Review Article

Biotechnological initiative for management of Mungbean Yellow Mosaic Virus in Mungbean

Abstract

Mungbean (*Vigna radiata)* is one of the most important legume crops of Asiatic region. The average yield of mungbean is quite low due to its susceptibility against mungbean yellow mosaic virus (MYMV). Mungbean yellow mosaic virus disease (MYMD) is caused by MYMV, which is transmitted through whitefly (*Bemisia tabaci*). The controlling of this devastating disease is mainly depends upon spraying of insecticides, which cause serious ill effect on humans and soil health. Breeding for its resistance is one of the best strategies for developing MYMV resistant genotypes in mungbean. Several types of molecular markers have been used in marker assisted breeding (MAB) in mungbean. Among them SSR markers are widely used and a plethora of scientific reports advocates the use of the SSR marker in developing MYMV resistnace in mungbean.

Keywords: Mungbean [*Vigna radiata*], Mungbean Yellow Mosaic Virus (MYMV), whitefly (*Bemisia tabaci*), SSR, Marker Assisted Breeding (MAB), RNAi, CRISPR-Cas9

Introduction

The mungbean [*Vigna radiata*] is one of Asia's most important pulse crops. Pulses are the main source of protein in the traditional vegetarian diet of the Indian population, ranking second after cereals and behind chickpea and pigeonpea. Greengram, also known as mungbean, has been grown in India for centuries. Southern China, Indochina, and Java were among the first places where it was introduced. It's been brought to East and Central Africa, the West Indies, and the United States in recent years. Grown in the irrigated northern plains in the spring and as a rabi crop in the southern and south-eastern parts of the country, where the winters are mild. The grains (whole or split) are used to make dal or flour, cattle feed is made from straw and husk, sprouts are made from germinated grains are also useed. MYMD is caused by the mungbean yellow mosaic virus (MYMV), which is propagated by whiteflies (*Bemisia tabaci*) and spreads quickly in the presence of a vulnerable host, a favourable environment, and a virulent vector. "The economic losses due to this virus account up to 85% in mungbean which is spreading faster towards newer areas" (Karthikeyan et al., 2014).

The initial signs of the disease appear as yellow specks or dots on immature leaves. Bright yellow patterns are intermingled with green sections on the leaf coming from the tip. In severe cases, the leaves turn completely yellow, and the infected plants become stunted, bearing few flowers and pods, and maturing late. In naturally infected sensitive cultivars, yield losses varied depending on the timing of infection. The symptoms of early infected plants were more severe than those of late infected plants. Plant height, fresh shoot weight, yield per plant, and seed weight all decreased as a result of chlorosis, stunting, and reduced branching. Shape, size and appearance of pods and seeds of diseased plants were considerably distorted, although seed germinability was unaffected. The degree of MYMV infection was adversely linked with yield and its components. MYMV infection of mungbean has a strong indirect influence on yield via lower seed weight, according to pathcoefficient analysis (Singh et al.). Host plant resistance has always been considered as the most practical and environment friendly approach for the effective management of MYMD (Mungbean central, 8th edition 2021), both male and female whiteflies can transmit the virus, but female adults were four times more efficient than male adults (Costa, 1976). MYMV is a ssDNA(+/-) virus that belongs to the Geminiviridae family. MYMD is a

major disease in northern and central parts. The integrated pest management strategy consists of cultural, mechanical, biological and chemical control measures.

In India, “MYMV was first reported from the mungbean fields of Indian Agricultural Research Institute (IARI), New Delhi during 1950s” (Nariani, 1960). Nariani was first to report that the disease is caused by virus and the virus was named as mungbean yellow mosaic virus (MYMV) by Nene (1968). Viral particles were observed by Thongmeearkom et al. (1981) and purified latter on by Honda et al.(1983). First complete sequence of MYMV was isolated from mungbean originating from India. In western or southern India, Thailand and Indonesia, MYMV is the most common isolate affecting mungbean crops. In central, eastern or northern India, Pakistan, Bangladesh, and Nepal, MYMV is the most common isolate affecting mungbean crops.

