# **Chapter 5 Towards Development of Climate Smart Mungbean: Challenges and Opportunities**



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**Abstract** Mungbean is a quantitative short-day plant and grown across environments, locations, and seasons. It has inherent intrinsic tolerance mechanisms to many of the environmental stresses. However, being grown so widely, suffers from high temperatures, terminal moisture stress, soil salinity, and photo-thermo period sensitivity. Significant advancements have been made in the past 3-4 decades towards the development of input responsive, high yielding, disease-resistant, and short-duration varieties in mungbean. However, breeding for abiotic stress resistance has largely remained untouched and consequently, these pose serious constraints to mungbean production. Abiotic stresses such as heat, drought, salinity, etc. have deleterious effects on the morphology, physiology, and reproductive ability of the plants and ultimately reduce their plasticity and adaptation to changing climates, thereby affecting the quality and quantity of the produce significantly. Ample genetic and genomic resources are now available in mungbean and related Vigna crops, which can be exploited for the development of climate smart mungbean cultivars. Through various breeding approaches, climate smart traits can be incorporated in mungbean which will lead them to adapt to changing climate and perform well across environments. This chapter focuses on the development of climate smart mungbean and highlights gaps which need to be filled to this effect.

**Keywords** *Vigna radiata* · Climate resilience · Genomic resources · Genetic transformation · Biotic stresses · Abiotic stresses

# 5.1 Introduction

More than a dozen pulse crops are grown globally which form an integral part of cropping systems in many countries and offer great significance in sustainability of cereal-based agriculture. Besides, these ensure food and nutritional security in pre-

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dominantly vegetarian countries like India. Among all pulses, mungbean or green gram (Vigna radiata (L.) Wilczek) has a unique position due to its shorter life cycle, high per day productivity and its use in numerous food preparations (Singh et al. 2017). It has a wider adaptability being cultivated in spring, summer and rainy seasons and low input requirements having an inherent ability to fix the atmospheric nitrogen in symbiotic association with *Rhizobium* bacteria. While mungbean is cultivated since ancient times in India, it is also widely grown in Southeast Asia and also in Africa, South America and Australia. In Australia, mungbean cultivation started in 1930s primarily for forage use and as a green manure crop to improve soil fertility in cereal-based farming systems (Chauhan and Williams 2018). In India alone, >4.0 million ha area is currently under cultivation of this crop and a phenomenal increase has been observed in area, production as well as productivity in the last five decades. The total area under this crop increased from 1.84 million ha in 1965–1966 to 4.50 million ha in 2016–2017 while the production increased from 0.55 to 2.83 million tons during the same period (Project Coordinator, MULLaRP Report 2018). With increased irrigation facilities through new irrigation projects, remunerative prices and availability of short-duration cultivars, this crop now occupies considerable area during spring and summer seasons in several parts of India (Gupta and Pratap 2016). Simultaneously, development of new cultivars with shorter maturity duration (60-65 days), high yield (1.0-1.5 t/ha), photo-thermo period insensitivity, synchronous maturity, and resistance to Mungbean Yellow Mosaic disease during the last two decades has promoted mungbean cultivation in summer season (Gupta and Pratap 2016). Summer mungbean can ideally be grown in irrigated tracts of northern and eastern India as catch-cum-cash crop diversifying rice-wheat system, canal command areas of Gujarat and Madhya Pradesh as a bonus crop and new delta areas of Cauvery basin of Tamil Nadu sandwiched between two rice crops. Success in summer cultivation has not only increased the mungbean production but also helped in defeating malnutrition, crop diversification, sustaining agricultural production and increasing household income of poor farmers of India.

Nevertheless, with an expansion in mungbean area in different climates, challenges have also increased manifolds towards the development of widely adaptable climate smart varieties which can perform well across seasons and environments. Despite an average yield potential of >1.2 t/ha for most of the released mungbean varieties, the average productivity is still < 0.7 t/ha in India and < 1.0 t/ha in several other mungbean growing countries. The yield gap is not likely due to subsistence farming but due to several biotic and abiotic factors including insect pests, diseases, and postharvest losses as well as heat, drought, salinity and photoperiod sensitivity. Mungbean Yellow Mosaic Virus and leaf crinkle during Kharif season in northern part of India and powdery mildew in southern coastal part of India during winter season cause considerable losses. Among the insect-pests, defoliators such as hairy caterpillars, semilooper and sucking pests such as thrips and aphids are common. Activity of thrips starts at the bud stage and poses serious problem when the crop is in flowering stage, resulting in considerable flower drop. Thrips also transmit Groundnut Bud Necrosis Virus (GBNV). To make mungbean a remunerative crop, such varieties are required to be developed which can perform reasonably well even

in unfavorable climates. Designing new plant types which are resilient to changes in climate and can perform uniformly well across a series of environments will solve the problems of food and nutritional security.

## 5.2 Climate Change and Mungbean

The present trend of climate change indicates drought or drought-like situations occurring more frequently and rainfed agriculture is expected to suffer most as a result of water crisis due to delayed monsoon, uneven distribution and above all, complete failure of rains as a result of climate change (Singh et al. 2013). Reduction in yields is predicted to be more pronounced when drought and high temperature will interact together, and the damaging effects of both the stresses will be far more severe than their individual effects. Among pulses, mungbean being a warm season crop and grown under irrigated conditions is likely to be affected less by climate change due to its relatively higher tolerance towards high temperature and assured the availability of water during most of the cultivation period. However, the impact of climate change on mungbean may be serious when its reproductive phase coincides with terminal heat and drought stresses, especially during spring/summer seasons. In Vigna crops, the thermal regimes do not change drastically from vegetative to reproductive phase as the total crop duration is very short. In contrast, the cool season pulses (rabi crops) witness a clear-cut phase transition from one thermal regime to another when these crops shift from cool temperature vegetative phase (November–January) to reproductive stage at high temperature (February–March). Therefore, winter pulses such as chickpea, lentil, and field pea are more sensitive to abrupt changes in the temperature coinciding with podding stage as compared to crops like mungbean. Various abiotic stresses, such as temperature, drought, and salinity affect the growth of legumes at different developmental stages (Suzuki et al. 2014). Abiotic stresses result in a series of morphological, physiological, biochemical and molecular alterations, which negatively influence plant growth, productivity, and yield (Bita and Gerats 2013).

## 5.3 Sources of Climate Smart Traits in Mungbean

Germplasm resources are valuable repositories of useful genes which can be exploited for the development of improved cultivars in crop plants. While germplasm collection of pulse crops in India was initiated at the beginning of twentieth century by Botanical Section of the Imperial Agricultural Research Institute at Pusa (Bihar), the systematic efforts were made after the establishment of the All India Coordinated Pulses Improvement Project (AICPIP) in 1966–1967. Later, exploration and germplasm collection was continued by National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India and the state agricultural universities of India. Simul-

<b>Table 5.1</b> Current status of germplasm resources (wild and related species) at global	Crop	Global holdings	National holdi Indigenous	ngs at NB Exotic	PGR Total
and national level	Mungbean	24,918	3567	537	4104
	Black gram	3767	3127 + (13)	6	3146
	Rice bean	-	1883	179	2062
	Wild Vigna	-	490	-	490

Adopted from Singh et al. (2017)

taneously, germplasm collections were also made by United States Department of Agriculture (USDA) and several other international research institutes. Globally, the mungbean germplasm collections are maintained at different places including Indian Council of Agricultural Research (ICAR)-NBPGR; the University of the Philippines; The World Vegetable Center (erstwhile Asian Vegetable Research and Development Center, AVRDC), Taiwan; the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences; and the Plant Genetic Resources Conservation Unit of the University of Georgia, USA (Ebert 2013). The University of Philippines and the Rural Development Administration (RDA), Korea also hold duplicates of the mungbean germplasm collection of AVRDC-The World Vegetable Center. The current status of germplasm resources available at national and global level in *Vigna* species are given in Table 5.1. Many of these germplasm accessions are being utilized in national crossing programme for introgression of traits like resistance to diseases and insect pests, wider adaptability, earliness, high yield, large seed, long pod, heat tolerance, etc. (Table 5.2).

