

# Exploring the World of Mungbean: Uncovering its Origins, Taxonomy, Genetic Resources and Research Approaches

## Abstract

The mungbean is one of the important leguminous crops, holds great importance in agriculture and nutrition in Asian countries. It is a vital source of protein, playing a key role in vegetarian diets and offering versatility in culinary applications. The crop wild relatives and landraces are invaluable sources of novel genes and alleles, augmenting mungbean resilience against both biotic and abiotic stresses. Researchers have meticulously examined the roles of additive and dominant gene effects in governing vital traits, including seed weight, seed yield, plant height, days to flowering, and yield components. However, despite their potential, these genetic resources remain underutilized due to several constraints, encompassing interspecific hybridization barriers, limited trait evaluation data, and the absence of advanced breeding tools. Announcement of the VC1973A draft reference genome utilizing next-generation sequencing, have facilitated rapid DNA marker development, gene mapping, and the identification of candidate genes for complex traits, predominantly within the last decade. Reports on GBS analysis of mungbean relatively limited and mostly includes investigating population structure and LD in mungbean. This review offers a comprehensive overview of the origin, taxonomy, gene pool dynamics, and the pivotal role played by mungbean CWRs in ensuring agricultural sustainability and elevating crop improvement initiatives. Additionally, it discusses about genotypic characterization of CWRs, advances in sequencing technologies, QTLs mapping for various economically important traits and validation techniques that aid in confirming the QTLs found through GWAS.

**Key words:** Mungbean, Gene pool dynamics, Molecular genetic diversity, QTL, GWAS

## 1. Introduction

Mungbean originating from the Indo-Burma region and believed to have undergone domestication in India holds great importance in agriculture and nutrition (Jain and Mehra, 1978). The leguminous crop is a vital source of protein, playing a key role in vegetarian diets and offering versatility in culinary applications. Mungbean diversity spans Asia, encompassing regions from the northern mountains to the southern peninsula and northeast areas, with additional diversity hubs located in the Indo-Gangetic Plains (Bisht *et al.* 1998). It is a major pulse crop in Asia with India alone accounting for more than 60% of world with cultivation area of approximately 4.5 million hectares and achieving a total production of 2.5 million tons (Anonymous, 2021). Mungbean breeding initiatives prioritize two key objectives: firstly, the improvement of mungbean nutritional profile by addressing deficiencies in essential sulphur-containing amino acids such as methionine and cystine. This enhancement aims to elevate the protein quality and overall nutritional value of mungbean. Secondly, the focus is on increasing mungbean yields, a critical endeavour to ensure food security and meet the growing global demand for mungbean in a variety of culinary applications. Additionally, breeding efforts are dedicated to developing mungbean varieties that can thrive in various environmental conditions, including drought-prone areas, promoting sustainable agriculture and resistance to pests and diseases (Nair *et al.*, 2020; Fiorani and Schurr, 2013). Breeding for early maturity is also a priority, as it empowers mungbean to thrive in double and intercropping systems, particularly when paired with cereals. This shorter growing period not only makes mungbean an attractive choice for crop rotation but also significantly contributes to enhancing soil fertility and promoting agricultural sustainability (Ilyas *et al.*, 2018; Yaqub *et al.*, 2010). “In order to improve productivity and stabilize crop production, there is a need to develop varieties resistant to biotic and abiotic stress factors. The advanced plant breeding techniques, enhanced genetic designs, agro-

biotechnology, and genomics play a pivotal role in the improvement of mungbean” (Nair *et al.*, 2020). Particularly, the genotyping-by-sequencing approach has revolutionized extensive genotyping by leveraging single-nucleotide polymorphisms (SNPs). This method acts as a bridge, effectively reducing the gap between genotype and phenotype (Narum *et al.*, 2013). It enables genetic selection at a genome-wide scale, enhancing our ability to link specific genetic traits with observable characteristics in plants (Brachi *et al.*, 2011). Gene pool which is defined as a reservoir of genetic diversity refers to the total set of genetic information and variations within a population of individuals of the species. It is critical for evolutionary processes, as it represents the genetic diversity available within a population, enabling natural selection, genetic drift, and other mechanisms to shape the traits and characteristics of future generations (Pratap and Kumar, 2011). However, despite their potential, these genetic resources remain underutilized due to several constraints, encompassing interspecific hybridization barriers, limited trait evaluation data, and the absence of advanced breeding tools. Genome-wide association study (GWAS) have evolved over the last few years into a powerful tool for comprehensive analysis of the entire genome, enabling the identification of specific genes and genetic markers associated with economically significant traits. Genome wide association studies can be a key to harness the potential of the unexplored germplasm (Sokolkova *et al.*, 2020). In summary, this review provides a comprehensive overview of the origin, taxonomy, gene pool dynamics, and the pivotal role played by mungbean CWRs in ensuring agricultural sustainability and demonstrates the potential of GWAS as a potent instrument for unravelling the genetic foundations of complex traits in mungbean.

## **2. Origin, evolution and taxonomic classification**

“Mungbean is native to Asia's Indo-Burma region where it was probably domesticated, mungbean is said to have its roots in the Indian gene centre” (Jain and Mehra, 1978). “*Vigna radiata* var. *sublobata* has been named as the wild progenitor species of mungbean and it is an Indian species” (Chandel *et al.* 1984). “In addition to the Tarai region and sub-Himalayan tract, the wild *V. radiata* var. *sublobata* is occasional in India's western and eastern peninsular tracts. India is also the primary centre of mungbean diversity” (Arora and Nayar, 1984). “The diversity of mungbean can be found widely across the continent, from the Mountains in the north to the southern peninsula and north-eastern region. The Indo-Gangetic Plains are regarded as a secondary centre of mungbean diversity” (Bisht *et al.* 1998). “In early days, emigrants and traders from Asia brought mungbean seed to the Middle East, East Africa, Latin America, some regions of South America, and Australia” (Poehlman, 1991). “Among the Asiatic *Vigna* species, it is one with the greatest distribution and its production is steadily rising” (Kim *et al.* 2015). In South and Southeast Asia, the crop is currently grown in countries including India, Pakistan, Bangladesh, Sri Lanka, Myanmar, Philippines, Thailand, Laos, Vietnam, Cambodia, Indonesia, Malaysia, Taiwan and South China. It was cultivated as the Chickasaw pea in the USA as early as 1835. In various areas of Australia, it has also been reintroduced as well as grown to a lesser amount in several parts of Africa and the USA. However, it did not develop into a significant commercial crop in these nations. In India crop is primarily cultivated in states such as Rajasthan, Maharashtra, Gujarat, Odisha, Bihar, Andhra Pradesh, and Madhya Pradesh.

Mungbean is a member of the subgenus *Ceratotropis* of the genus *Vigna Savi* in the family *Fabaceae*. Before several of the genus species migrated to the new genus *Vigna*, the mungbean was once known as *Phaseolus aureus* (Lambrides and Godwin, 2007). The wide collection of domesticated plants and wild relatives found in Asia and Africa constitute the genus *Vigna*. According to Takahashi *et al.* (2016), it had about 100 species spread throughout 7 subgenera and 19 sections, of which seven are the most widely grown worldwide. The two species are native to Africa (*Vigna subterranean* L. and *V. unguiculata* L.) and the remaining five species are known to have originated on the Indian subcontinent in

the Asian group (*V. mungo* L., *V. radiata* L., and *V. aconitifolia* Jacq.) and in Asia's Far East (*V. angularis* Wild and *V. umbellata* (Vavilov, 1926). There are four direct subspecies of the species *Vigna radiata*, namely *Vigna radiata* subsp. *radiata*, *V. radiata* var. *radiata* (L.) R. Wilczek, *V. radiata* subsp. *sublobata* (L.) R. Wilczek, and *V. radiata* var. *setulosa* (Dalzell) Ohwi & H. Ohashi.

