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Mungbean (*Vigna radiata* (L.) R. Wilczek): Progress in Breeding and Future Challenges

Anil Kumar [a*], P. Yadav [a], Seema [b], R. R. Kumar [c], A. Kushwaha [d], Md. H. Rashid [a], N. Tarannum [a] and S. Chakraborty [a]

[a] P.B.G, B.A.U., Sabour-813210, Bihar, India.
[b] Agronomy, B.A.U., Sabour-813210, Bihar, India.
[c] MBGE, B.A.U., Sabour-813210, Bihar, India.
[d] Foods and Nutrition, CoHS, GBPUAT, Pantnagar, Uttarakhand, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors AK, PY and RRK wrote the first draft of the manuscript & final shaping of the manuscripts. Author AK managed literature searching and revision of manuscript and authors MHR, NT and SC managed the literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Mungbean (*Vigna radiata* (L.) R. Wilczek) is a short duration farmer preferred warm-season pulse crop. The crop has shown a balanced growth worldwide, especially in developing countries. Mungbean being a great source of protein with higher folate and iron levels attracts high demand and price on the market making the farmers happy and satisfied. Moreover, it can fix atmospheric nitrogen through symbiosis with nitrogen-fixing bacteria, making it perfect for rice based cropping systems and intercropping with other crops. Despite having so many benefits, mungbean has been a neglected crop compared to other pulses with limited efforts aiming at its breeding and development. Higher productivity, breeding for biotic and abiotic stresses resistance and nutritional quality improvement are future challenges for mungbean breeders. Several researchers are working in the direction of collecting and maintaining mungbean genetic resources. Mutation breeding and genetic engineering has also been enhanced in mungbean varietal improvement. Genomic information is limited compared to other legume species. However, the recent successful sequencing of mungbean genome has opened new vistas into the crop's R&D. It is a self-pollinated pulse with small genome size, which could be used as a model for studying other legumes.

**Corresponding author: E-mail: dranilbau@gmail.com;*

Mungbean breeders at present times aim to identify useful alleles from diverse germplasm and markers closely associated with desirable traits. The high-throughput marker genotyping system has now made it feasible to pinpoint the exact gene locations and mutations contributing target phenotypes. In this review we present the current status of conventional and molecular breeding of the crop and summary of efforts in the utilization of genetic information and genomic resources for further mungbean improvement.

Keywords: Mungbean; functional and translational genomics.

1. INTRODUCTION AND IMPORTANCE

Mungbean (*Vigna radiata* (L.) R. Wilczek), commonly known as green gram, is an essential legume crop grown and consumed globally. It is a rich source of protein, carbohydrates, starch, vitamin C,isoflavonoid (vitexin), folate, iron and zinc [1,2]. It is believed that farmers prefer this pulse even in the present times of climate change due to its three benefits *viz*., nutrition, benefit to soil and short duration [3,2]. In addition to this, mungbean has low inputs requirement, is suitable for cereal-based crop rotation and inter-cropping with sugarcane and maize and with good performance under heat and drought stress. Considering these benefits, the demand for this pulse has increased. Various varieties of mungbean are used differently worldwide based on consumer requirement of a particular region. They are used as dry grains (dahl), vegetable bean sprouts, flour, soups, porridge, transparent noodles made of starch, mungbean paste used as an ingredient of sweets and cakes.

2. PRODUCTION STATUS

Mungbean is suitable for semi-arid to sub humid lowland tropics and subtropics with average annual rainfall of 600–1000 mm. Being grown during the warm season in absence of frost condition, it has a crop duration of 90–120 days from planting to maturity. The optimum temperature for planting is 15 °C and 20–30 °C during crop growth [4]. The flowering of the crop gets affected by day length. Short days accelerate flowering, while long days delay them [5]. Water stress during the reproductive stage has negative impact on flowering leading to decreased total yield [6]. Excessive rainfall increases humidity making congenial conditions for diseases and pest infestation [4].