Information on gene pools helps the plant breeders in effective utilization of different species for introgression of desirable traits in cultivates species from even distant backgrounds (Pratap et al. 2015a, b). Wild relatives of Vigna can offer sources for imparting resistance to several biotic and abiotic stresses besides improving yield and quality traits (Pratap et al. 2012a) (Table 5.3). Realizing the importance of wild relatives, extensive exploration-cum-collection trips have been organized and collections of wild Vigna accessions have been maintained at ICAR-NBPGR, New Delhi and ICAR-Indian Institute of Pulses Research (IIPR), Kanpur. All these collections have also been evaluated for various plant traits. In a collection of 206 accessions of 14 wild Vigna, species-wide genetic diversity was observed for 45 morphological characters (Bisht et al. 2005). The sub-gene pool of wild types in accession PLN 5 of V. radiata var. Sublobata (Singh and Ahuja 1977) and IW 3390 of V. mungo var. sylvestris (Reddy and Singh 1993) has been identified as potential sources of MYMV resistance, and TC 1966 of V. radiata var. sublobata was identified to carry bruchid tolerance gene (Tomooka et al. 1992). In cowpea, resistance to post-flowering insect pests including legume pod borers and pod-sucking bugs was reported in V. vexillata (Fatokun 1991). Similarly, variation for yield components and Mungbean Yellow Mosaic Virus (MYMV) resistance was reported in V. mungo var. sylvestris and a few accessions of the wild progenitor V. radiata var. sublobata (Singh 1990). A wild

Trait(s)	Accession(s)	Country of origin
Wide adaptability, earliness, and resistance to Tungro Mosaic Virus	EC 118889, EC 118894, EC 118895, EC 162584, EC 158782, EC 159734	Taiwan
Resistance to charcoal rot, Leaf Crinkle, tolerance to drought, flood, photoperiod insensitivity	EC 318985-319057	Taiwan
High yielding	EC 391170-75	Indonesia
Large seeded, long podded with shiny green seed coat	EC 393407-10	Bangladesh
Heat tolerant, short and long duration	EC 397138, EC 396394-396423	Thailand
High yielding	EC 390990-93	Taiwan
High yielding	EC 428862	Nepal
Resistance to MYMV	EC 564801-818, EC 565626-633	Taiwan
Early maturity	EC 512780-793	USA
Resistance to powdery mildew	EC 605445	Australia

 Table 5.2 Promising trait-specific germplasm of mungbean

 Table 5.3 Potential sources of alien variation in Vigna spp

Character	Species	References
Low trypsin inhibitor activity	V. tenuicaulis	Konarev et al. (2002)
Chymotrypsin absence	V. grandiflora	Konarev et al. (2002)
High methionine content	V. radiata var. sublobata	AVRDC (1987), Babu et al. (1988)
High photosynthetic efficiency and drought tolerance	V. radiata var. sublobata	Ignacimuthu and Babu (1987)
Drought tolerance	V. aconitifolia	Jain and Mehra (1980)
Heat tolerance	V. aconitifolia	Tomooka et al. (2001)
	V. riukinensis	Egawa et al. (1999)
Insect resistance	V. unguiculata ssp. dekindtiana var. pubescens	Ehlers and Hall (1997)
YMV resistance	V. radiata var. sublobata	Singh and Ahuja (1977)
High tolerance to saline and alkaline soils	V. radiata var. sublobata	Lawn et al. (1988)
High no. of seeds/plant and pods/plant	V. radiata var. sublobata	Reddy and Singh (1990)
Resistance to Yellow Mosaic Virus	V. radiata var. sublobata	Reddy and Singh (1990), Pal et al. (2000)
	V. trilobata	Nagaraj et al. (1981)
	V. umbellata, V. trilobata, V. mungo	Pandiyan et al. (2008)
Photo-thermoinsensitivity	V. umbellata, V. glabrescens	Pratap et al. (2014)

accession of *V. radiata* var. *sublobata*, PLN 15, was found to be the potential donor for pods per plant and seeds per pod (Reddy and Singh 1990). Resistance to MYMV has also been reported in *V. umbellata*, *V. trilobata and V. mungo* (Nagaraj et al. 1981; Singh et al. 2003). *Vigna mungo* var. *silvestris* has been reported to be immune to bruchids (Fujii et al. 1989; Dongre et al. 1996). Rice bean (*V. umbellata*) was identified as highly useful being a cultivated species and also because many of its accessions show complete resistance or immunity to the bruchids; therefore, gene transfer from rice bean into mungbean and urdbean may be comparatively easy. IC251442 of rice bean and IC 251372 of *V. glabrescens* were reported to be photo-thermo period insensitive (Pratap et al. 2014) and may be utilized for the development of widely adaptable varieties. Hybridization between the cultivated Vignas and their wild relatives in secondary and tertiary gene pools is constrained by crossability barriers and therefore, their successful utilization in crop improvement programmes requires special efforts such as deploying embryo rescue, colchicine treatment, reciprocal crossing, hormonal manipulations, and use of bridge species (Pratap et al. 2015a, b).

## 5.4 Physiological Characteristics and Crop Phenology

Mungbean, despite being a warm season crop, is grown in diverse climates. Therefore, several physiological and phenological factors influence its yield and stability. Mungbean has epigeal germination and the cotyledons come out of the soil to support the growing plant. Therefore, soils with deficient moisture and hard texture may limit the initial growth of the plant restricting its overall growth and development. Likewise, high initial growth vigor is advocated to be one of the criteria for good summer crop as the crop may suffer from terminal moisture and high temperature stress, especially at the time of flowering and pod formation (Pratap et al. 2013a) and high initial vegetative growth may support the plant at such times. Nevertheless, this has been reported to have no direct relationship with final yield in mungbean (Tekrony and Egli 1991). Variable germination of seeds also has a direct relation with optimum plant stand as a plant stand of about 30–35 plants/m<sup>2</sup> is the optimum to obtain maximum yield in mungbean. Poor plant stand, due to poor germination as one of the factors, is expected to affect the final yield of mungbean negatively, especially in marginal environments (Harris et al. 2005). After successful establishment, the yielding ability of a plant depends upon the ability of the crop to produce and partition dry matter into grain yield which is directly dependent upon several developmental stages in a plant which are further dependent upon its response to photoperiod and temperature (Chauhan and Williams 2018). Plant canopy, leaf area index, biomass accumulation, light interception, conversion of absorbed radiation into assimilates, and partitioning of the assimilates into roots, leaves, pods, and seeds are the major physiological determinants of grain yield in mungbean. A manipulation in the efficiencies of these processes is ultimately dictated by the response of plants to varying photoperiods, available moisture, and changing temperature regimes.