### 3. Mungbean plant Genetic Resources

#### 3.1 Mungbean Gene Pool

“There is generally no barrier to cross compatibility between domesticated forms and their nearest relatives, nevertheless the cross compatibility between *Vigna* species is not well characterised. Interspecific barriers might be readily overcome because there are few studies on such wide hybridization for expanding the genetic basis of *V. radiata* employing *V. mungo*, *V. umbellata*, and *V. Trilobata*” (Pandiyani *et al.*, 2008). “It is possible for *V. radiata* to interbreed with other *V.* species, including *V. mungo*, *V. radiata* var. *sublobata*, *V. radiata* var. *setulosa*, *V. trilobata*, *V. trinervia*, *V. hainiana*, and *V. dalzelliana*. In other instances, cross-barrier issues, in particular those brought on by chromosomal pairing incompatibilities, have also been noted” (Pandiyani *et al.* 2008). “Mungbean gene pool was categorised as *V. radiata* var. *radiata* and its closest wild relative, *V. radiata* var. *sublobata* in gene pool 1 (GP-1). GP-2 contains *V. umbellata*, *V. grandiflora*, *V. stipulacea*, *V. subramaniana*, *V. tenuicaulis*, and *V. mungo*. GP-3 also includes *V. aconitifolia* and *V. angularis*. However, some research teams have deviated from this classification of gene pools, placing wild forms of *V. radiata* var. *setulosa*, *V. aconitifolia*, and *V. umbellata* in GP-1, GP-2, and GP-3, respectively” (Pratap *et al.* 2014). Recent hybridization studies have shown that, despite the species being classed in GP-3, *V. glabrescens* produces fertile offspring when crossed directly with *V. radiata*. This suggests that *Vigna* gene pool classification has to be reviewed and traditional crossing-based investigations together with molecular methods will yield reliable grouping of *Vigna* species.

#### 3.2 Mungbean Crop Wild Relatives (CWRs)

Wild relatives of the mungbean crop are found throughout Asia. Indian Gene Centre is home to numerous wild varieties of *Vigna* include those of the following: *V. radiata* var. *sublobata*, *V. umbellata*, *V. mungo* var. *sylvestris*, *V. vexillata*, *V. hainiana*, *V. aconitifolia*, *V. trilobata*, *V. stipulacea*, *V. dalzelliana*, *V. trinervia* var. *bourneae*, *V. marina*, *V. khandalensis*, *V. pilosa*, *V. capensis*, *V. grandis*, *V. angularis* var. *nipponensis*, etc. In the Indian National Genebank at ICAR-NBPGR, New Delhi, more over 2,000 collections of wild *Vigna* species are preserved ex situ. A few *Vigna* species, such as *V. aconitifolia* and *V. umbellata*, are active cultivators in many Indian states and can be found in both wild and cultivated forms. *Vigna* CWRs provide more vital functions outside serving as genetic resources; they are employed for things like good grain, green manure, green pods, tubers, cover crop, forage, ornamental, adverse environments, and abiotic and biotic stressors (**Table 1**). In particular for resilience to biotic and abiotic stresses, wild species and landraces are important sources of novel genes and alleles. Due to barriers of interspecific hybridization, a lack of evaluation data on specific traits, a lack of advanced breeding tools, negative alleles flanking desirable alleles, and the tendency in breeding projects to emphasize short-term outputs, these have largely remained underutilized.

**Table 1 Different species of mungbean and their purpose of growing**

No.	Functions	Sources
1	Food grain	<i>Vigna. umbellata</i> , <i>V. trilobata</i> , <i>V. marina</i> , <i>V. stipulacea</i>
2	Green manure	<i>V. stipulacea</i> , <i>V. hosei</i> , <i>V. parkeri</i>
3	Green pods	<i>V. minima</i> , <i>V. umbellata</i> , <i>V. subterranea</i>
4	Tubers	<i>V. vexillata</i> , <i>V. adenatha</i> , <i>V. angivensis</i> , <i>V. ambacensis</i> , <i>V. reticulate</i> , <i>V. fischeri</i> , <i>V. monophylla</i>

5	Cover crop	<i>V. trinervia</i>
6	Forage	<i>V. luteola</i>
7	Ornamental	<i>V. Caracalla</i>
8	Dry harsh	<i>V. aridicola and V. aconitifolia</i>
9	Swampy marshland habitat	<i>V. adenantha and V. luteola</i>
10	Sandy saline seashore	<i>V. marina</i>
11	High altitudes	<i>V. nakashimae and V. angularis var. nipponensis</i>
12	Limestone outcrops	<i>V. exilis</i>
13	MYMD-resistant cultivars	<i>V. radiata var. sublobata and V. umbellata</i>
14	Powdery mildew resistance	<i>V. stipulacea</i>
15	Resistance for legume pod borer	<i>V. vexillata</i>
16	Adzuki bean weevil, cowpea weevil and storage weevil resistance	<i>V. umbellata, V. minima, V. reticulata, V. vexillata, V. luteola, V. oblongifolia, V. minima, V. reflexo-pilosa, and V. radiata var. sublobata</i>

### 3.3 Mungbean Genetic Resources collections across the world

For the breeding of new crop cultivars as well as for gene mining in genomics research, genetic resources are crucial. Considerable genetic resources are available for mungbean across the country (Table 2). These extensive collections of mungbean germplasm make it possible to identify novel, beneficial genes and explore how they work in breeding through genomic research.

**Table 2 Mungbean germplasm accessions conserved in the gene banks across the world**

No.	Institute/Country	Accessions conserved	official website
1	The World Vegetable Centre in Taiwan	10,438	<a href="https://genebank.worldveg.org">https://genebank.worldveg.org</a>
2	ICAR-National Bureau of Plant Genetic Resources, India	11,000	
3	Plant Genetic Resources Conservation Unit, University of Georgia, USA	3,931	<a href="http://www.genesys-pgr.org">www.genesys-pgr.org</a>
4	University of The Philippines, Philippines	1,385	<a href="http://www.genesys-pgr.org">www.genesys-pgr.org</a> )
5	Rural Development Administration (RDA), Korea	1,091	<a href="http://genebank.rda.go.kr">http://genebank.rda.go.kr</a>
6	Japan	1,455	<a href="http://www.gene.affrc.go.jp">www.gene.affrc.go.jp</a>
7	Russia	877	<a href="http://www.genesys-pgr.org">www.genesys-pgr.org</a>
8	United Arab Emirates	408	<a href="http://www.genesys-pgr.org">www.genesys-pgr.org</a>

### 3.4 Mungbean Germplasm introduction

The ICAR-NBPGR is the designated nodal institution in India's single-window system for the exchange of small samples of plant germplasm (including genetically modified crops) intended for study. Under the guidelines of the Plant Quarantine (Regulation of Import into India) Order, 2003, of the Destructive Insects and Pests (DIP) Act of 1914, it controls the import of seeds and planting material for research. Plant introductions from around the world include genetic stocks, elite strains, related species, improved varieties, and plant germplasm. According to the needs of the breeders or researchers, introductions are made periodically. Since 1976, more than 4,000 different mungbean accessions have been introduced into India from countries including Bhutan, Bangladesh, Australia, Czech Republic, Ghana, Germany, Ethiopia, Indonesia, Italy, Brazil, Canada, China, Japan, Netherlands, Philippines, Russia, Sri

Lanka, Surinam, Sweden, Madagascar, Malaysia, Nepal, Taiwan, Thailand, Uruguay, Uzbekistan, and USA.