Mungbean Production and Area

The world mungbean area is about 7.3 million hectares and the world production is about 5.3 million tons (2015-17), India and Myanmar produce about 30%, China 16% and Indonesia 5%. India is its primary origin and is mainly cultivated in East Asia, Souteast Asia and the Indian subcontinent. It is the third important pulse crop of India grown in nearly 16 percent of the total pulse area of the country. It contains protein rich seed with 20-25 percent protein and sometimes plants are cut and plouhed into the soil to enrich. India is the major producer of mungbean in the world and grown in almost all the states. It is grown in about 4.52 million hectares with the total production of 2.56 million tonnes with a productivity of 566 kg/ha and contributing 10 percent to the total pulse production.

Table 1: Area and production of mungbean in India (source: Greengram Outlook Report- January to May 2021)

Year	Area (million hectares)	Production (million tonnes)
1980-81	2.85	0.98
1090-91	3.36	1.37
2000-01	3.00	1.00
2010-11	3.60	1.80
2015-16	3.80	1.60
2018-19	4.76	2.37
2020-21	4.52	2.56

Important mungbean producing states in the country are Rajasthan, Madhya Pradesh, Maharashtra, Karnataka, Bihar and Andhra Pradesh (Table 2). Bihar ranks 5th in mungbean production with 1.18 lakh tonnes under an area of 1.69 lakh ha with productivity of 698 kg/ha. Rajasthan and Madhya Pradesh states showed an increased in the area grown over the two decades while the states Andhra Pradesh, Bihar, Karnataka and Maharashtra showed an decreased area grown over two decades. The reason behind the decline in mungbean production is that the improved irrigation facilities, which allows to grow intensive crops such as rice and wheat. So, the government is incentivizing Minimum Support Price (MSP) of mungbean which increased from Rs. 4350 in 2014-15 to Rs. 6000 in 2020-21.

Table 2: Area (1000 ha) and production (1000 tonnes) of major producing states of mungbean in India (source: Greengram Outlook Report- January to May 2021)

	2000-01		2010-11		2015-16		2018-19	
Name of states	Area	Production	Area	Production	Area	Production	Area	Production
Rajasthan	458	79	1050	653	1364	596.9	2466.78	1222.23
Madhya Pradesh	90	23	99	35	295	131.2	291	280.25
Maharashtra	714	244	558	374	366	69	481.1	203.79
Karnataka	451	185	402	111	348	43.9	421.04	142.57
Bihar	187	108	172	104	169.2	94.4	169.63	118.45
Andhra Pradesh	520	184	167	52	212	137	121	84.71

Molecular Marker

Using conserved nucleotide sequence information from known candidate genes from other plant species, such markers might be constructed. Only a few markers for viral resistance genes have been found in crop species in recent years. In this study, functional markers were developed to screen Mungbean Yellow Mosaic Virus (MYMV) resistant mungbean germplasms for use in MYMV resistance introgression through molecular breeding, avoiding the labor intensive anmethod consuming phenotyping procedure used to screen MYMV reaction using artificial feeding.

Single Sequence Repeats (SSRs) are quickly becoming a type of DNA marker preferred over Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) in its use for the analysis of genetic material, because SSR markers are locus specificity and their high reproducibility, widely dispersed throughout the genome, ease of PCR analysis, highly polymorphic due to variation in repeating units, constitute a powerful tool for genetic analysis, and highly informative due to the co-dominant nature. SSRs are therefore the markers of choice for genetic studies because of their hypervariability, their codominant nature. Chen et al., 2015 developed 500 novel SSRs (eSSRs) based on expression sequence tags (ESTs) and genomic SSRs (gSSRs) from mung bean transcriptome and genomic sequences for evaluation of diversity (Akbergenov et al., 2006).