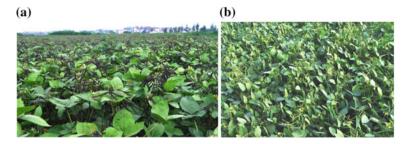


Fig. 5.1 Popular mungbean cultivars a Samrat and b Virat. Both these cultivars have narrower leaves and vertical arrangement

The time taken by a mungbean cultivar from sowing to maturity and also harvest is the prime determinant of the quantity and quality of the produce and it also determines the cropping system in which this crop can fit (Chauhan and Williams 2018). Temperature and photoperiod as well as growing conditions affect the crop duration and also its other phenological processes and therefore, the crop may behave variably in different seasons. Mungbean is a quantitative short-day plant and has broad, trifoliate leaves which generally have an overlapping and horizontal orientation. Therefore, narrower mungbean leaflets are expected to allow better light interception. The most popular Indian cultivars of mungbean, viz., "Samrat" and "Virat" (Fig. 5.1) have narrower leaves and vertical orientation allowing better light interception and this may be the reason why these despite being short-duration cultivars (55–60 days) yield high. Lee et al. (2004) reported a mungbean cultivar with narrower leaves and higher light interception to have a higher yield potential as compared to mungbean with broader leaves. Rachaputi et al. (2015) observed that sowing of mungbean at narrow rows of 0.5 m can accelerate closer canopy development and achieve better light interception as compared to wider rows of 1 m. Kuo et al. (1977) reported inadequate leaf area development as one of the limiting factors to yield increase in mungbean. Drought is also reported to affect leaf expansion and appearance rates (Lawn 1982).

#### 5.4.1 Salinity

Legumes are highly salt-sensitive crops and high concentrations of Na and Cl ions around the root zone in water-scarce areas limit the geographical range of legumes in arid and semiarid climates where evapo-transpiration exceeds precipitation (HanumanthaRao et al. 2016). Salinity affects the crop growth and yield in three ways: (a) osmotic stress, (b) ion toxicity, and (c) reduced nodulation and therefore reduced nitrogen-fixing ability. Salinity stress has been reported to cause a significant reduction in mungbean yield (Abd-Alla et al. 1998; Saha et al. 2010). This can occur through reduction in seed germination, root and shoot growth and seedling vigor and

the yield reduction level may vary in different genotypes (Promila and Kumar 2000; Misra and Dwivedi 2004). Other pronounced symptoms, viz., enhanced chlorosis, necrosis, and decreased content of chlorophyll and carotenoids are also noticed in mungbean (Gulati and Jaiwal 1993; Wahid and Ejaz 2004). NaCl stress has been reported to have more deleterious effect on roots than shoots, with a sudden dip in root growth associated traits (Friedman et al. 2006; Saha et al. 2010).

During germination under saline conditions, high osmotic pressure of saline water is created due to capillary rise leading to more salt density at seed depth than at lower soil profile, which reduces time and rate of germination (Mudgal et al. 2010). In mungbean seedlings, high salt concentration causes increased  $H_2O_2$  content in both roots and leaves, hence salts should be removed to ensure proper growth and development (Saha et al. 2010). Chakrabarti and Mukherji (2002) reported a decrease in total leaf area and stomatal opening due to high salinity. Likewise, Arulbalachandran et al. (2009) reported a reduction in total chlorophyll content, sugar, starch, and peroxidise enzyme activity in roots and shoots of three different species of *Vigna (V. radiata, V. mungo* and *V. unguuiculata*). Germination percentage, seedling growth rate as well as photosynthesis were observed to decrease with increasing levels of NaC1 in all the species while the growth decrease was higher in mungbean as compared to the other two species.

Increased salinity was also observed to have a profound effect on nodulation and nitrogen metabolism in mungbean. Salt stress significantly affected initiation, weight, and nitrogen-fixing ability of the root nodules and also lead to inhibition of root colonization by Rhizobium (Mudgal et al. 2010). The ill effect on nitrogen metabolism was more prominent on above ground parts as compared to roots (Munns and Tester 2008).

Maas (1986) observed that soil salinity also delays and reduces flowering and yield of crop plants. Mungbean shows decreased plant growth, photosynthesis as well as yield at higher levels of salinity which leads to delayed pod ripening during the spring season (Sehrawat et al. 2013a, b, c, d). Excessive salt may lead to injury to leaves by entering into the transpiration stream and subsequently may lead to reduced photosynthesis (Hossain and Fujita 2010). Misra and Dwivedi (1995) reported that a salt tolerant mungbean cultivar was characterized by higher levels of total soluble carbohydrates than a salt-sensitive cultivar irrespective of salinity level. While less intense salinity level in the field affects the mungbean crop a little, moderate and uniform salinity leads to restricted vegetative and reproductive growth. Nevertheless, intense form of salinity leads to drastic reduction in plant growth and vigor, flower initiation, and yield.

## 5.4.2 Temperature

Temperature is an important factor affecting seed yield and quality in legumes. For subtropical and tropical crops, heat stress occurs when the temperatures cross 32-35 °C (Bita and Gerats 2013). For cool season legumes, a daily maximum tem-

perature above 25 °C is considered as the upper threshold for heat stress (Wahid et al. 2007). High temperatures can adversely affect growth, reduce yield, and truncate crop cycles (Araujo et al. 2015). Photosynthesis may be adversely affected by heat stress (Crafts-Brandner and Salvucci 2002) and carbon assimilation associated chloroplast functions coupled with impaired vigor, cellular respiration, N fixation, and metabolism may be affected (Buxton 1996). Mungbean grows well at a mean temperature of 28–30 °C (Carberry 2007; Chauhan et al. 2010). High temperature stress is reported to affect reproductive development in mungbean (Tzudir et al. 2014; HanumanthaRao et al. 2016) and it negatively affects flower initiation, pollen viability, stigma receptivity, ovule viability, ovule size, fertilization, fruit set, seed composition, grain filling as well as seed quality (Barnábas et al. 2008). The sudden rise in the temperature beyond 35 °C causes an increase in the respiration rates and unusually high degradation of stored starch as major chloroplasts carbon source was observed. As a result, failure to set pods, reduced or incomplete grain development at high temperature could be partly due to the inadequate supply of carbon and nitrogen from leaves or by a decrease in the activity of sucrose synthase, the key enzyme playing a crucial role in grain development. Poor partitioning of carbon and nitrogen at high temperature leads to low harvest index and low productivity in mungbean.

Abscission of reproductive organs was ascribed as the primary determinant of yield reduction in heat stress in many grain legumes by Rainey and Griffiths (2005). While terminal high temperature stress is a serious problem in spring/summer grown mungbean, early stage heat stress is observed to occur in *kharif* season crop. These lead to a drastic reduction in crop yield due to pollen sterility, lack of fertilization, flower drop, and embryo/endosperm degeneration leading to the development of undersized seeds. On contrary, low temperature stress, especially at the time of sowing and early growth stage of spring crop, may lead to delayed and reduced germination and slow plant growth. Low temperature stress at early growth stage and sudden rise in temperature during reproductive phase which usually occurs in spring grown crop in northern India has a deleterious effect on mungbean and seriously hampers its growth and development. During flower development, male and female organs are sensitive to high temperature, especially at temperatures beyond 30 °C (Sita et al. 2017). Heat stress severely affects flower bud initiation, and this sensitivity may prevail for 10–15 days (Hedhly et al. 2009; Bita and Gerats 2013). Heat stress influences the reproductive stage by decreasing the number and size of flowers, deforming floral organs, resulting in loss of flowers and young pods, and hence reduction in seed yield (Morrison and Stewart 2002) as reported in mungbean (Tickoo et al. 1996).