It is mainly grown by farmers of South, East, and Southeast Asia with small land holdings. Although, India is the largest producer and consumer of mungbean accounting for >50% of global annual production, the average yield is merely 420 kg/ha which is very low compared to global average of 721 kg/ha [7]. Over the past few years, area of mungbean in India has increased, currently being approx. 3.8mha giving produce of about 1.6 mt. Among the Indian states, Rajasthan (31%) leads in area and production, followed by Maharashtra (11%). Myanmar being world's largest exporter of mungbean accounts for 70% of mungbean imported from India. There is 1.2 m ha area of the crop in Myanmar currently giving almost equal produce compared to India (1.59mt).

2.1 Taxonomy, Genetic Resources and Domestication

Mungbean is a self-pollinated diploid crop with chromosome number of 2n=2x=22 [8]. This warm-season, frost intolerant, fast-growing pulse crop belongs to the genus *Vigna,* subgenus *Ceratotropis* in the papilionoid subfamily of the Fabaceae. The key aim of breeders is to accumulate beneficial alleles from various parental lines into a new plant variety. Therefore, the availability of mungbean genetic resources having rich genetic variation is fundamental for success of crop improvement programs. The first step in finding superior alleles or the individuals carrying them is to secure a germplasm pool with high genetic diversity. A number of research institutes have been established for mungbean germplasm conservation activities and cultivar development. Germplasm of mungbean is being maintained at AVRDC-The World Vegetable Centre, Taiwan; National Bureau of Plant Genetic Resources (NBPGR) of the Indian Council of Agricultural Research (ICAR); the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences; the Plant Genetic Resources Conservation Unit of the University of Georgia, USA and the University of the Philippines [9]. AVRDC- World Vegetable Center, Taiwan, holds the largest *Vigna* germplasm collection of the world made up of 11,832 accessions of mungbean (10,673 *Vigna*species, 881 *V. angularis*, 278 *V. unguiculata*), as vital resources for cultivar

improvement through inter-specific hybridization. The institute maintains a core collection of 1481 accessions with a mini-core collection of 296 accessions based on phenotypic and molecular characterization [10]. On the basis of archaeological evidence, diversity data and morphological studies, it is believed that mungbean was domesticated in India ~3500 years ago [11,12,13]. The wild form of mungbean, *Vigna radiata* var. *sublobata*, is however, indigenous to the subtropical and tropical regions of Australia and widely distributed throughout Africa and Asia [14]. Studies based on protein variation and enzyme diversity conclude that modern mungbean cultivars are a result of multiple rounds of domestication [15].

2.1.1 Current state of mungbean breeding

Indeterminate growth habit, late and non-synchronous maturity, abiotic and biotic stresses are factors that cause depletion in mungbean harvest index [16,17]. Slightly delayed harvesting also causes major yield loss as mature and dried pods shatter and get exposed to pest and pathogen attack. Multiple harvests are performed to prevent such loss which results in additional costs beared by producers. Thus, synchronous maturity is a key objective of mungbean breeding programs that can result in cost-effective harvesting. Although, the genetic basis of this trait is still unknown [18,19].

Elaborate on the biotic and abiotic stresses as well Mungbean breeding also aims at resistance to biotic stresses mainly diseases like powdery mildew, *Cercospora* leaf spot, mungbean yellow mosaic virus (MYMV) and insect pests like mungbean pod borer, bruchids and bean flies [20]. Do we have any information on the genetic basis of resistance to the diseases and insect pests in mung beans that you have mentioned? Certainly yes, just make a brief of what is known

2.2 Traditional Breeding

In any crop species, wild resources are mined and utilized for useful genes as the cultivated germplasm has lost a number of useful alleles during domestication andmodern breeding programs [21,22,23]. Abruchid-resistant mungbean cultivar, indicate code or name of that cultivarwas successfully developed utilizing TC1996, a wild mungbean relative resistant to bruchid beetles (*Callosobruchuschinensis* and *C. maculatus*) [24,25]. Also, MYMV resistant cultivars (indicate their code names or cultivar names if possible) have been developed from *Vigna mungo*, another wild relative species of mungbean [26,27]. Such success stories have made researchers to further realize the importance of conserving and maintaining germplasm in order to sustain mungbean genetic resources.