The amplified bands generated by SSR, PCR amplification were evaluated based on the presence (1) or absence (0) of bands for each primer and were used to calculate a genetic similarity matrix using the coefficient of Jaccard similarity using numerical taxonomy and multivariate system analysis (NTSYSpc) version 2.1. Cluster analysis was performed on molecular data using the unweighted pair group method using the arithmetic mean Algorithm (UPGMA), from which dendrograms describing the similarity between varieties were extracted and plots using NTSYSpc markers. The present study found that SSR markers are very effective in detecting genetic diversity present among the genotypes studied and correlate with the disease resistance phenotype. Investigation, an attempt was made to identify the mungbean resistance genotypes against MYMV and molecular characterization of mungbean genotype with SSR markers. “The result showed the presence of narrow genetic diversity in Indian mungbean

genotypes and would help the mungbean breeders in selection of suitable parents for breeding purposes and genetic mapping studies"(Akbergenov et al.,2006).

Mechanism of MYMD caused by MYMV

The whitefly (*Bemisia tabaci*), an insect vector for MYMV, transmitted MYMD to the mungbean crop. The whitefly *(Bemisia tabaci*), a polyphagous pests of Asian descent that caused problems on over 1,000 plant species not only by having to suck the sap but also by serving as a vector for several infectious infections. It has the ability to disseminate over 300 viral species from several virus genera, including 90% of the Begomovirus. While feeding on the plant's phloem sap, the whiteflies' mouthparts are adapted to retain the virus through their stylet. After entering the vector, the virus circulates continuously and is injected with salivary secretion during its next feeding on a healthy plant. The virus passes (but don'tt proliferate) through the whitefly's foregut, midgut, hindgut, hemolymph, and salivary glands before being released into the plants. The vector requires at least 15 to 60 minutes for virus acquisition and inoculation by phloem sap, and 15 to 30 minutes for inoculation via phloem sap. For successful viral transmission, an 8 hour minimum latent time between acquisition and inoculation is required. The ability of a whitefly to transmit a virus is directly related to the time it takes to acquire it, while the vector's gender and age also have an impact on the virus's transmission efficiency. The virion's minimum acquisition access period and maximum retention time determine the persistent mode (usually 3 days for male whiteflies and 10 days for female whiteflies).

The virus can be transmitted to whitefly nymphs by contaminated leaves, but it cannot reach the eggs. Furthermore, neither the male nor the female whitefly can maintain infectivity indefinitely. Begomovirus whitefly specificity is determined by the interaction of the highly conserved virus coat protein with receptors in the whitefly's gut and salivary glands, and any changes to the virus coat protein (CP) change their vector preferences. Molecular chaperone proteins, HSP70 (70-kDa heat shock proteins), and other proteins encoded by whiteflies contribute in the effective circulative transmission of viruses. According to Murugan and Nadarajan (2012), there is no link between the existence of leaf trichomes in blackgram and the activities of whiteflies, and therefore however, there is no such report for mungbean resistance to YMV. Begomoviruses are a type of virus that can infect humans to reduce the lifetime and fecundity of whiteflies in order to increase their transmission; the genetic composition and evolution of whiteflies is also influenced by their behaviour and feeding habits.

Resistance mechanisms of Plants

Mungbean crop diversity and MYMV afflicted area have continuously risen since the mid-1990s, Because resistance is controlled by a number of factors including plant genotype, ambient meteorological conditions, MYMV stains, whitefly biotypes, and the availability of other carriers, any single MYMD strategic plan may not be effective. Other MYMD management

issues include (i) the lack of a distinct molecular infection mechanism for various MYMV strains; (ii) the development of the several viruses strain specific resistance lineages; and (iii) the reduction of the vector population just under the criterion in field conditions. The lack of long-term resistance in mungbean after forty years of MYMD resistance breeding could have been attributed to ground germplasm assessment, which overlooked the natural existence of several begomoviruses as well as the abundance of whitefly organisms. As a result, any effective MYMD management plan in mungbean should consider MYMV strains, biotypes of whiteflies and geographic dispersion in the research region, as well as artificial screening via forced feeding and agroinoculation**.**