Reduced fertilization is a common problem associated with heat stress in many food legumes due to disruption of meiosis and fertilization (Kaushal et al. 2013; Jagadish et al. 2014). High temperature may arrest fertilization by inhibiting the development of male (Jain et al. 2007) and female gametophytes (Snider et al. 2009). Reduced fertilization efficiency due to heat stress has been attributed to increasing oxidative stress, reduced carbohydrates, ATP concentration in gynoecium, and decreased leaf photosynthesis in mungbean (Suzuki et al. 2001). Temperature fluctuations during seed filling have been reported to drastically reduce yield (Kaur et al.

2015a, b). High temperature stress causes yield loss in legumes (Canci and Toker 2009; Kumar et al. 2016) and other crops due to poor seed development (Hall 2004).

Male sterility has also been observed in many heat-stressed food legumes, including mungbean (Kaur et al. 2015a, b) and impaired pollen development has been a vital reason linked to yield losses due to heat stress (Wassmann et al. 2009). Anthers developing under high temperature showed cell proliferation arrest, distended vacuoles, altered chloroplast development, and mitochondrial abnormalities (Sakata et al. 2010). Heat stress decreases the accumulation of carbohydrates in pollen grains and stigmatic tissue by changing the partitioning of the assimilates and the proportion between symplastic and apoplastic loading of the phloem (Taiz and Zeiger 2006), which affects pollen viability (Kaushal et al. 2013). Heat stress decreases the activity of sucrose synthase and many cell wall and vacuolar invertases in developing pollen grains; as a result, the turnover of sucrose and starch turnover are impaired to reduce the accumulation of soluble carbohydrates in mungbean (Kaur et al. 2015a, b).

Temperature exceeding beyond 42 °C during summer, causes hardening of mungbean seeds due to incomplete sink development. Based on multilocation evaluation at Vamban (Tamilnadu) and Durgapura (Rajasthan) in India, 12 promising genotypes (IPM 02-16, IPM 9901-10, IPM 409-4, IPM 02-3, PDM 139, IPM 02-1, IPM 2-14, IPM 9-43-K, PDM 288, EC 470096, IPM 2K14-9, IPM 2K14-5) were identified which have been confirmed to be tolerant to heat and drought. Based upon sucrose synthase activity and protein profiling as biochemical markers, a few promising mungbean varieties were identified as heat tolerant which have been validated by repeated field trial across diverse agroclimatic zones prone to be affected by recurrent high temperature stress. These genotypes are PDM 139 (Samrat), IPM 02-1, PDM 288, IPM 05-3-21, ML-1257.

## 5.4.3 High Temperature and CO<sub>2</sub>

High  $CO_2$  induces closure of stomata and inhibits photosynthesis in mungbean. High temperature x  $CO_2$  interaction studies revealed negative impact on mungbean plants. Results indicated formation of leaf starch at high  $CO_2$  leading to poor assimilate export from source to sink and grain filling was adversely affected. The high level of carbon dioxide is however beneficial after setting of strong sinks, i.e., developing grains with high sucrose synthase activity.

The rate of light-saturated photosynthesis Pmax (PFD 1000  $\mu$  mol photons m<sup>-2</sup> s<sup>-1</sup> at 20 °C) at elevated carbon dioxide (500 ppm) increased both at vegetative as well as grain filling stage (Table 5.4). At the podding stage, when sink demand is high, both photosynthesis and transpiration tremendously increased under elevated CO<sub>2</sub> and without any water-limiting conditions. However, high CO<sub>2</sub> level during vegetative stage contributed towards increased water use efficiency as compared to ambient CO<sub>2</sub> (300 ppm). High photosynthesis accompanied by increased transpiration and

Crop stage	CO <sub>2</sub> cond (ppm)	lition	Photosynthetic rate $(mmol m^{-2} s^{-1})$	Stomatal con- duc- tance	Transpiration rate	Ahs/Cs	Pn/Gs
Vegetative	Ambient	380	6.45	0.027	0.85	0.006	310.53
Vegetative	Elevated	500	8.96	0.030	1.33	0.009	738.71
Podding	Ambient	380	6.54	0.012	0.40	0.007	750.20
Podding	Elevated	500	13.84	0.235	3.86	0.023	227.20

**Table 5.4** Relative changes in photosynthetic rates, stomal conductance and transpiration in mungbean at vegetative and podding stage

stomatal conductance under elevated CO<sub>2</sub> supported high sink demand during grain filling.

If the duration of mungbean crop is reduced by 8–10 days without significant yield penalty, the losses caused by these stresses can be avoided in its major production base. Keeping this in view, two extra early maturing mungbean genotypes were developed by the ICAR-IIPR, Kanpur which matured in 50–55 days during Summer as well as rainy seasons (Pratap et al. 2013b). The variety IPM 205-7, popularly known as Virat was developed from the cross IPM 2-1 X EC 398889 and the genotype IPM 409-4 was developed from the cross PDM 288 X IPM 3-1. Both these genotypes also showed high resistance to vellow mosaic disease and were registered with ICAR-NBPGR as unique germplasm (Pratap et al. 2012b). Virat has been developed using identified heat-tolerant germplasm line EC 398889 and an early maturing and high yielding variety "Samrat". Samrat has synchronous podding and rapid grain filling. Physiological and molecular characterization of the heat-tolerant line EC 398889 differed significantly in respect to heat sensitive line LGG 460 when tested with the marker CEDG147 and pollen germination tested at 43 °C. One of the simplest approach to develop combined tolerance to drought and heat is to shorten the crop duration which may help escape terminal heat stress during summer season, induce synchronous podding, helping in single harvest and faster grain filling and integrating traits like osmotic adjustment or deep root system to avoid intermittent drought at early stages and terminal heat >40 °C during pod filling. A number of green gram accessions have been evaluated for heat tolerance and an exotic line EC 398889 has been identified having high levels of heat tolerance as compared to LGG 460. Molecular characterization of both of these accessions revealed significant differences for a specific marker that confirmed with high rate of in vivo pollen germination when pollen exposed to temperature above 44 °C for 2 h (Pratap et al. 2015a, b).

## 5.4.4 Drought

Mungbean can tolerate moderate temperature and soil moisture deficits and therefore has a definite role to play in drought-prone areas. A fairly regular supply of moisture is desirable for mungbean during growing period while complete dry conditions are required at harvest. Severe drought reduces vegetative growth, flower initiation, and pod set (Morton et al. 1982). It has the ability to extend its roots deeper in the soil in response to drought. The moderate soil moisture is needed for early growth till the onset of flowering and podding. Intermittent drought situations are very critical for this crop, particularly under dry conditions where air water deficit is higher and soil moisture loss is faster due to high evaporation demand. Soil water holding and the crop's water retention capacity both determine the ability of a mungbean cultivar to escape the drought stress before the reproductive stage. The mungbean has low water retention capacity by virtue of having low or inability for osmotically adjusted when subjected to drought, relatively higher lethal leaf water potential (less negative) in response to drought, as a result, the crop quickly loses turgor and stress symptoms may appear recurrently during early growth stages. Plant encounters recurrent transient drought stress when leaf water potential falls below -1.5 MPa but recovers thereafter when load of solar radiation and temperature are diminished. It may not be possible to revive the crop once it reaches lethal leaf water potential nearly to -2.5 MPa.

Some cultivars are more drought tolerant than others which could be due to enhanced ability to close the stomata in the leaves and reduce the rate of growth and leaf expansion during period of severe water stress. There is a variation in the root system in the cultivars of mungbean which can be exploited in breeding programme to develop varieties with delayed dehydration. Drought tolerance rating or sensitivity of pulses are as follows.

