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Begomoviruses of soybean and their management

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Abstract

Soybean (*Glycine max* L.) is a member of the family Fabaceae. About 85% of the world's soybeans are processed annually into soybean meal and oil. This oil seed crop has been reported to be infected by a number of plant viruses, which cause huge losses to the farmers all over the world. These viruses not only reduce the yield of the crop but also the oil content. More than 111 viruses or their strains have been reported worldwide infecting soybean. Of these viruses, begomoviruses are the important ones. They cause several types of symptoms in the plants and are transmitted by white flies (*Bemisia tabaci*) in nature. These viruses can be identified by polymerase chain reaction using the purified viral DNA or total DNA from the virus infected plants. Several strains of geminivirus have been confirmed using sequencing as a powerful tool to establish the homology of the gene sequences. The management of geminiviruses is a problem. Development of resistant varieties and vector control are the main strategies. Marketing of soybean is also one of the important fields of concern so that the farmers could get the good amount for their produce.

Keywords: Soybean; Geminivirus; Symptoms; Gene sequence; Management

1. Introduction

Soybean (*Glycine max* L.) is a member of the family Fabaceae. It has its origins in China and is widely grown around the world. Soybean has an important place in world's oilseed cultivation scenario, due to its high productivity, profitability, and vital contribution towards maintaining soil fertility. The crop also has a prominent place as the world's most important seed legume contributing 25% to the global vegetable oil production, about two thirds of the world's protein concentrate for livestock feeding and is a valuable ingredient in formulated feeds for poultry and fish.

Only 2% of soybean protein is consumed directly by humans in the form of soy food products such as tofu, soy hamburger, or soy milk analogs. All but a very small percentage of the other 98% is processed into soybean meal and fed to livestock, such as poultry and pigs [1]. The major soybean producing nations are the United States, Brazil and Argentina. The three countries dominate global production, accounting for 80% of the world's soybean supply. Global production of soybean has grown at a compound annual growth rate (CAGR) of 2.78% from 215.69 million metric tons in 2004-05 to 283.79 million metric tons in 2013-14.

About 85% of the world's soybeans are processed annually into soybean meal and oil. Approximately 98% of the soybean meal is crushed and further processed into animal feed with the balance used to make soy flour and proteins. Of the oil fraction, 95% is consumed as edible oil and the rest is used for industrial products such as fatty acids, soaps and biodiesel.

Soybean contributes significantly to the Indian edible oil pool. Presently soybean contributes 43% to the total oilseeds and 25% to the total oil production in the country. Currently, India ranks fourth in respect to production of soybean in

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the world. The crop helps earn valuable foreign exchange (Rs. 62000 millions in 2012-13) by way of soya meal exports. Soybean has largely been responsible in uplifting farmer's economic status in many pockets of the country. It usually fetches higher income to the farmers owing to the huge export market for soybean de-oiled cake.

Soybean production has been reduced heavily due to many biotic factors e.g. bacteria, fungi, insect, nematodes and virus, which not only reduce the crop yield but also reduce the quality of soybean crop globally. Soybean is exposed naturally to many virus infections with different modes of transmission. More than 111 viruses or strains, belonging to different virus genera and families, are able to infect soybean naturally or under experimental conditions [2]. Among these viruses, it is a natural host for 33 potentially important viruses [3]. Of these viruses infecting soybean, 27 are considered a threat to the soybean industry [4,5]. Among all viruses, begomoviruses (Family Geminiviridae) are the most fatal factor for soybean production in Indian sub-continent [6]. Legume infecting begomoviruses of family Geminiviridae are transmitted by *Bemisia tabaci* and generally cause yellow mosaic disease (YMD), which severely hamper production potential of legumes including soybean [7].

The viruses infecting soybean present unique challenges to soybean producers, crop consultants, breeders, and other professionals. They cause serious economic threat to soybean production impacting growth and yields without being widely recognized. Yield losses caused by soybean viruses can exceed 50% depending on the plant variety, infecting virus, and the growth stage of soybean when infected. However, the variety of symptoms produced by viral diseases, symptom suppression during certain soybean growth stages and limited familiarity with viral disease symptoms contribute to difficulties in diagnosis.