2.3 Molecular Marker-Assisted Breeding

Molecular markers are tags that help breeders to identify loci or genomic regions associated with desirable traits. Finding the phenotypic trait governed by a segment of DNA through the use of molecular markers is more accurate compared to traditional breeding [28]. Be it genetic diversity studies, construction of linkage maps or identifying QTLs for desirable traits, molecular makers are always preferred as they are cost effective, precise and reduce time and input resources. The recent availability of whole-genome sequencing andhigh-throughput genotyping systems have aided molecular genetics studies [29,30]. Markers have been used for many resistance studies in mungbean and have been successfully deployed in mungbean improvement. Protein markers were used for studies like phylogenetic relationship between *Vigna* species, detection of polymorphism between MYMV-resistant and susceptible genotypes [31,32]. Since these markers were limited in quantity, researchers gave DNA-based markers upper hand. Restriction fragment length polymorphism (RFLP) markers were the first to be used in mungbean for traits like the genetics of bruchid resistance, seed weight, seed size and powdery mildew [33,34,35]. Molecular marker studies in mungbean became common with the introduction of random amplified polymorphic DNA (RAPD) markers. They were used to assess genetic diversity in germplasm, cultivars and map biotic resistance traits like MYMV and bruchid beetles [36,37,38,39]. Utilizing an F_2 mapping population (cross between *V. radiata*ssp. *radiata*and *V. radiata*ssp. *sublobata)*, Lambrides et al. [40] integrated RFLP and RAPD markers to construct a genetic map having 12 linkage groups. Chattopadhyay et al. [41] used a combination of RAPD and inter simple sequence repeat (ISSR) markers to assess genetic diversity in the crop. The AFLP markers further improved diversity and trait mapping studies [42-44].

Simple sequence repeat (SSR) markers are highly reproducible and informative markers of breeders' choice as they are co-dominant, highly

polymorphic and easy to generate. The first SSR markers reported in mungbean were generated from 6 SSR sequences with 5 different types of motifs [45]. Adzuki bean shares close phylogenetic relationship with mungbean. Thus, the SSR markers from adzuki bean were successfully detected during genetic diversity assessment various cultivated and wild mungbean accessions with higher allelic polymorphisms in the latter [46].The SSR markers were then generated from *Vigna* species genomic and transcriptomic sequences [47,48,49,50]. A genetic map with 150 SSR markers across 11 linkage groups was constructed in mungbean [51].SSR markers were also used in mungbean with partial linkage maps to identify traits like resistance to powdery mildew, *Cercospora* leaf spot, phytic acid content, etc., [52,53,54] and assess germplasm genetic diversity [55]. Prior to the emergence of high-throughput genotyping and next generation sequencing (NGS), limited polymorphic genetic markers were available to quantify population genetic diversity. Limit in the number of markers can create biased QTL identification due to inappropriate allelic diversity representation in genome [56]. SNPs are now most preferably used genetic markers as they are single base, biallelic, co-dominant and uniformly distributed across the genome [57]. Transcriptome sequencing and Illumina Hi-Seq sequencing were utilized for study of two mungbean cultivars (Seonhwanogdu and Jangannogdu) genomes to study resistance to stink bug (*Riptortusclavatus*) and adzuki bean weevil (*Callosobruchuschinensis*) [58,59]. Single-nucleotide polymorphic (SNP) markers were detected in mungbean followed by the completion of whole genome sequence of cultivar VC1973A [60]. Following the draft genome assembly, transcriptome assemblies were analysed from 22 accessions of 18 *Vigna* species providing better insights into the evolution of *Vigna* species. Utilizing a high-density genetic map, a total of 2748 scaffolds were anchored on 11 pseudo-chromosomes spanning 431 Mbp. This genotyping-by-sequencing (GBS) based genetic map comprised of 1321 SNP markers developed from an F_6 recombinant inbred lines (RILs) population. The population had 190 RILs derived from a cross between Seonhwanogdu (*VC1973A*) and Gyeonggijaerae5 (V2985). Estimated genome size covered in this map is about 80% (579 Mbp). Also, 22,427 high-confidence protein-coding genes were annotated which is great compared to previous low-resolution linkage maps and fragmental genomic sequence information. Across the mungbean genome, a total of 2,922,833 SNPs were revealed in wild and cultivated varieties with 6.78 per 1 kbp frequency. 63,294 SNPs were located in protein-coding sequence (CDS) regions, while 30,405 showed non-synonymous changes. Moreover, from 342,853 insertions/deletions (InDels), 55,689 were located around genic regions, causing frame-shifts in a1057 genes. These developments have increased the likelihood of vast number of markers' production which can lead to more projects of GBS and re-sequencing exploring new dimensions in the crop [60,55]. It has improved the state of mungbean breeding.