Lathyrus > Horsegram > Cowpea > Pigeonpea > Chickpea > Lentil > Mungbean> blackgram > Fieldpea > Rajmash

The lethal water potential is defined as the water status of leaf at the point where plant cannot survive any longer. Comparative studies showed that turgor loss in pulses occurs at much lower leaf water potential than wheat and potato indicating the high tolerance of pulses to drought (Table 5.5). However, as compared to pigeonpea, mungbean has four times less dehydration tolerance which needs to be improved further.

The degree of osmotic adjustment (OA) has also been shown to be correlated with yield under dryland conditions in pulses. From Table 5.6, it is clear that among pulses chickpea, pigeonpea and peanut are more tolerant to drought as compared to mungbean. Genetic diversity of OA can be exploited to inherit drought tolerance trait in mungbean as water demand is proportionately less if OA increases. Moreover, OA increases only when drought is intensified.

Species	Crop	Lethal water potential (MPa)	Dehydration tolerance
Pigeonpea	Legume	-7.0 to -8.2	Very high
Groundnut	Legume	-3.4 to -8.2	Very high
Soybean	Legume	-5.0	High
Mungbean	Legume	-1.9	Moderate
Cowpea	Legume	-1.8 MPa	Moderate
Sorghum	Cereal	-3.0 MPa	High
Wheat	Cereal	0 to -2.0 MPa	Moderate

Table 5.5 Lethal leaf water potential for a range of grain legumes

 Table 5.6
 Range of osmotic adjustment in grain legumes as compared to cereals and vegetables

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Species (pulses)	Range of osmotic adjustment (MPa) in leaves	Degree of dehydration postpone- ment	Species (cere- als/vegetables)	Range in osmotic adjustment (MPa)	Degree of dehydration postpone- ment
Groundnut	0.2 to 1.6	Very high	Sorghum	0.8 to 1.7	Very high
Pigeonpea	0.1 to 1.3	High	Wheat	0.2 to 1.5	High
Soybean	0.3 to 1.0	High	Barley	0.2 to 0.5	Moderate
Chickpea	0.0 to 1.3	High	Maize	0.1 to 0.4	Moderate
Lentil	0.0 to 0.6	Moderate	Potato	0.0 to 0.25	Low/sensitive
Mungbean	0.3 to 0.4	Moderate	Lupin	0.1 to 0.5	Moderate
Blackgram	to 0.5	Moderate	Fieldpea	0.0 to 0.4	Moderate
Cowpea	0.0 to 0.4	Moderate	Faba bean	0.0 to 0.2	Low/sensitive
			Lathyrus	0.0 to 0.1	Low/sensitive

## 5.5 Classical Genetic Studies

Several attempts have been made to understand the genetics of quantitative and qualitative traits related to climate resilience in mungbean besides studying the inheritance of resistance to biotic and abiotic stresses (Table 5.7). Since the first genetic studies on inheritance of color of ripe pods and seed coat surface by Bose (1939), numerous attempts were made to study the inheritance of morpho-physiological traits. Seed coat color is a highly variable trait in mungbean. Khattak et al. (1999) reported monogenic inheritance of this trait and black, black-spotted and dull-green seed coat colors were reported to be dominant over green, nonspotted and shiny green color, respectively. The inheritance of black and green seed colors was found controlled by a single gene (B), black being dominant over green (Chen and Liu 2001). For twining habit, a single dominant gene (T) was reported to be responsible (Khattak et al. 1999). However, Pathak and Singh (1963) reported a single recessive gene for this trait. Semi-spreading habit was reported to be dominant over erect habit and governed by

Trait	Inheritance	Reference
Plant type and growth habit	Single dominant/recessive gene, Semi-spreading is dominant over erect habit	Pathak and Singh (1963), Khattak et al. (1999)
Pubescence	Single dominant gene	Murty and Patel (1973)
Nodulation	Additive and non-additive gene action	Singh et al. (1985)
Leaf traits	Single dominant gene, large leaflet is dominant over small leaflet; lobbed is dominant over entire type	Singh and Singh (1995), Talukdar and Talukdar (2003)
Flower color	Single dominant gene	Bose (1939)
Pod color	Single dominant gene	Sen and Ghosh (1959), Murty and Patel (1973)
Pod shattering	Single dominant gene	Verma and Krishi (1969)
Seed coat color	One or few genes; mottling governed by single gene	Khattak et al. (1999), Chen and Liu (2001), Lambrides et al. (2004)
Seed coat surface	Two complementary genes	Sen and Ghosh (1959), Murty and Patel (1973)
Hard seededness	One or few dominant genes involved	Lambrides (1996), Humphry et al. (2005)
Preharvest sprouting	Additive and non–additive gene action; high $G \times E$ interaction	Durga and Kumar (1997)

 Table 5.7 Inheritance and gene action of economically important traits in mungbean

a single dominant gene (Pathak and Singh 1963). For indeterminate growth habit, a single dominant gene which inherited independently from leaf shape was reported to be responsible (Talukdar and Talukdar 2003). Anthocyanin pigmentation is reported to be associated with drought resistance and heat tolerance in mungbean. On contrary, purple pigmentation on stem, petiole, and veins of the leaves was reported to be controlled by a single dominant gene "Ppp1" with pleiotropic effect. There are variable reports for inheritance of yield components in mungbean and it has been reported to be controlled by additive as well as nonadditive gene action in different studies (Dasgupta et al. 1998; Khattak et al. 2002). For seed weight, small seed has been reported dominant over large size (Sen and Murty 1960; Fatokun et al. 1992; Humphry et al. 2005) For leaf traits, narrow lanceolate leaf has been reported to be controlled by two recessive genes, "nl1" and "nl2". Several reports suggest that the trifoliate leaf is dominant over the entire leaf and this trait is governed by a single dominant gene (Chhabra 1990; Talukdar and Talukdar 2003). However, monogenic control was reported for pentafoliate leaf (Chhabra 1990). There are also a few reports of two dominant genes, "Tlb1" and "Tlb2" with duplicate gene action for trilobed leaves (Sareen 1985).

Pubescence has been ascribed to impart resistance to insect pests in many crop species. Pubescence of pods was reported to be dominant over nonpubescence and governed by independent duplicate genes (Khadilkar 1963). Seed hardiness is mostly observed in summer grown crops where the temperatures during pod formation and seed filling stage may go beyond 40 °C. Humphry et al. (2005) reported four loci to be responsible for hard seededness through quantitative trait locus (QTL) analysis among which two QTLs of hard seededness were found co-localized with the loci conditioning seed weight. For inflorescence type, the simple types were reported to be controlled by two dominant genes and compound types are double recessive and number of clusters controlled by single gene (Sen and Ghosh 1959; Singh and Singh 1970)

Among biotic stresses, resistance to MYMV in *Vigna* species is reported to be governed by two recessive genes, however, in few cases, resistance has also been reported due to a single dominant/recessive gene. The bacterial pustule in mungbean is due to a dominant gene. The discordance in the nature of inheritance could be ascribed to racial differences in these studies. The allelic relationships have been studied in the case of MYMV only. Resistant lines of mungbean, Tarai local, L-80, LM-214, and LM-294-1 had nonallelic genes for resistance to MYMV (Shukla and Pandya 1985). Resistance to bruchids in mungbean is dominant and is governed by few major genes (probably two) with some modifiers (Sarkar and Bhattacharya 2014).