1. RNA silencing

The widespread finding that fresh leaves of diseased plants are mostly disease free is due to a process known as "recovery," in which the same or similar viruses cannot damage the newer plant leaves. Another plant defence system known as RNA silencing is responsible for the recovery process. Plants use RNA silencing as a fundamental antiviral defence strategy. Small RNAs destroy bigger RNAs in a sequence specific manner in this mechanism. Depending on their source, small RNAs are classified as micro RNAs or short interfering RNAs (siRNAs). MicroRNAs are made by trancribing non protein coding DNA followed by some procedure. siRNAs are produced by cleavage of dsRNA. RNA silencing has two modes of actions depending upon the target. In first case, post transcriptional gene silencing, the messenger RNA of virus is targeted and degraded resulting in immunity against that virus. In second case cytosine residue of viral DNA or histone protein is methylated, which makes the DNA compact and transcription is blocked this is called transcriptional gene silencing. Although there is no confirmation of symptom recovery in MYMD, it has been shown that resistant genotypes breakdown MYMV RNAs more quickly than susceptible genotypes. In resistant genotypes, transcriptional gene silencing has been documented in the area of MYMV replication start site (Yadav and Chattopadhyay, 2011).

2. Insecticide

Managing whiteflies is difficult since they attack in groups and even a single attack can severely harm a plant. "Two indigenous cryptic species, Asia II-1 and Asia II-8, are claimed to be prominent in Northern and Southern Indian environments, respectively" (Nair et al., 2017). Because the sensitivity of different whitefly species to different insecticides varies greatly, comprehensive knowledge regarding the abundance of whitefly species in any given region is crucial for the judicious use of pesticides. The use of widespread insecticide combinations in the early stages of growth proved efficient for whitefly control since it eliminates the vector while also protecting the plant from further attack. "To control the whitefly population, field sanitation, plucking of affected leaves, water sprays, and avoiding an excess of nitrogen fertiliser all are suggested" (Karthikeyan et al., 2014). Furthermore, seed hydro-priming for 8 hours was found to be efficient in decreasing the occurrence and severity of MYMV infection in mungbean.

3. Mutation Breeding

Mutation breeding is a rapid technique to increase genetic variation for a variety of attributes in crop plants, including MYMD resistance. In mungbean, gamma irradiation doses of 10–30 KR were found to be very effective in obtaining desired mutants for characteristics such as earliness, Synchronous maturity, and MYMD resistance. When performing mutation breeding, breeders usually choose one or a few target characteristics to improve. "During the M2 and subsequent generations, single plant selections were carried out under disease pressure conditions to find the plant(s) with MYMD resistance and high yield through the selection of various other traits such as fertile branches per plant, pods per plant, and seed yield per plant, among others. These mutant lines may be released as such as a variety or the characteristics may be incorporated in other varieties through backcross breeding" (Pratap et al., 2020). Mungbean improvement began in the 1970s at Pakistan's Nuclear Institute for Agriculture and Biology (NIAB), with an emphasis on genetic manipulation (gamma irradiation) and crossing to produce better crop and MYMV resistant varieties. As a result, the mutation breeding strategy tends to show potential not only for MYMD resistance but also for improving production without radically altering the genetic configuration.

4. Based on Pathogen Derived Resistance (PDR) strategy

The ectopic production of viral genome sequence as RNA or protein to promote resistance to homologous (related sequence) or heterologous (unrelated sequence) viruses is referred to as PDR. It can be used to produce a number of MYMV genes in mungbean, including coat protein (CP), protease, membrane protein (MP), replicase, and others. In geminiviruses, CP and Rep gene expression is mostly used for PDR, but because to mungbean resistance to Agrobacterium-mediated transformation, this method is still being successful.