### **3.5 Mungbean Genetic Resources Characterization and Evaluation**

“Germplasm utilization in crop improvement programs begins with characterization and evaluation. The process of characterizing plant germplasm includes description and establishment of diagnostic characteristics. Usually, characterization traits are qualitative and are highly inheritable. Germplasm evaluation involves describing how a plant germplasm expresses under optimum or adverse environmental conditions in order to determine its potential useful variability available within the germplasm. Crop improvement relies heavily on evaluation of traits, which are quantitative in nature. The evaluation aims to identify trait-specific germplasm to breeders and researchers. In 1993 at NBPGR, 1,532 accessions were characterized and evaluated for 19 qualitative and 19 quantitative traits” (Kawalkar *et al.*, 1996). “Further, during 2016–18 around 1,500 indigenous and exotic collections were characterized and evaluated for 27 agro-morphological traits. According to these morphological observations and statistical analyses, the cultivated mungbean gene pool exhibits a very good degree of variation for economically important traits like seed weight, flowering period, pod length, number of seeds per pod, and seed size. Similarly, at World Vegetable Centre, Taiwan has characterized and evaluated global mungbean collections of 5,234 accessions for 8 agro-morphological traits includes primary leaf width, primary leaf length, days to 50% flowering, plant height at flowering, plant height at maturity, pod length, seeds per pod and 1,000 seed weight. They witnessed decent amount of phenotypic variability for certain characters in these global mungbean accessions expressed in terms of Shannon’s diversity index 0.82 (average of all traits)” (Schafleitner *et al.*, 2015). “Several research groups have been characterized mungbean germplasm for understanding phenotypic variability, genetic variability, genetic divergence, and trait association” (Bisht *et al.*, 1998; Chattopadhyay *et al.*, 2008; Sangiri *et al.*, 2007; Yimram *et al.*, 2009; Rahim *et al.*, 2010; Tantasawat *et al.*, 2010; Abna *et al.*, 2012; Singh *et al.*, 2014; Hakim, 2008). Moreover, the germplasm of the mungbean crop has been tested for resistance to major biotic and abiotic stresses like MYMD (Akhtar *et al.*, 2011; Sudha *et al.* 2013a, b), bruchids (Somta *et al.*, 2007; Somta *et al.*, 2008), cercospora leaf spot, and powdery mildew (Chankaew *et al.*, 2011) and nematodes (Mukhtar *et al.*, 2017). Many traits-specific mungbean germplasm have been found as a consequence of the mungbean characterization and evaluation studies.

## **4. Conventional and molecular breeding approaches**

### **4.1. Genetic studies**

According to Rohman *et al.* (2003), the additive gene effect regulated 100 seed weight, seed yield per plant, plant height and days to flower to a greater extent. In a related manner, Mak and Yap (1980), found that the genetic additive variation that controlled seed weight and pod length was more than the genetic dominant variation. However, the genetic influence of dominance variance was more significant for the number of pods per plant and the number of seeds per pod. Additionally, it was discovered that seed yield and yield components are controlled by the additive gene action (Khattak *et al.*, 2002). One important agronomic trait, the number of pods per plant is found to be regulated by additive gene action (Yohe and Poehlman 1975; Malhotra *et al.*, 1980, Reddy and Sreeramulu 1982), partial dominance to overdominance (Singh and Singh 1971). “Additionally, for the expression of this trait, non-additive effects were more evident than additive effects” (Singh and Singh 1971; Alam *et al.*, 2014). “Many researchers have demonstrated additive gene action controlling the number of seeds per pod, which is a significant yield component” (Yohe and Poehlman 1975; Reddy and Sreeramulu 1982; Malhotra 1980; Alam *et al.*, 2014). Additionally, partial dominance to overdominance (Singh and Jain 1971) epistasis regulation of this trait has been documented (Malik and Singh 1983).

## 4.2. Genome Diversity analysis

Only a few studies have looked at the molecular genetic diversity of large mungbean collections. In a study conducted by Sangiri et al. (2007), a total of 615 mungbean accessions from various distribution areas, comprising 415 cultivated, 189 wild, and 11 weedy accessions, were examined using 19 Adzuki bean SSR markers. While there were roughly twice as many alleles in wild mungbean (257) as in cultivated mungbean (138), the gene diversity (HE) in cultivated mungbean was only 65.01% of that in wild mungbean. The most diversified cultivars of mungbean were discovered in Pakistan and India. South Asia has the highest diversity of wild mungbean (HE = 0.68), but it was not significantly more diverse than Australia (HE = 0.59). These findings support hypotheses of Tomooka et al. (1992). However, the genetic makeup and phenotypic characteristics of the Australian wild mungbean are significantly different from those of the Asian and African wild mungbean (Rebetzke and Lawn, 2006; Sangiri *et al.*, 2007). Hence, when breeding cultivated mungbean, the wild mungbean can be employed to increase the gene pool of cultivated mungbean.

Based on SSR profiles and phenotypic information, a core collection of 52 cultivated and 49 wild accessions was created (Sangiri *et al.*, 2007). In a separate study, 695 AFLP and 15 SSR markers were used to analyse the diversity of 705 cultivated mungbean accessions from 26 countries, with over 60% coming from Korea (Moe *et al.*, 2011). By using AFLP and SSR markers, researchers were able to estimate the gene diversity of the germplasm at 0.69 and 0.36, respectively. AFLP markers were used to create a core collection of 222 accessions (Moe *et al.*, 2011). Based on geographic data and eight agronomic parameters, a core collection of 1,481 mungbean accessions was created from 5,234 cultivars and kept at World Vegetable Center (Schafleitner *et al.*, 2015). Moreover, a mini-core collection of 297 accessions was created after this core collection was molecularly examined using 20 SSR markers. The core and mini-core collections had 122 and 166 alleles, respectively and HE of 0.49 and 0.57, indicating a modest level of genetic diversity. Using 5,288 SNP markers obtained by genotyping-by-sequencing (GBS), this mini-core collection was further characterised. The results showed that the collection is composed of four genetic clusters that are often connected to the geographic origins of the mungbean. Mungbean from other regions were subdivided and clustered together, whereas those from South Asia (India and Pakistan) were clustered with accessions from West Asia but fragmented into various sub-clusters. The research community can use the core/mini-core sets developed by Sangiri et al. (2007) and Schafleitner et al. (2015) to identify QTLs and genes using GWAS. Integrating the core collection produced by Sangiri et al. (2007) and the mini-core collection provided by Schafleitner et al. (2015) can boost genome diversity, use of genetic resources in mungbean genomics research, and genetic advances. Recently, the diversity of 276 accessions of mungbean germplasms (233 cultivated and 42 wild) from 23 different countries was evaluated using genome-wide SNP variants that were explored by GBS (Ha *et al.*, 2021). Compared to wild mungbean, cultivated mungbean had a lower nucleotide diversity and Indian cultivated mungbean showed the closest relationship to wild mungbean. Furthermore, it is evident that domestication and breeding have resulted in a significant loss of genetic diversity in cultivated mungbean. A major source of tolerance to biotic and abiotic stresses is wild mungbean germplasm (Rebetzke and Lawn, 2006), which have becoming increasingly important due to climate change. To make use of their inherent allelic variation for breeding climate-resilient mungbean, the genomes of wild mungbean should be studied using WGS (whole-genome sequencing) and whole-genome resequencing. In a recent GWAS study, using mini-core collection established by the World Vegetable Center comprising of 296 mungbean accessions SNPs associated maturation were found localized on chromosome 2 with strong linkage, in which genes encoding zinc finger A20 and AN1 domain stress associated protein (SAP) are located (Sokolkova et al. 2020).