## 5.6 Traditional Breeding Strategies

While breeding for developing climate smart mungbean, the inherent physiological attributes should be taken into consideration. The first step is to ensure why the crop is sensitive to a particular stress and what are the associated traits that are lacking or have a reduced expression. The second step is to explore available genetic variation for important traits associated with drought, heat and other climate variable traits and their intregression in the desired genetic background for improving the tolerance level. As suggested in Table 5.3, there is ample scope to improve OA, water use efficiency (WUE), biomass, and photosynthetic rates in mungbean as these features are inherently low in this crop. While numerous improved varieties have been bred in mungbean till date, most of these were developed for improvement of yield traits and resistance to major diseases. Limited attention was paid towards the development of climate smart mungbean. The early phase of systematic varietal development programme in mungbean targeted improving locally adapted but genetically variable populations, mainly by the methods of pure line and mass selections (Singh et al. 2017). This led to the development of several pure line varieties which became highly popular. The emphasis was gradually shifted towards hybridization and selection, later followed by distant hybridization and consequently, >150 mungbean varieties have been bred in mungbean till date in India. The first variety of mungbean, "Type 1" was developed in the year 1936 from a local selection of Muzaffarpur, Bihar and has

been extensively utilized in hybridization programme to develop mungbean varieties T 2, K 851 and T 44 and Sunaina. Being a short-duration variety and possessing good seed quality, "T 44" became very popular in Spring/Summer season. Pusa Baisakhi was used later to develop PIMS 4 and Jyoti. Two varieties of mungbean ML 1 and ML 5 were developed from PAU, Ludhiana during the early 1970s and these were further used to develop ML 131, ML 267, ML 337 and ML 23. Large-seeded varieties of mungbean viz., Pusa Vishal, Pant moong 5 and SML 668 were developed from the selection of AVRDC material. Another landmark early duration variety, "Samrat" was also developed as a selection from local material and this variety became highly popular among the farmers.

The recent period has seen the development of largely the photo- and thermo period tolerant varieties in mungbean. Lately, the focus of breeders shifted towards development of short-duration, photo- and thermo period-insensitive varieties of mungbean coupled with resistance to major biotic stresses, viz, yellow mosaic disease and powdery mildew, which contributed significantly to the national mungbean production. For example, KM 2241, HUM 16, MH 2-15, and TMB 37 were other varieties developed through intraspecific hybridization and these became very popular among the farmers in short time (Singh et al. 2017). The variety PKV AKM 4 developed from a cross between BM4 X PS 16 has also been recommended for two zones, viz., Central Zone and South Zone of the country. IPM 02-3 was developed using IPM 99-125 and Pusa Bold 2 and recommended for both spring and Kharif seasons. IPM 410-3 (Shikha) has been recommended for Summer season in North West Plain Zone as well as Central zone while this has been performing very well in Kharif season also in North Hill Zone. Later other varieties for high yield, YMV resistance were released which were the products of intraspecific hybridization. IPM 2-14 is one such highly promising variety which has been released for spring cultivation in South Zone of the country and gained tremendous popularity. Another variety DGGV-2 developed from the cross Chinamung x TM-98-50 and Pusa 0672 developed from  $11/395 \times ML$  267 were released for South Zone and North Hill Zone, respectively. Distant hybridization has also led to the development of three mungbean cultivars viz., HUM 1, Pant Moong 4 and IPM 99-125 in India. These cultivars have improved plant types in addition to high yellow mosaic resistance and synchronous maturity.

#### 5.7 Genomic Resources

The availability of high-throughput and cost-effective next-generation sequencing (NGS) platforms as well as high-throughput genotyping technologies have facilitated the generation of massive genomic data for model as well as crop legumes. These platforms have been vital in producing the genome sequence assemblies for the mungbean (Kang et al. 2014). Whole genome-resequencing data are also becoming readily available for mining superior alleles. Genomic resources of mungbean, viz., whole genome/transcriptome sequences (Kang et al. 2014), chloroplast genome (Tangphatsornruang et al. 2010) and mitochondrial genome sequence (Alverson et al. 2011) and available which are invaluable resource for mungbean research community. These resources may be tremendously useful in designing climate smart mungbean cultivars. A number of quantitative trait loci (QTL) have been reported in mungbean which can be of tremendous use in incorporating various yield and related traits for genetic improvement (Table 5.8).

#### 5.7.1 Nuclear Genome

The development of molecular markers is critical for crop improvement programmes. Moreover, molecular markers are important for integrating useful alleles of wild genetic resources, such as MYMV and bruchid resistance, into domesticated mungbean (Chen et al. 2013). Although molecular marker resources are limited for mungbean, there have been several efforts to identify the genomic regions related to domestication-related traits, including seed size and seed germination (Isemura et al. 2012). Similarly, transcriptomics/gene expression studies, using a range of platforms, have been valuable for identifying candidate genes associated with tolerance/resistance to different stresses as well as several agronomic traits (Campbell et al. 2014; Brasileiro et al. 2015).

Sequencing of Vigna radiata genomic DNA was carried out using 454 Life Sciences technology on the Genome Sequencer (GS) FLX System (Tangphatsornruang et al. 2009). A total of 470,024 quality filtered sequence reads was generated with the average read length of 216 bases covering 100.5 Mb. Assembly of the obtained nucleotide sequence reads was performed using the Newbler, de novo sequence assembly software (Margulies et al. 2005; Kang et al. 2014) sequenced domesticated V. radiata var. radiata, its polyploid relative V. reflexo-pilosa var. glabra and its wild relative V. radiata var. sublobata. For V. radiata var. radiata, the pure line VC1973A was chosen for genome sequencing and a high-quality draft genome sequence of the diploid V. radiata var. radiata VC1973A with an estimated genome size of 579 Mb (1.2 pg per 2C) was constructed. A total of 22,427 genes were identified in the genome including 160 Vigna gene clusters and 1850 genes encoding transcription factors (TFs). Another 2310 noncoding genes were predicted, including 629 transfer RNAs, 280 ribosomal RNAs, 537 microRNAs, 717 small nucleolar RNAs, 110 small nuclear RNAs, and 37 regulatory RNAs. In contrast, the allotetraploid V1160 has a total of 41,844 genes, almost twice the number of mungbean genes. The estimated genome size of polyploid Vigna genome (968 Mb) is almost twice that of mungbean genome (579 Mb). The availability of this genome sequence can serve as a model to understand mungbean domestication.

Table 2.0 INIAJOI	Tame 2.0 Major ATES reported III IIIMIBUCAR IN VALUUS LARS				
S.No.	QПL	Trait (s)	Location/linkage group	Amplifying marker	Reference
1	2 QTL (qPMR-1 and qPMR-2)	Powdery mildew/2 QTLs	LG-2 (20.10-57.1%)	RFLP	Kasettranan et al. (2010)
2	20 QTL collectively	Days to Ist flower, days to first pod maturity, days to harvest, 100 seed wt, no. of seed/pod, pod length		SSR	Kajonphol et al. (2012)
3	4 QTLs, 11 QTLs	Hard seededness, seed weight	1	RFLP	Humphry et al. (2005)
4	46 QTLs	Seed permeability (4 QTL)	LG-1, LG-2, LG-3, LG-4 (33.7%),	SSR	Isemura et al. (2012)
		Pod dehiscence (2 QTL)	LG-1, LG-7 (10.8–12.7%)		
		PDRW (3 QTL)	LG-1 (20%), LG-6, LG-7		
		Seed size related traits (5–7 QTL)	LG-8 (15.1–22.7%), LG-2 (11.4–16.6)		
		Pod length (5 QTL)	LG-2 (20.5%), LG-7, LG-8 (28.5%)		
		Pod width (4 QTL)			
		Primary leaf width (1 QTL)	1		
		Stem thickness (1 QTL)	LG-2 (10.2%)		
		Branch number (3 QTL)	LG-2 (22.3%), LG-4, LG-6		
		Flowering time (4 QTL)	LG-2 (32.9%), LG-4, LG-6, LG-11		
		Days to pod maturity (6 QTL)	LG-2 (20.3%), LG-4 (19.9%), LG-6, LG-7, LG-9, LG-11		
		Seed number/pod (2 QTL)	LG-1 (7%), LG-2 (9.1%)		
		Total number of pod (4 QTL)	LG-2, LG-4, LG-7 (5.8–12%)		
5	1 major QTL (QCLS)	Cercospora leaf spot resistance	LG-3 (65.5-80.53%)	SSR	Chankaew et al. (2011)
6	1 major QTL (qPMC72V18-1)	Powdery mildew	92.4%	ISSR, ISSR-RGA	Poolsawat et al. (2017)
7	1 QTL (qMYMV)	MYMV	LG-5 (47.43%), LG-10	SSR	Kitsanachandee et al. (2013)
8	1 QTL	Bruchid resistance	LG-8, 3.6 CM	RFLP	Young et al. (1992)
	,				'