5. CRISPR/Cas9 Virus Resistance Mechanism in Mungbean Crop

Using sgRNAs intended to target viral genomic DNAs, CRISPR/Cas9 technology was used to edit the plants and develop resistance over Begomovirus infection. A number of recent genome editing research has focused on a variety of commercially significant food crops required for conventional agriculture. This method has been found to be effective in enhancing crop productivity, disease resistance, and grain quality, among other features. The CRISPR/Cas9 approach uses a three-step procedure to identify and target a pathogen's genetic material: (i) acquisition, (ii) expression, and (iii) interference. Unwanted foreign DNA is received as a spacer from viruses or plasmids, which is subsequently divided into small fragments, recognised, and integrated into the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) locus. CRISPR loci have been duplicated and used to generate CRISPR RNA (crRNA), which directs effector endonuclease genes to target virus components via simple complementarity. The protospacer adjacent motif (PAM) is a short (2–5 bp) sequence of conserved nucleotides used to

identify a DNA fragment (spacer) CRISPR-mediated pathogen immunity is compromised by mutations in viral genomes and PAM. With the use of the Cas9 protein (CRISPR associated protein 9) and the trans-activating crRNA (tracrRNA) molecule, CRISPR/Cas9 expression involves efficiently transcribing the big pre-CRISPR RNA (pre- crRNA) obtained from the CRISPR locus and converting it into multiple crRNAs. Through base complementarity, the tracrRNA binds to the crRNA repeat area and facilitates pre-crRNA processing in crRNA. The active crRNAs become part of the CRISPR-associated antiviral protection complex (CASCADE), which helps in the identification and base-pairing of a specific target area of foreign DNA. A single Cas9 protein is required for DNA interference in the CRISPR/Cas9 technique.

During the interference step, the crRNA directs the Cas9 protein into the target location of the foreign DNA, causing it to break down and providing immunity against pathogen attacks. Cas9 is a massive protein with numerous domains (the RuvC domain at the amino terminus and the HNH nuclease domain in the middle) and two short RNA segments, crRNA and tracrRNA. Cas9 increases adaptability, takes part in precrRNA processing that leads to crRNA, and conducts specific DNA double-strand breaks (DSBs) triggered by tracrRNA with RNAse III-specific double-stranded RNA. CRISPR/Cas9 structures can be created and developed in a relatively straightforward, cost-effective, and intellectual property free manner. The CRISPR/Cas9 tool, crRNA, and tracrRNA components can be combined to form the sgRNA, which directs Cas9 to target specific DSBs. The configuration of sgRNAs is identical to that of sgRNAs, making genome editing simpler. The CRISPR/Cas9 technique was developed to produce DNA cleavage in vitro at various locations. This approach has recently been used to change genomes in bacterium, fungus, viruses, yeast, and a variety of other organisms after achieving successful selective mutagenesis. A CRISPR/Cas9-mediated genetic manipulation tool has been recently successfully used in cowpea (*Vigna unguiculata*) to disrupt the symbiotic nitrogen fixation gene by targeted the symbiosis receptor related kinase gene, resulting in a 67 percent mutagenesis effectiveness and perfect nodule formation inhibition (Arora et al., 2017). As a result of CRISPR/Cas9 in vigna, functional genomics investigations for a range of other traits, especially YMD resistance, are likely to spread quickly to other vigna species. Cpf1, like Cas9, is a form of CRISPR nuclease that is more efficient and has a reduced off target effect, making it a better option for altering the genomes of diverse plants, including mungbean for MYMD resistance. As a result, multiple viral resistance should be a goal of CRISPR based genome editing techniques (Cong et al., 2013). The CRISPR/Cas9 technology can potentially be utilised for targeted mutagenesis to identify host elements that regulate plant resistance (Shan-e-Ali Zaidi et al., 2016).

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