2.4 Functional and Translational Genomics

The transcriptome data and molecular markers developed from it are highly informative as they are based on variations present in expressed genome regions. After the use expressed sequence tag (EST) sequences in crops, data mining has become a fast, cost-effective way of developing markers contributing to functional genomics studies in mungbean. Moe et al. [58] used 12,596 EST sequences from cv. Jangannogdu that identified 2299 SSR motifs in 1848 EST sequences. 97 PCR primer sets were successfully designed and amplified in two mungbean cultivars, TM96-2 and TARM-18 from the same which contained approx. 45% and 55% SSR motifs located in CDS and untranslated regions (UTRs), respectively. The NCBI mungbean database was used to identify EST-SSR markers via data mining without bearing extra sequencing costs [61]. An SSR-enriched library was constructed from six mungbean genotypes *viz.* ACC41, VC1973A, V2709, C01478, C01558, and C01579 that identified 308,509 SSR motifs [62]. To characterize and validate SSR markers detected from in-silico EST-SSR markers, the mungbean transcriptome was sequenced using Illumina paired-end sequencing [50]. These studies resulted in the development of various markers leading to advances in linkage map and QTL mapping analysis, thus facilitating mungbean breeding.

Translational genomics can be defined as the complete study of genomic information from a species utilized to analyse other species [63]. Genomic information can be applied from model species to crops that have rarely been studied previously, thus helping breeders improve various other crops with ease [64]. Currently post

the genomic sequences for mungbean has been available, studies are being performed to characterize the mungbean genome using translational genomics. Genome-wide comparisons between mungbean and *Arabidopsis* identified flowering genes in mungbean [65]. Out of 207 flowering genes in *Arabidopsis,* 129 genes were found homologous to mungbean genes. Also, comparison between mungbean and soybean genomes identified five putative flowering-related genes of mungbean in homology with soybean flowering genes [65]. Comparative and synteny analysis between mungbean and soybean found several mungbean QTLs and putative genic loci associated with important traits *viz.* plant height, flowering, seed weight, seed oil content, seed size/germination, etc. (60,9, 66]. Translational genomics studies in mungbean will expedite molecular studies leading to functional characterization of desirable genes.