Table 5.8 Major OTT s reported in munchean for various traits

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### 5.7.2 Chloroplast Genome

Chloroplast genome of Fabaceae family is known to have undergone more rearrangements than other angiosperms. Based on 454 pyro-sequencing technology, gene content and structural organization of mungbean chloroplast (cp) genome were reported to be similar to common bean, *Phaseolus vulgaris* (Tangphatsornruang et al. 2010). With an average AT content of 64.82%, they reported mungbean chloroplast genome to be 151.27 kb in length including pair of IRs of 26.474 kb separated by small single copy region of 17.427 kb and large single copy region of 80.896 kb. The genome contains 108 unique genes and 19 of these genes are duplicated in the IR. Of these, 75 are predicted protein-coding genes, 4 ribosomal RNA genes, and 29 tRNA genes.

## 5.7.3 Mitochondrial Genome

Mitochondria are rod-shaped organelles considered to be the power generator (ATP) of the cells and generally harbor circular double-stranded DNA molecules of variable sizes, structure, and sequence complexity. Based on shotgun Sanger sequencing, mungbean mitochondrial genome was reported to be protein-gene-poor 401.262 kb in length with that lacks large, recombinationally active repeats and *promiscuous* sequences from chloroplast and nuclear genomes (Liu et al. 2016). The contents of A, T, C, and G in the NM92 mitogenome were found to be 27.48%, 27.41%, 22.63%, and 22.48%, respectively. The NM92 mitogenome encoded 3 rRNAs, 16 tRNAs, and 33 proteins. Eight protein-coding genes (*nad1*, *nad2*, *nad4*, *nad5*, *nad7*, *rps3*, and *rps10*) certain introns. Among them, three (*nad1*, *nad2*, and *nad5*) are trans-spliced genes. A phylogenetic tree was reconstructed using the 21 protein-coding genes of 16 crops.

## 5.7.4 Marker-Assisted Backcrossing (MABC)

Success of a breeding programme greatly relies upon the selection efficiency. Selecting desirable plants from segregating and subsequent-segregating progenies that contain appropriate and desirable combinations of genes is a critical component of plant breeding (Ribaut and Betran 1999). Plant breeders typically work with hundreds or even thousands of populations which often contains large numbers (Ribaut and Betran 1999; Witcombe and Virk 2001). Marker-assisted selection may greatly increase the efficiency and effectiveness in plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes of interest have been identified, prior to field evaluation of large number of plants, breeders may use specific DNA marker alleles as a diagnostic tool to identify the plants carrying genes. Molecular marker-assisted backcross breeding (MABC) deploys foreground selection (FGS) and background selection (BGS) using genome-wide SSR markers for recovery of recurrent parent genome. This is an environment independent, precise, and quick approach for the development of cultivars of the trait of interest (Varshney et al. 2010). Therefore, a plant breeder would prefer to exercise marker-assisted backcrossing (MABC) for development of superior cultivar with desired traits. While considerable success has been reported in deploying MABC in legumes like chickpea (Pratap et al. 2017), this technology is yet to be successfully deployed in crops like mungbean. Nevertheless, success in chickpea has encouraged taking molecular marker-assisted breeding as a routine tool in crop improvement programme in mungbean also.

## 5.8 Genetic Transformation

Development of highly reproducible regeneration protocol is a prerequisite for widespread application of in vitro tissue culture techniques in legume improvement programmes (Pratap et al. 2018). Success of this technique also depends upon well characterized and cloned genes for target traits. Advancements in genetic engineering of crop plants have ensured recovery of improved plants with genes introgressed in them from across the species barrier (Pratap et al. 2018). Nevertheless, as legume species are largely recalcitrant to in vitro techniques, routine transformation protocols are limited in most of these species. Though efficient protocols for shoot regeneration have been worked on and established for mungbean since long (Gulati and Jaiwal 1992, 1994; Chandra and Pal 1995; Amutha et al. 2003; Khatun et al. 2008; Yadav et al. 2010a, b; Mookkan and Andy 2014), they vary based on genotype and age and type of explant(s). Different explants respond in a variable manner to phytohormones with change in genotype. Variables like explant type (hypocotyl, apical meristem, cotyledonary nodes, excised embryo, etc.), age of explant, basal media (MS with MS salts, B5 with B5 salts, MS with B5 salts), phytohormones (IAA, BA, zeatin, TDZ)alone and in combination, presence of supplements (AgNo<sub>3</sub>) decide the success and efficiency of standardized protocol. Literature reveals reports on both direct organogenesis as well as indirect organogenesis for regeneration. In Vigna, regeneration through callus has rarely been reported indicating that genetic factors affect regeneration ability. Literature on in vitro regeneration in mungbean is abundant, but its further utilization for genetic transformation and related studies is relatively less.

The first successful recovery of mungbean transgenic plants was reported from cotyledonary node explants in the mungbean cv. K-851 using *Agrobacterium tumefaciens* strain LBA4404 harboring pTOK233 vector carrying  $\beta$ -glucouronidase (*gusA*) and neomycin phosphotransferase II (*npt*II) marker gene at an overall efficiency of 0.9% (Jaiwal et al. 2001). However, transmission of transgenes (*GUS and npt*II) to the progeny was not confirmed. In another report, mungbean transgenic plants were regenerated via direct organogenesis from primary leaf explants of 10-day-old seedlings cv. K-851, cocultivated with disarmed *A. tumefaciens* strain C-58 harboring a pCAMBIA–1301 plasmid comprising  $\beta$ -glucouronidase (*GUS*) and hygromycin

phosphotransferase (*hpt*) genes (Mahalakshmi et al. 2006). Hygromycin-selected shoots were rooted and transferred to glasshouse to produce seeds. Presence and stable inheritance of gus gene were confirmed by PCR and Southern hybridization and histochemical GUS assay confirmed the stable gene expression. However, lower regeneration efficiency of primary leaf explants and lot of escapes on hygromycincontaining medium may limit the use of these explants for routine introduction of desirable genes to mungbean. Tazeen and Mirza (2004) worked on varieties from Islamabad and regenerated shoots via callus. 2,4-D in B5 media was used to induce callus in explants. Sahoo et al. (2016) developed transgenic mungbean having AtNHX1 for salinity tolerance using cotyledonary node as explant with MSB5 media having BAP as sole phytohormone. TDZ was initially used in preculturing. An average transformation efficiency of 2.07% was documented. Reports of use of embryonic axis attached to cotyledon are also reported for transformation work. Mahalakshmi et al. (2006) reported an efficient genotype independent transformation protocol giving an efficiency of 65-75% based on GUS assays. They had used primary leaves cut at node as choice explant, from both 4- and 10-day-old seedlings, and regenerated them post-transformation on B5 media having only BAP as the phytohormone.