### 4.3. Linkage disequilibrium (LD) analysis

The distance over which LD decays determines the quantity and density of markers needed for an association mapping analysis. The long history of domestication is reflected in the LD patterns of the mungbean (Fuller, 2007) and as a self-pollinated species, it is anticipated that genome-wide recombination will occur to a lesser extent than in cross-pollinated species (Flint-Garcia *et al.*, 2003). The  $r^2$  of cultivated mungbean decreased to half its maximum value at ~100 kb compared to that of the wild species which decreased by ~60 kb. The wild variety of mungbean has a higher level of allelic diversity, making it a valuable source of genetic diversity for the cultivated gene pool (Noble *et al.*, 2018).

According to research on closely related species, LD usually degrades by 150 kb in cultivated soybean, 75 kb in wild soybean and between 450 and 550 kb in chickpea (Bajaj *et al.*, 2015). Contrarily, the LD degraded quickly in *Medicago truncatula*, a leguminous plant utilised in genomic studies and declined to around half of its original value within 3 kb (Branca *et al.*, 2011), which is comparable to the model species *Arabidopsis thaliana*: 3–4 kb (Koornneef *et al.*, 2004). Cultivated and wild rice reported the highest variation between the highly cultivated varieties *O. japonica* at ~200 kb and *O. indica* ~65 kb in contrast to wild varieties *O. rufipogon* and *O. nivara* which exhibit rapid decay by ~10 kb (Xu *et al.*, 2012), similar to sorghum: improved inbreds ~19.7 kb and land races ~10.3 kb (Mace *et al.*, 2013). These analyses emphasise the significance of wild accessions and genetically varied populations in facilitating access to novel exotic alleles as well as the significant impact of domestication has had on modifying genome dynamics.

### 4.4. Linkage mapping for important agro- economic traits

To elucidate the genetic basis of complex traits like stress tolerance and grain yield, a large sample size is necessary nearly hundreds to thousands of accessions are required. With the advent of high throughput technologies, it is now possible to sequence thousands of genomes in a short interval. As compared with genotyping, fieldwork phenotyping is still laborious and environment specific involving the measurement of various traits at different points of time in large scale experiments across different environments. To address this concern recently, some sensor-based technologies have been developed for calculating biomass traits, including spectral reflectance of plant canopies and near-infrared spectroscopy on agricultural harvesters (Montes *et al.*, 2007). Phenotyping technology advancements in the future will hasten crop genetic mapping and gene discovery.

Revealing genetic basis of vital economic traits, such as flowering time, maturity date, plant height, pods per plant, seeds per pod, seed coat colour, grain size, and disease resistance are important to introgressing and manipulating these traits to accomplish the breeding objectives. During early days, linkage mapping is the basic tool to map genomic loci underlying important traits of interest. However, so far only few numbers of linkage maps have been constructed in mungbean (Lambrides *et al.*, 2000; Humphry *et al.*, 2002) in spite of great efforts still, saturated and comprehensive genetic linkage map of all eleven chromosomes has not been constructed (Kim *et al.*, 2015). It is essential to unravel the molecular genetic mechanism involved in controlling various agro-economic traits in mungbean. Isemura *et al.* (2012) generated a mungbean linkage map by using 430 SSR markers, and discovered 4 QTLs on linkage group (LG)2, LG4, LG6, and LG11 governing the number of days to first flowering (FLD). The QTL on LG2 had the largest effect (phenotypic variation explained=32.9%). They also reported 6 QTLs linked with the days to first pod maturity from first flowering on LG2, LG4, LG6, LG7, LG9, and LG11. Kajonphol *et al.* (2012) reported 4, 3, and 3 QTLs related to FLD, days to first pod maturity (PDDM) and days to harvest (PDDH) by using 152 SSR markers and these ten QTLs were spread across LG2, LG4, and LG11. Using 101 SSR markers, three QTLs for days to 50% flowering and two for days to 50% maturity were identified and mapped to LG4A, LG4B, and LG7 by

Sompong et al. (2012). Using 56 SSR markers, Somta et al. (2015), have found 5 QTLs related to days to flowering in two dry seasons and one rainy season. They believed that the detection of QTLs was impacted by the difference in photoperiod regime between the dry and wet seasons. 100-seed weight QTLs investigated by Fatokun et al. (1992), which were previously mapped to LG6 and LG2, were repositioned to LG1 and LG8 respectively. Despite these progresses, there are two major constraints to QTL mapping. First, there is only a limited number of recombination events occur in the mapping population; for instance, normally one or two recombination's took places per chromosome, which causes poor mapping resolution unless very large-scale populations are used. Second, Genetic variation within a species is accounted for only by a small fraction of the sequence divergence between selected parents, only QTLs at which the two parents differ can be identified. Some alternative populations have been developed and employed to get around these drawbacks. Therefore, Nested association mapping (NAM) was created to enable joint linkage-association analysis with high power and high resolution (McMullen *et al.*, 2009). Similarly, The Multiparent Advanced Generation Inter-Cross (MAGIC) population was created to enhance the allelic diversity within a mapping population. The QTLs accounting for 10% of the phenotypic variance can be found using the MAGIC population. Further, a group of researchers crossed 8 Arabidopsis germplasms and produced a set of 6 RIL populations known as the Arabidopsis multiparent RIL (AMPRIL) population (Huang *et al.*, 2011). The AMPRIL population's QTL analysis demonstrated that this genetic resource was able to identify QTLs that accounted for 2% or more of a trait's variation.

#### **4.5. Genome wide association studies (GWAS)**

Completion of the mungbean reference genome sequence using high throughput sequencing technologies have allowed detection of genomic polymorphism in a wide-scale collection of mungbean accessions (Kang *et al.*, 2014). Through GWAS, genetic loci associated with variation in mungbean seed coat colour and coat lustre was discovered by (Breria *et al.*, 2020). Recently, 2,912 SNPs and 259 gene presence and absence variant (PAV) events associated with 33 agronomic traits were revealed by GWAS in mungbean (Liu *et al.*, 2022), performed GWAS for nine agronomic traits and analysed phenotypic variance across six environments, and resequenced 558 accessions to analyse genetic variation.

Using 264,550 SNPs, GWAS was conducted on agronomic traits such as plant height (PH), days to flowering (DF) and seed size (100 SDW) in a USDA collection of 482 accessions of mungbean by Sandhu and Singh, (2021). For each trait, they have identified three SNP loci on various chromosomes. For instance, SNP1\_11367629 on chromosome 1 and SNP 5\_4604047 on chromosome 5 had  $R^2$  values above 25% for DF, whereas the  $R^2$  values for the other SNPs were only about 1%. Only SNP1\_11367629, with a  $R^2$  of about 30%, appeared to be appropriately detected for trait PH, whereas the others, with  $R^2$  -values of 0%, were probably false positives. On chromosomes 1 and 7, the major loci for 100 SDW were located, and each QTL was responsible for 10–13% of the variation in seed weight. Particularly, SNP 1\_11367629, situated in *LOC106774729* encoding receptor like protein kinase FERONIA (FER), was linked with all three traits. FER has varied functions regulation of flowering time, root hair development, hypocotyl and root elongation and plant growth and development (Deslauriers and Larsen, 2010; Wang *et al.*, 2020; Duan *et al.*, 2010; Zhu *et al.*, 2020). They have looked into 83 kb region covering *LOC106774729* and discovered five more FER genes close to *LOC106774729*. Hence, it will be challenging to identify the FER genes that cause these traits. They also performed a region-wide scan around SNP 5\_4604047 and discovered that a Phytochrome gene is roughly 25 kb away from this marker. But, none of the DF QTLs found in this investigation. Whereas, in the same region VrPHYA, a putative candidate gene for DF have been reported by Hwang et al. (2017). However, it is unknown how the seed