2.5 Genetic Transformation and Regeneration in Mungbean

Traditional breeding has achieved limited success in developing new varieties of mungbean owing to narrow genetic variation in the crop. Similar set of parental lines have been used repeatedly for varietal development. In addition to the effective utilization of germplasm, biotechnological tools are also a powerful tool to overcome drawbacks in development of elite mungbean breeding lines. Tissue culture allows regeneration of plants from transformed plant cells [67]. First genetic transformation report in mungbean was conducted by Jaiwal et al. [68] using hypocotyls and primary leaves. A generalized plant regeneration protocol using primary leaf explants was developed in mungbean showing ~90% survival rate [69]. Cotyledonary node explants were used for the successfully expression of the insecticidal α-amylase inhibitor-*1*gene from and the bialaphos resistance (*bar*) gene in mungbean [70]. Improvement for other important mungbean traits from various crops like *Phaseolus vulgaris*, mustard have been worked out in different studies for traits like disease resistance, drought and salt stress tolerance [71,72,73]. Regeneration protocols have been reported in mungbean through embryogenesis and organogenesis [74,75, 76,77]. Very few regeneration studies which used cotyledonary node explants were successful [78] (Amutha et al. 2006) [72,79]. Limited studies could report production of stable transformed mungbean transgenic having stably inherited transgenes [69,70,71,72,73]. The recalcitrant nature in mungbean tissue culture is the reason for lagging of transgenics report in the crop [80,78]. Application of recent biotechnological tools like genetic engineering, clustered regularly interspaced short palindromic repeats (CRISPR) to food crops can be positive tools in mungbean cultivar development overcoming the limitations of traditional breeding [81].

2.6 Mutation Breeding

In case of traditional breeding methods, the key bottleneck to enhancing yields in mungbean is the low genetic variability in germplasm and continuous decline in the genetic diversity (add reference). Thus, artificial mutations provide the way forward for crop improvement. Physical and chemical mutagens are considered effective ways to induce mutations subsequently creating genetic variability. Several mutagens like ethyl methane sulfonate (EMS), sodium azide (SA), hydrazine hydrate (HZ) and gamma rays have been used in mungbean. Various mungbean genotypes treated with EMS and gamma rays lead to new cultivars with higher yields and increased resistance to bean fly infestation [82,83]. The use of EMS and HZ as mutagens in mungbean showed significant increase in the number of branches, pods and seed yield in mutants [83]. Mutants obtained from SA, EMS, and gamma rays displayed a wide range of morphological and physiological features like variegated leaves, synchronous pod maturity, etc [84,85,86].

Mungbean suffers serious yields losses from pathogens like viruses, fungi, bacteria and nematodes affecting tissues including seeds, leaves, flowers, roots and stems. Seed germination, shoot development, flower development are other detrimental pathogen impact ultimately reduced yields. MYMV is one of the most studied mungbean viruses with its full reference genome available [87]. The MYMV-infected plants show yellow discoloration leading to approx. 85% yield losses [88]. Development of fully-resistant cultivars hasn't been successful yet in mungbean, although targeted mutagenesis and sequencing techniques can be a path forward for breeders to identify the casual variations induced by mutagens via forward genetics analysis [60]. The cultivars released through mutation breeding increases genetic diversity by being used as breeding materials for conventional plant breeding leading to the

genetic improvement of mungbean. Mutation-assisted plant breeding is capable of creating customized crop cultivars to sustain in the era of global climate change and food insufficiency. So do we have any examples of cultivars released resulting from mutations?

3. CONCLUSION AND PROSPECTS

Due to its farmer preference and high nutritional content, mungbean has gainedits importance among other pulses. Earlier mungbean received less attention being a crop of interest only for developing countries and the lack of genomic information. The reference genome sequence of mungbean published in 2014 encouraged breeders and researchers to study the genetic and genomic backgrounds of ergonomicallyimportant traits in the crop. In recent times, there has been subsequent increase in genomic resources of mungbean, thus generating huge amount of valuable data. Various markers and QTLs have been developed and identified based on the currently available genomic information and phenotypic data. Researchers are screening valuable resources to find QTLs and locating casual genes for traits of their interest. Furthermore, the germplasm collections can be utilized for investigating allelic variations; genome-wide association studies (GWAS) to locate candidate genes. Mungbean has all the properties to be a model plant system for carrying out genomic studies in legumes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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