Sonia et al. (2007) reported an improved protocol of genetic transformation of mungbean (cv. Pusa 105) using phosphinothricin as selective agent and Phaseolus *vulgaris*  $\alpha$ -amylase inhibitor-1 ( $\alpha$ AI-1) gene for resistance to bruchids. Vijayan and Kirti (2012) generated transgenic mungbean (cv. ML-267) plants from cotyledonary node explants using kanamycin selection for enhancing resistance against seedling rot pathogen, Rhizoctonia solani. Yaday et al. (2012) reported standardization of different parameters for efficient Agrobacterium-mediated transformation in mungbean cv. ML267 using double cotyledonary node as explant of choice. A transformation efficiency of 4.2% was reported. They regenerated mungbean on MSB5 media having BAP as the lone phytohormone. Sahoo et al. (2016) employed Arabidopsis thaliana tonoplast Na<sup>+</sup>/H<sup>+</sup> anti-porter (AtNHX1) gene in transgenic mungbean (cv. K-851) for incorporating enhanced salt tolerance based on kanamycin monosulphate selection. These transgenic lines exhibited enhanced tolerance to salt as confirmed by physiological and biochemical studies. Baloda et al. (2017) developed plants with salinity and drought tolerance plants by introducing a gene for an osmoprotectant glycine betaine.

#### 5.9 Agronomic Manipulations

In most of the mungbean producing countries, consistent yields are obtained mainly by multiple harvesting of pods from multiple flushes. However, this kind of production is unsuitable for intensive and mechanized production systems where the rowto-row spacing is much higher than the manually harvested crops. Experimentation has proven that mungbean sown at narrow row spacing yield better. Even in mechanically harvested fields, the mungbeans planted at a row spacing of 50 cm produced better yields in 95% of the seasons in different locations in Australia (Rachaputi et al. 2015). Narrow row spacing reduces evaporative soil losses, especially during summer season and increases overall water use through transpiration and therefore water use efficiency (Chauhan and Williams 2018). Therefore, narrow row spacing may be especially beneficial during summer cultivation of mungbean. This has been well demonstrated by reducing row spacing in mungbean cultivar Virat which yielded better at a spacing of  $15 \times 7$  cm as compared to  $30 \times 10$  cm spacing. Increasing plant population may be anther avenues which may substantially increase its yield although the response to changes in plant density depends to a great extent on the specific characteristics of a variety (Muchow and Charles-Edwards 1982; Pookpakdi and Pataradilok 1993). In such a situation, while branching may be an important trait for maximizing grain yield and crop plasticity, limiting the number of branches may be necessary to maximize yield under closer planting (Chauhan and Williams 2018).

Growing mungbean in a season which has consistent day length and minimal day and night temperatures fluctuations is also one of the strategies to increased mungbean yield. Spring season provides such an opportunity, especially in northern and central parts of India where vast amounts of land are vacant after the harvest of wheat, potato, chickpea, and rapeseed mustard and temperatures during this season are not too high. Nevertheless, terminal temperature and moisture stress may adversely affect mungbean during its reproductive phase, leading to flower drop, less number of pods, shriveled, and hard seeds. Spring mungbean is becoming common in India and there has been tremendous increase in area and productivity in Spring/Summer mungbean in India during the last decade (Gupta and Pratap 2016) and in other parts of Asia (Ali and Kumar 2004).

#### 5.10 Perspectives

Mungbean is a quantitative short-day and warm season plant. However, it is grown across several environments and climatic conditions and accordingly, the breeding efforts have been directed towards the development of varieties suitable to specific niches. In the past two decades, several achievements have been made towards the development of input responsive, high yielding, biotic and abiotic stress resistant, and short-duration varieties in mungbean. The biggest achievement has been made towards reducing the crop duration from 100-120 to 55-65 days which has made it possible to cultivate mungbean in several niches including rice fallows. The reduced duration has made mungbean a suitable candidate to grow as a catch crop during spring/summer season and also as a noncompeting intercrop in cash crops like sugarcane. Development of photo-thermo period-insensitive varieties like Shikha and IPM 2-3 ensured that a few promising varieties could be cultivated over large area without a need to change the varieties in different seasons. Likewise, synchronous maturity in modern-day varieties such as Samrat, Virat, IPM 2-3, HUM 1, HUM 12, MH 421, Pant Mung 5, Pusa Vishal, etc. made it possible to harvest the crop in a single go, thereby saving time and money involved in multiple pickings and also

reduce the drudgery involved. The impact of such varieties has also been realized well in production as well as productivity of mungbean which showed a significant increase despite a reduction in its area. Simultaneously remarkable progress was also made in collection, evaluation, characterization, and documentation of germplasm Wild *Vigna* accessions were also collected and evaluated to great extent. There have also been remarkable success stories in transferring alleles from wild *Vigna* relatives to cultivated mungbean backgrounds and a few cultivars have been developed.

However, there are still a few gray areas which need attention, especially while developing a widely adaptable mungbean cultivar which may also suite to changing climates. Terminal heat stress is the major problem in spring/summer mungbean. While shortening crop duration is one of the strategies adopted to escape terminal heat stress, the crop length cannot be further shortened as it will lead to yield penalty. Therefore, developing heat-tolerant genotypes will help in mitigating the effects of high temperature. Breeding short-duration (52–55 days) varieties for spring/summer season with minimum yield penalty, longer duration genotypes (65-75 days) for Kharif season, and varieties with high initial growth vigor for rice fallow will promote this crop in new areas (Singh et al. 2017). Waterlogging at the early growth stage and preharvest sprouting at the time of maturity are the major limitations in Kharif grown mungbean. Soil salinity poses a significant threat in northern and western parts of India. The problem of storage pest, buchid, still remains largely untouched. A major thrust is required on incorporation of preharvest sprouting and bruchid resistance, pyramiding of genes for resistance to major insect pests (thrips, jassids and pod borer) and diseases (MYMV, powdery mildew and Cercospora leaf spot) for which resistance levels are not very high in cultivated germplasm. A number of cultivars have been developed which are resistant to yellow mosaic disease in recent past. However, its vector, whitefly (*Bemisia tabaci*) is considerably affected by prevailing environmental conditions. Natural transmission of YMD happens through whitefly, however, it can also be transmitted in the plants through Agrobacterium-mediated infectious clones. Temporal and spatial variations in reactions of mungbean cultivars to YMD are of common knowledge. This type of behavior of the cultivars may be attributed to one or more factors including mixed infection of the viruses, changing virus population, influence of weather, mixing of seeds of two or more cultivars, etc. Most of the resistant genotypes available so far have not been screened specifically against identified viruses and hence they may react differently against each or in combination of viruses. Meager information is available on gene/s expression pattern in YMD susceptible/resistant mungbean cultivars. Keeping in view the gap in the existing knowledge, there is a strong need to map yellow mosaic disease causing viruses in mungbean production hot spots, identify the host factors suppressing the virus multiplication, and ultimately silence the viruses causing yellow mosaic disease through genome editing.

The application of molecular marker technology for exploitation of favorable alleles in the wild *Vigna* relatives will provide an excellent opportunity for advances in mungbean improvement. Cost-effective, polymorphic, and reproducible markers such as SSRs, SNPs, etc., are available in plenty in mungbean now and can be deployed towards the development of improved cultivars employing marker-assisted

breeding approaches. Establishing marker-trait association will enable the scientists to manipulate abiotic and biotic stresses constraining crop productivity. Simultaneously, high-throughput sequencing will accelerate the development of new genomic resources. These will together be useful in developing climate smart cultivars of mungbean.

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