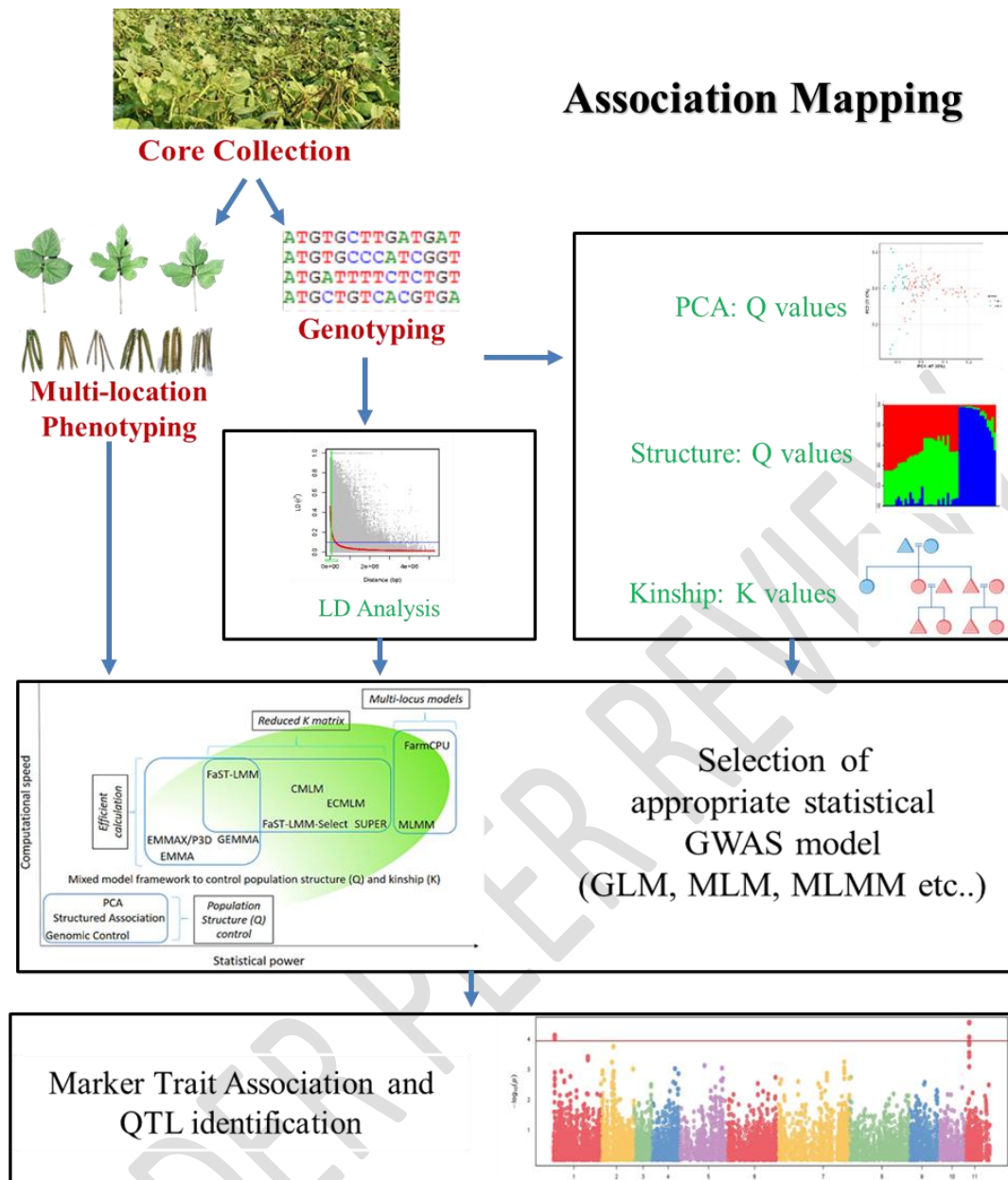
weight QTLs discovered by this GWAS and those discovered via biparental mappings relate to one another.

Mungbean seeds can have a green, black, yellow, or brown seed coat. Many genetic models with up to five genes have been proposed for the inheritance of seed coat colour in mungbean, despite the fact that it is a qualitative feature (Dikshit *et al.*, 2020). GWAS analysis carried out using 18,171 SNP markers on 9 wild mungbean accessions and 209 cultivated from 23 countries and identified a single SNP located in *Vradi04g08950* on chromosome 4 encoding a pectin esterase, which associate with variation in seed coat color red, green, and blue (R, G, B) color (Daovongdeuan, 2017). Using 22,230 SNPs, GWAS was carried-out for seed coat colour variation in 16 wild mungbean accessions and 466 cultivated accessions from different locations and identified 9 SNPs on four chromosomes, which are linked to colour variation (Noble *et al.*, 2018). Five SNPs that are grouped together and cover a 22 kb region located on chromosome 4, harboured a gene that codes for a transcription factor MYB113, which play a role in the synthesis of anthocyanins. This is the putative candidate gene for black colour seeds and SNPs present on chromosome 5 linked with gene coding flavonoid 3'-hydroxylase, which governs yellow seed color in soybean (Noble *et al.*, 2018). Another study utilised GWAS to discover SNPs linked to seed coat colour at locations 4,639,979 and 17,694,108 on chromosomes 2 and 4, respectively (Sandhu and Singh, 2021). Black mungbean seeds have anthocyanins in their seed coats, however green, yellow, and brown mungbean seeds don't (Pandey *et al.*, 1989). Nevertheless, when we looked at the 22 kb region described by Noble *et al.* (2018), they didn't identify any MYB genes. Instead, they have found the MYB gene *Vradi04g08920* (VrMYB16) coding MYB16 and gene *LOC106758748* (VrMYB90) coding MYB90, which are situated around 38 and 10 kb away from the region, respectively. MYB90 and MYB16 belong to R2R3-MYB a transcription factor that controls important biological process in crop plants such as biosynthesis of anthocyanin. Plants produce the colours purple, red, blue, and black owing to pigments called anthocyanins. It has been shown that MYB16 prevents the production of anthocyanins in apples (Xu *et al.*, 2017), While MYB90 has been demonstrated to control anthocyanin synthesis in the tissues of various plant species, including mungbean (Qi *et al.*, 2020; Lin *et al.*, 2022). Gene overexpression, haplotype analysis and Map-based cloning revealed that VrMYB90 is responsible for mungbean hypocotyl anthocyanin production via the up regulation of *VrDFR*, which encodes dihydroflavonol 4-reductase enzyme at the last stage of synthesis, but still gene not caused the black seed coat colour (Lin *et al.*, 2022). Hence, it is conceivable at this stage that *VrMYB16* regulates the mungbean black seed colour. It is interesting that a small amount of black mungbean seeds were included in the germplasm used in the GWAS conducted by Daovongdeuan (2017) and Noble *et al.* (2018), which led to the discovery of false associations. In addition, wild mungbean seeds are typically dull, brownish-black, or black. Nevertheless, when the seed texture is removed, the glossy seed green or mottled green-black colour is visible (Watt *et al.*, 1977; Sarikarin *et al.*, 1999). In light of this, caution must be taken while phenotyping seed coat colours. To identify the exact association between *VrMYB16* and seed coat colour, more genomic research is required.

43 SNP markers from 35 chromosome regions associated to potassium, calcium, iron, sulphur, manganese, phosphorus, and zinc concentrations in seeds were found by the GWAS of a limited sample of germplasms consisting 95 cultivated mungbean accessions (mainly from India and Pakistan) with 6,486 SNPs (Wu *et al.*, 2020). Several of the SNPs had associations with multiple minerals, pointing possible pleiotropic effects. The important SNP markers typically explained between 10 and 34% of the variations in minerals. 26 putative candidate genes linked to numerous biological functions were discovered for mineral uptake, metal translocation and metal binding.

