sublobata. The map included 13 linkage groups with an average distance of 3 cM, and a highly conserved marker order was reported between mung bean and *Lablab* purpureus. Transfer of bruchid beetle resistance allele (Somta et al. [2008](#page-30-0); Tomooka et al. [1992](#page-31-0)) and yellow mosaic disease resistance allele (Basak et al. [2005;](#page-22-0) Gill et al. [1983\)](#page-23-0) from wild mung bean is an example of marker-assisted breeding in mung bean.

RAPD markers were applied to assess the genetic diversity among uncultivated and cultivated Vigna species, namely V. angularis, V. umbellata, V. radiata, V. aconitifolia and V. mungo (Kaga et al. [1996\)](#page-25-0). A genetic map was prepared using RFLP and RAPD markers using F2 populations obtained by crossing V. radiata ssp. radiata and V. radiata ssp. sublobata. Lambrides et al. [\(2000](#page-26-0)) grouped all the 67 accessions in 12 linkage groups having 691.7 cM intervals. Kaga and Ishimoto [\(1998](#page-25-0)) also used RFLP and RAPD markers to prepare a linkage map and identified the genes accountable for bruchid resistance. Genetic maps showing the information on several morphophysiological and agronomic traits of cultivated and wild accessions of mung bean have been constructed (Isemura et al. [2012;](#page-24-0) Wang et al. [2016\)](#page-31-0) that will facilitate the understanding of important traits of interest in both cultivated and wild mung bean accessions. RAPD and inter-simple

sequence repeat (ISSR) markers were used to assess genetic diversity in mung bean germplasm (Chattopadhyay et al. [2005\)](#page-22-0), wherein ISSR markers were found to be competent as compared to RAPD markers. Yu et al. [\(1999\)](#page-32-0) employed simple sequence repeat (SSR) to assess microsatellite efficacy as genetic markers in mung bean and 61 simple repetitive DNA sequences having 23 motifs were recognized as prospective microsatellites.

Mung bean gene pools comprising 415 cultivated, 189 wild and 11 intermediate accessions were assessed to study the presence of genetic diversity using 19 SSR markers (Sangiri et al. [2008](#page-29-0)), and wide polymorphism was recorded among wild and cultivated pools. The study suggested that Australia and New Guinea were the diversity core for wild mung bean. In view of the higher diversity in mung bean accessions from South Asia, it was suggested that the crop may have been domesticated in South Asia (Sangiri et al. [2008](#page-29-0)). SSR markers linked to Cercospora leaf spot (Yundaeng et al. [2021\)](#page-32-0) and powdery mildew diseases (Chankaew et al. [2013;](#page-22-0) Kasettranan et al. [2010](#page-25-0)) have been identified, and quantitative trait loci (QTL) maps were prepared using these markers. A genetic linkage map was constructed, and a genetic analysis of domestication-related traits in mung bean was done using 430 SSR and EST-SSR markers (Isemura et al. [2012](#page-24-0)). The markers were grouped into 11 linkage groups with a total distance of 727.6 cM, and 105 QTLs including 38 domestication-related gene traits were distinguished. The study also revealed some useful QTLs for seed size, pod dehiscence and pod maturity in mung bean.

With the developments in next-generation sequencing, the attention of researchers has shifted to finding single nucleotide polymorphisms (SNPs). SNP markers are biallelic, codominant and universally distributed across the entire genome (Brumfield et al. [2003\)](#page-22-0). Mung bean cultivars were sequenced to search for resistance to Riptortus clavatus and Callosobruchus chinensis (Moe et al. [2011\)](#page-26-0), and 2098 SNPs were reported. Raturi et al. [\(2012a\)](#page-28-0) characterized 44 genotypes of mung bean based on nuclear ribosomal DNA and RAPD polymorphism to assess the genetic diversity and relationships and reported 82% polymorphism with wide intraspecific variations. The study also revealed internal transcribed spacer (ITS) length variations, SNPs and insertions/deletions at the number of sites in nuclear rDNA region. Genome sequence of mung bean and its comprehensions into evolution within Vigna species were carried out (Kang et al. [2014](#page-25-0)), and genomic evidence of allopolyploid event was reported on the basis of de novo assembly of a tetraploid Vigna species (V. reflexo-pilosa var. glabra).

EST-based SSR markers have been exploited to study functional genomics in mung bean (Chavan and Gacche [2014](#page-22-0); Chen et al. [2015](#page-22-0); Moe et al. [2011](#page-26-0)). SSR motifs were recognized in 1848 EST sequences in mung bean, and it was observed that about 45% and 55% of these motifs were situated in coding and untranslated regions, respectively (Moe et al. [2011\)](#page-26-0). Biotin-labelled oligo-probes and streptavidin-coated beads were applied to prepare an SSR-enriched library from mung bean genotypes, and 308,509 SSR motifs were identified (Wang et al. [2016\)](#page-31-0). Illumina paired-end sequencing technology was used for transcriptome sequencing of mung bean genes, and identification of EST-SSR markers (Chen et al. [2015](#page-22-0)) and more than 103 million high-quality cDNA sequences was done.

Mung bean genome has been characterized using translational genomics to obtain genomic information from well-studied species (Isemura et al. [2012;](#page-24-0) Kim et al. [2014\)](#page-25-0). The flowering gene in mung bean was recognized with the help of genomewide evaluation between mung bean and *Arabidopsis*. It was observed that out of 207 genes that were related to flowering in Arabidopsis, 129 were homologous to mung bean genes (Kim et al. [2015b](#page-25-0)). In another study, it was also observed that these genes were near to the SSR markers on a genetic map (Isemura et al. [2012](#page-24-0)). Mung bean genome was also compared to the soybean genome, and it was noticed that five flowering-related genes in mung bean were homologous to soybean flowering genes (Kim et al. [2015b](#page-25-0)). The studies may lead to the functional characterization of genes of interest in mung bean. Application of several biotechnological tools may facilitate the introduction of beneficial genes in promising mung bean lines to increase genetic variability.

5.14 Conclusion and Prospects

Being an important leguminous crop owing to its high nutritional contents, several studies have been carried out in mung bean addressing yield-related traits including resistance to different diseases and domestication-related traits. The lack of genomic information has led to stagnation in mung bean breeding. However, after the publication of the reference genome sequence of mung bean in 2014, breeders have got a better opportunity to understand the genomic and genetic background of several agronomically important traits of the crop. Preparation of wild mung bean pool from diverse origins and environmental conditions is essentially required to conserve the genetic diversity of the crop. The yield of more than 20 quintals per hectare, maturity period between 60 and 75 days, higher harvest index, photoperiod insensitivity, resistance to major insect pests/diseases, compact canopy and synchronous maturity are some of the important objectives for crop improvement in mung bean. The inclusion of the ideotype approach may also be considered to attain sustainable yield.

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