Moreover, the GWAS for mungbean resistance to biotic and abiotic stressors was conducted. In a USDA mungbean collection of 482 accessions, GWAS for resistance to Fusarium wilt disease, caused by *F. oxysporum* was done. 264,550 SNPs identified, 3 loci associated with resistance (Sandhu and Singh, 2021). But, the highest  $R^2$  value among these loci is only 6.8%. In 144 mungbean accessions (mainly from India, but also from Thailand, Taiwan, and Bhutan), a GWAS of phosphorus use efficiency with 55,634 SNPs found 136 SNPs of 77 annotated genes related with response to low-P and optimum-P concentrations in hydroponic conditions; Root architecture and leaf area were associated with 84 and 29 SNPs, respectively (Reddy *et al.*, 2020). GWAS was carried out for salt stress tolerance in minicore collection of World Veg mungbean with 5,288 SNPs, discovered 2 genomic regions on chromosomes 7 and 9 linked with tolerance (Breria *et al.*, 2020). Though, QTL regions about 1 Mb it is very large, gene *Vradi07g01630* coding the ammonium transport protein, was considered to be candidate gene for identified QTL on chromosome 7, although, *Vradi09g09600* and *Vradi09g09510* encoding dnaJ domain proteins and OsGrx\_S16-glutaredoxin subgroup II, respectively. It is believed to be putative candidate genes for QTL on chromosome 9 (Breria *et al.*, 2020). Further, genomics studies should be carried out to clarify the salt stress tolerance mechanism in mungbean because salt stress tolerance is a complicated trait that impacts plants at all stages of growth and development.

Along with above mentioned traits, GWAS has been performed for pod colour, hypocotyl colour, leaf drop at maturity and seed coat lustre (Sandhu and Singh, 2021). These results demonstrate that the GWAS method in crop plant is a reliable and practical approach that complements traditional biparental cross mapping and has the capacity to simultaneously map many traits. GWAS outcomes are probably further utilized to study the genetic basis of plant morphology, physiology and yield. Figure 1 shows the general procedure for the GWAS followed in mungbean.



**Figure 1** General procedure for the GWAS followed in mungbean

#### 4.6. High throughput genotyping and Next-Generation Sequencing (NGS)

Crop genome differences can be identified by genotyping each cultivar separately. The genotype is an individual's full hereditary information, often defined to be the allele pattern at multiple molecular markers. An individual's phenotype is the observable features that are governed by both the genotype and the environment. Classical genetics uses linkage mapping in a recombinant population or association mapping in a natural population to pinpoint the genotype that produces a phenotype.

There are different molecular marker types that correspond to various genotyping strategies. The use of Polymerase chain reaction (PCR)-based markers and then allele scoring on an agarose gel built the framework for quantitative trait locus (QTL) mapping and gene cloning. For instances, The SSRs markers can potentially be used for genetic improvement, testing genetic purity (Harisha *et al.*, 2021) and construct linkage maps of mungbean (Tangphatsornruang *et al.*, 2009); still, all of the SSRs were not allied with linkage groups. However, the genotyping methods that are based on PCR markers are quite difficult, costly, and time consuming, when high-density genotyping is desirable for a huge number of

individuals. Through the improvement in NGS, researchers have concentrated on searching SNPs to be utilised as genetic markers, as SNPs are single-locus, co-dominant, biallelic markers that are ubiquitous and abundant throughout the genome and are freely used for genotyping (Brumfield *et al.*, 2003).

NGS is a technology that enables quick, affordable, and thorough investigation of the genomes of single species and complex populations by allowing the parallel sequencing of millions of small DNA fragments. NGS has transformed genome research across all disciplines and throughout all kinds of organisms. NGS platforms include Ion Torrent, Roche 454, Illumina/Solexa, and SOLiD sequencing, among others. These platforms, which first became accessible in the late 2000s and early 2010s, vary in terms of read length, template preparation, sequencing chemistry, and outputs per run (Metzker, 2009). Parts of the mungbean nuclear genome and transcriptome were sequenced in the early stages of NGS, which resulted in the discovery of a wide range of SSRs and single nucleotide polymorphisms (SNPs) and the development of new SSR markers (Tangphatsornruang *et al.*, 2009; Moe *et al.*, 2011; Van *et al.*, 2013; Gupta *et al.*, 2014). The development of highly dense genetic linkage maps for mungbean using GBS (Elshire *et al.*, 2011) demonstrates the power of NGS in the detection and genotyping of huge numbers of SNPs at a significantly reduced cost (Kang *et al.*, 2014; Hwang *et al.*, 2017; Mathivathana *et al.*, 2019; Ha *et al.*, 2021). Moreover, NGS was used to comprehensively sequence the mungbean chloroplast genome (Tangphatsornruang *et al.*, 2010).

Whole-genome sequencing (WGS) represents NGS's most significant contribution to mungbean genomics. On the chromosomal level, a drafted reference genome of the superior mungbean breeding line "VC1973" from WorldVeg centre was created using Illumina/Solexa and Roche 454 sequencing (Kang *et al.*, 2014). 80% of the projected entire mungbean genome, which is 579 Mb, was sequenced, resulting in 2,748 scaffolds with a N50 length of 1.62 Mb. A linkage map of 1,321 SNP markers was used to construct 11 pseudochromosomes covering 314 Mb, or 73% of the assembled sequence. Only 22,427 protein-coding genes in total were found, of which 18,378 were found on the pseudochromosomes. The entire genome of the wild mungbean accession "TC1966," which is also known as "VC1973A," was also sequenced (423 Mb, or roughly 84% of the expected genome size). Mungbean "VC1973A," "V2984," and "TC1966" genome sequences were compared, and millions of SNPs and hundreds of thousands of indels were found (Kang *et al.*, 2014). SNPs, indels, and drafting genome sequences are excellent tools for mining and mapping genes. The WGS of the breeding line "RIL59" was published, a few years following the release of the draft genome sequence of "VC1973A." (Liu *et al.*, 2016). Illumina sequencing was used to sequence a 455.2-Mb portion (88%) of the RIL59 genome (517.9 Mb), which was then assembled into 2,509 scaffolds with a N50 of 676.7 kb and an average sequence length of 181.4 Mb. There were estimated to be 36,939 genes that code for proteins. Using SALF sequencing, a high-density genetic map comprising of 4180 SNPs spanning 151.39 Cm was constructed and 69-bp sequence deletion was identified in the coding region of *Vradi05g03810*, the gene that encodes a probable resistance-specific protein, was found to be the most likely key candidate gene in mung beans (Chen *et al.*, 2022). In a recent study, haplotype analysis using 124 mungbean accessions indicated that 10 SNPs identified in exon 3 of *VrMYB90* gene responsible of anthocyanin accumulation, may lead to an abolished expression and an absence of anthocyanin accumulation in the hypocotyl of Sulv1 and KPS2 (Lin *et al.*, 2022).

Advanced genomic analysis can be used to systematically discover and define the functions of genes because it is based on a comprehensive and high-quality reference genome sequence. Therefore, the advent of third generation of sequencing, such as single-molecule real-time (SMRT) and nanopore sequencing provides sequence reads with unprecedented

length in less time and with greater accuracy. For the development of contiguous and chromosome-scale genomic sequences, such technology is very helpful (Amarasinghe *et al.*, 2020). Recently, the "Sulv1" mungbean cultivar's high-quality chromosome-scale genome assembly was created by combining second- and third-generation sequencing with Hi-C analysis (Yan *et al.*, 2020). In the Sulv1 genome assembly, 473.7 Mb, or 87.8% of the expected genome size, was assembled. 99.32% of the sequences were assigned to 11 pseudochromosomes, and 33,924 predicted protein-coding genes were found in the scaffold N50 of 42.4 Mb. Using SMRT sequencing more recently, the genomic sequence of VC1973 was improved (Ha *et al.*, 2021). The latest genome assembly has a N50 length of 5.2 Mb and just 0.4% gap, covering a total sequence of 476 Mb from 557 scaffolds. The sequence, which was arranged into 11 pseudochromosomes and comprised 30,958 genes, accounted for 87.5% of the VC1973A genome. A comparison between the new and previous genome assemblies revealed many disagreements between them based on the mapping of common SNP markers. However, comparative genomic research between the newly assembled mungbean genome and the genomes of closely related legumes showed that the new assembly was more reliable than the old one. Mungbean genomes differ significantly in the number of genes identified in VC1973A, RIL59, and Sulv1. There is an advantage to having multiple reference genomes for mungbean when analysing gene discovery and genome structural variations (e.g., presence-absence, copy number, and chromosomal rearrangements). Different genomes of a species differ in gene content and repetitive portions of the genome (Della Coletta *et al.*, 2021). Sulv1 and the wild mungbean TC1966 genome sequences, as well as the improved VC1973A genome sequence, have not yet been made public. Nevertheless, WGS still does not cover a sizable amount (12%) of the VC1973A, RIL59, and Sulv1 genome sequences. In order to maximise genome coverage, further genome sequencing is required. Hence, Pangenome construction may help to solve the issue of mungbean genome variation. Further references mungbean genome sequences are being created and a pangenome project for the mungbean crop has been started by Nair *et al.*, (2019).

Transcriptome sequencing provided by NGS can be used to study functional elements of the genome, molecular constituents of cells and tissues, and phenotypic variations. Prior to 2000, only one mungbean transcriptomics study available. The number of transcriptomics sequencing research on mungbean dramatically rose when the cost of NGS became cheap in the middle of the 2010s (Moe *et al.*, 2011; Chen *et al.*, 2014; Li *et al.*, 2016). The vast majority of these transcriptome investigations were concerned with responses to biotic and abiotic stressors, such as drought, waterlogging, heat, chilling temperature, bruchids (*C. chinensis*), and Fusarium wilt (*Fusarium oxysporum*).

Crops such wheat, maize and barley have big genomes and whole-genome resequencing for these crops is still costly. There are now numerous options to sequencing the whole genome. One procedure is to create restriction-site-associated DNA tags (e.g., using EcoRI or SbfI) and sequence them to find polymorphic markers. Another parallel method is to cut the genomic DNA and ligate the genomic fragments to bar-code adapters to make a multiplex sequencing library and this whole procedure is known a Genotyping-by-sequencing (GBS) (Elshire *et al.*, 2011).

#### **4.7 Genotyping by Sequencing (GBS)**

GBS today offers breeders new opportunities with inexpensive, multiplexed sequencing, and genome-wide scanning platforms (Kim *et al.*, 2015). The broad applicability of GBS techniques for genetic analysis has also been successfully demonstrated in a variety of important crops, including global accessions of rice, maize, sorghum, pearl millet, barley, wheat, and others. However, reports on GBS analysis of mungbean relatively limited and mostly includes investigating population structure and LD in mungbean (Noble *et al.*, 2018; Ha *et al.*, 2021). Today's advancements in bioinformatics, along with the accessibility of

affordable and customizable genomic technologies, make GBS the perfect tool for mungbean genotyping, SNP finding, and genetic diversity mining. Another emerging tool is GBS-SNP-CROP, which uses a clustering strategy to build a population-tailored "Mock Reference" based on the same GBS data used for downstream SNP calling and genotyping (Melo *et al.*, 2016). This shows the importance of GBS technology, which reduces time and money on pre-breeding research projects by giving breeders useful data on population structure, allelic richness, and gene mapping. As a result, GBS technology may be a useful genomic tool for orphan crop cultivar development. However, GBS has limitations in SNP distribution and number (Elshire *et al.*, 2011; Morris *et al.*, 2013).

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**Table 3 : Comparative analysis of the result with different previous study.**

<b>Traits</b>	<b>Parents</b>	<b>Populations</b>	<b>DNA markers</b>	<b>Number of QTLs and name(s)</b>	<b>References</b>
Flowering time	(JP229096 × JP211874)	BC <sub>1</sub> F <sub>1</sub>	SSR	4; Fld5.2.1-, Fld5.4.1-, Fld5.6.1-, Fld5.11.1+	(Isemura <i>et al.</i> , 2012)
	(KUML29-1-3 × W021)	F <sub>2</sub>	SSR	4; Fld2, Fld4.1, Fld4.2, Fld11	(Kajonphol <i>et al.</i> , 2012)
	(V1725BG × AusTRCF321925)	F <sub>2</sub>	SSR	3; DFL4.1, DFL4.2, DFL7.1	(Sompong <i>et al.</i> , 2012)
	(KPS1 × V4718)	F <sub>2</sub>	SSR	5; qDFL2.1, qDFL2.2, qDFL4.1, qDFL5.1, qDFL6.1	(Somta <i>et al.</i> , 2015)
	(VC2917 × ZL)	RIL	SSR	8; qFLD4.1, qFLD4.2, qFLD4.3, qFLD4.4, qFLD4.5, qFLD4.6, qFLD4.7, qFLD4.8	(Liu <i>et al.</i> , 2017)
	(VC1973AG × V2984)	RIL	SNP	2; Dff3-1 (Df3-1, FI4-1), FI4-1	(Hwang <i>et al.</i> , 2017; Ha <i>et al.</i> , 2021)
Dates to maturity	(JP229096 × JP211874)	BC <sub>1</sub> F <sub>1</sub>	SSR	6; Pddm5.2.1-, Pddm5.4.1-, Pddm5.6.1-, Pddm5.7.1+, Pddm5.9.1-, Pddm5.11.1+	(Isemura <i>et al.</i> , 2012)
	(KUML29-1-3 × W021)	F <sub>2</sub>	SSR	3; Pddm2, Pddm4.1, Pddm4.2	(Kajonphol <i>et al.</i> , 2012)
	(KUML29-1-3 × W021)	F <sub>2</sub>	SSR	3; Pddm2, Pddm4.1, Pddm4.2	(Kajonphol <i>et al.</i> , 2012)
	(V1725BG × AusTRCF321925)	F <sub>2</sub>	SSR	3; DMT4.1, DMT7.1	(Sompong <i>et al.</i> , 2012)
	(VC1973AG × V2984)	RIL	SNP	2; SPM4-1, SPM7-1	(Ha <i>et al.</i> , 2021)
Plant height	(JP229096 × JP211874)	BC <sub>1</sub> F <sub>1</sub>	SSR	6; Stl5.1.1+, Stl5.2.1+, Stl5.3.1+, Stl5.6.1+, Stl5.9.1-, Stl5.10.1+	(Isemura <i>et al.</i> , 2012)
	(KPS2 × NM10-12)	RIL	SSR, AFLP	4; qPH1.1_combined, qPH2.1_combined, qPH3.1_combined, qPH4.1_combined	(Somta <i>et al.</i> , 2014)
	(VC2917 × ZL)	RIL	SSR	16; qPH2.1, qPH3.1, qPH4.1, qPH4.2, qPH4.3, qPH4.4, qPH4.5, qPH4.6, qPH4.7, qPH5.1, qPH5.2, qPH5.3, qPH5.4, qPH8.1, qPH8.2, qPH8.3	(Liu <i>et al.</i> , 2017)
	(VC1973AG × V2984)	RIL	SNP	2; Height4-1, Height5-1	(Ha <i>et al.</i> , 2021)

Seed dormancy	(Berken × ACC41)	RIL	RFLP	4; HsA, HsB, HsC, HsK	(Humphry <i>et al.</i> , 2005)
	(JP229096 × JP211874)	BC <sub>1</sub> F <sub>1</sub>	SSR	4; Sdwa5.1.1+, Sdwa5.2.1+, Sdwa5.3.1+, Sdwa5.4.1+	(Isemura <i>et al.</i> , 2012)
	(KPS2 × ACC41)	F <sub>2</sub>	SSR, InDel	2; Sdwa5.1.1+, Sdwa5.1.2+	(Laosatit <i>et al.</i> , 2022)
Pod shattering	(JP229096 × JP211874)	BC <sub>1</sub> F <sub>1</sub>	SSR	2; Pdt5.1.1-, Pdt5.7.1-	(Isemura <i>et al.</i> , 2012)
Seed weight	(TC1966 × VC3980A)	F <sub>2</sub>	RFLP	4	(Fatokun <i>et al.</i> , 1992)
	(Berken × ACC41)	RIL	RFLP	11; swA, swB1, swB2, swD, swE1, swE2, swF, swG, swI, swJ, swK	(Humphry <i>et al.</i> , 2005; Mei <i>et al.</i> , 2009)
	(JP229096 × JP211874)	BC <sub>1</sub> F <sub>1</sub>	SSR	7; Sd100wt5.1.1+, Sd100wt5.1.2+, Sd100wt5.2.1+, Sd100wt5.3.1+, Sd100wt5.7.1+, Sd100wt5.8.1+, Sd100wt5.11.1+	(Isemura <i>et al.</i> , 2012)
	(KUML29-1-3 × W021)	F <sub>2</sub>	SSR	6; Sd100wt2.1, Sd100wt2.2, Sd100wt4.1, Sd100wt8.1, Sd100w9.1, Sd100wt11.1	(Kajonphol <i>et al.</i> , 2012)
Seed yield	(KPS2 × NM10-12)	RIL	SSR, AFLP	3; qSY2.1_combined, qSY3.1_combined, qSY9.1_combined	(Somta <i>et al.</i> , 2015)
	(VC2917 × ZL)	RIL	SSR	4; qYLD1.1, qYLD4.1, qYLD8.1, qYLD8.2	(Liu <i>et al.</i> , 2017)

#### **4.8 Validation: A necessary adoption for future GWAS**

In spite of strong theoretical foundation and efforts to eliminate undesired noise (population structure) and use stringent probability cut-offs, false-positive associations will still happen owing to the huge number of statistical implications and other unaccounted factors such as small population size (Finno *et al.*, 2014), low-accuracy genotype calling at some loci (Browning and Yu, 2009), and synthetic associations. This necessitates an independent validation procedure, which is usually included in GWAS design. There are at least two approaches used in validation: one validating possible candidate's gene in different population, the associations would be considered to be more probable when found in separate studies and the other is by employing laboratory tests such as candidate gene over-expression, knock-out, genetic complementation, T-DNA mutants and targeting induced local lesions in genomes (TILLING) analysis of candidate genes at GWAS loci in crops using artificially induced mutants (ethyl methane sulfonate induced mutants or radiation-induced mutants) may be an effective method. Recently, to definitively identify causal genes and causal variations, additional experimental research, including transgenic analyses, will be required. Currently, association mapping in various panels or linkage mapping in RIL populations or F<sub>2</sub> populations are used to ensure cross-population validation. For example, consider the association mapping of rice chlorophyll content in a diverse panel of 529 individuals and three specialized F<sub>2</sub> populations to validate GWAS signal (Wang *et al.*, 2015). Further cross-population study could be stretched to cross-species analysis for homologous or analogous traits. This could enhance understanding of the differentiated, conserved and dynamics genetic architecture for any trait under study and these cross-species analyses has already been applied to rice (Huang *et al.*, 2011) and other cereals (Chen *et al.*, 2016; Liu *et al.*, 2017).

Beyond statistical implication, genetic and molecular testing is a trustworthy method to verify GWAS signals. Due to the poor throughput and case dependence of most wet experiments, this is still challenging. Moreover, new genome-editing tools promise a high-throughput strategy that is efficient. Recently, utilising the CRISPR/Cas9 technology in rice, two Chinese teams concurrently developed genome-wide targeted mutant libraries (Lu *et al.*, 2017; Meng *et al.*, 2017). Combining high throughput forward and reverse genetics methods, it is easier than ever to identify the functional gene within each GWAS peak. This information will be extremely helpful in addressing the crucial biological challenges.

#### **4.9 GWAS for Characterize non-phenotypic variation in natural accessions**

Apart from the phenotypic characteristics of ecological, economic interest other molecular components represent the inherent variation across individuals. Non-phenotypic variation could consist of the following, diversity in gene expression level, DNA methylation, and metabolite accumulation, which could influence phenotypic modifications via complicated pathways (Ferne *et al.*, 2009). Our understanding has amplified over the past decade as a result of research to characterize non-phenotypic variation in natural accessions. GWAS is the best method for finding eQTLs, methylation QTLs (mQTLs) and loci for different metabolism products. SNP data with the quick development of RNA-seq and MethylC-seq technologies have made it possible to map eQTLs and mQTLs at a scale that was previously unimaginable. In GWAS for eQTLs, the expression level of each gene is comparable to the phenotype data in an ordinary GWAS. Study conducted in *Arabidopsis* and maize revealed that a significant portion of the eQTLs were caused by polymorphisms in the gene or nearby (known as cis-eQTLs) (Li *et al.*, 2013; Schmitz *et al.*, 2013). On the other hand, several eQTLs were located in other parts of the genome (referred to as trans-eQTLs). In maize RNA was extracted from immature seeds and used to determine the gene expression levels using RNA-seq technology. In order to find potential cis-eQTLs a GWAS was conducted to search for associations between the expression level of each associated gene and nearby SNPs.

Among the GWAS loci, 41 show statistical association between SNPs and expression levels. These alterations in expression levels may reason for a significant amount of diversity in economic traits. Consequently, the finding of allelic expression differences and phenotypic variation will enhance our knowledge. DNA methylation is a covalent base alteration of nuclear genomes that can be precisely transmissible through cell divisions. In plants species, DNA methylation takes places on cytosines at CG, CHG, and CHH sites (where H = A, T, or C). Level change in DNA methylation across diverse natural accessions denoted to as natural epigenetic variation. It is believed that single-methylation polymorphisms are the most prevalent type of natural epigenetic variation. In a study, the genomes and methylomes of hundreds of different *Arabidopsis thaliana* accessions were sequenced population-wide. It was discovered that CG, CHG, and CHH single-methylation polymorphisms made up 23%, 13%, and 64% respectively, of all single-methylation polymorphisms (Schmitz *et al.*, 2013). After that, a GWAS was carried out to correlate the genetic and methylation variations. Association studies of the DNA methylation levels with genetic loci recognized 2,739 significant mQTLs. To better understand the genetically dependent methylation variation and the underlying mechanisms more research will be required.

## 5. Conclusion

The rich genetic resources of mungbean crop wild relatives like *V. umbellata*, *V. trilobata*, *V. glabrescens*, *V. radiata* var. *sublobata*, *V. mungo* var. *sylvestris*, *V. aconitifolia*, *V. marina*, etc., can play a very important role in the crop improvement. Conserved diversity, genomic resources, and advanced genomic tools are of immense importance in achieving the potential yield. Improving complex multigenic characteristics and simultaneously selecting for many traits may be more effective using genomic selection than with markers identified in QTL studies.

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