2. Characteristics of Geminiviridae

The worldwide expansion of agriculture has also resulted in the emergence and spread of numerous virus diseases and insect pests. Of particular importance are insect-transmitted viruses, especially in tropical and subtropical regions.

Viruses of Geminiviridae are insect-transmitted that have emerged over the past 30 years, as the largest group of plant viruses (in terms of number of species) and one of the most economically important viruses worldwide. These viruses cause extremely damaging diseases in a wide range of crops, including African cassava mosaic in Africa; Bean golden mosaic (BGM) in the Americas; Beet curly top in North America and the Middle East; Cotton leaf curl Kokhran in Asia; Maize streak in Africa, and Tomato yellow leaf curl in Africa, the Americas, Asia and Europe. In Brazil, BGM and tomato diseases caused by a complex of genera Geminiviridae are important diseases that lead to substantial yield losses in common beans and fresh market and processing tomato production. Begomovirus infection of soybean plants has been reported sporadically in Brazil and has generally not been considered to be of economic relevance [8]. Economic loss caused due to the infection of bean yellow mosaic viruses in legumes has been estimated to the tune of 300 million US $ [9]. Yellow mosaic disease (YMD) in soybean amounted to a yield loss of 105,000 metric tons [10]. The disease is caused by begomoviruses with bipartite genomes [11,12,13,14]. Although four species of begomoviruses have been reported to cause YMD of legumes in India [15], two species, Mungbean yellow mosaic India virus (MYMIV) and Mungbean yellow mosaic virus (MYMV) [16], are prevalent.

Geminiviruses comprise a biologically and genetically diverse family (Geminiviridae) of viruses. These viruses are twinned quasi-icosahedral virus particle (virion) of size ~22 X 38 nanometers and a small circular single-stranded DNA genome of ~2.5-5.2 kilobases (kb). The family name is derived from the distinctive twinned virions (the Latin word gemini means twin). Based upon genome structure, phylogenetic relationships, insect vector and host range, nine genera have been recognized by ICTV: Begomovirus, Mastrevirus, Curtovirus, Topocuvirus, Becurtovirus, Turncurtovirus, Eragrovirus, Grablovirusand and Capulavirus [17]. In nature, geminiviruses are transmitted by phloem-feeding insects, including various species of leafhoppers, a treehopper and whiteflies (*Bemisia tabaci*) and aphids. The subtropical northwestern region of Argentina (provinces of Tucumán, Salta, Jujuy, Santiago del Estero and Catamarca) suffers from a high incidence of the whitefly *Bemisia tabaci*, and the detection of begomoviruses is also common [18]. Among ten leaf samples, collected from different villages, nine were found to possess virus irrespective of the density of whiteflies in concerned region [19]. One sample was found to be virus free even though, it was having YMD symptoms and whitefly density in an area was moderate. It may be due to deficiency of micronutrients for YMD like symptoms in virus free plant. There was no correlation between the density of whiteflies and presence of virus in host plant.

These viruses are not transmitted through seeds, whereas many are graft-transmissible and some are mechanically (sap) transmissible. Begomoviruses can be subdivided into two major groups: those with a genome composed of two ~2.6 kb DNA components (referred to as DNA-A and DNA-B) as bipartite viruses, which are prevalent in the New World [(NW) e.g. southern states of the United States of America (USA), Mexico, the Caribbean and Central and South America], and those with a genome composed of a single ~2.9 kb genomic DNA, monopartite viruses, which are prevalent in the

Old World [(OW) e.g. Europe, Africa, Asia and Australia]. Many monopartite begomoviruses are associated with satellite DNAs (alpha satellites and beta satellites), some of which enhance pathogenicity [20].

3. Begomoviruses infecting Soybean

A number of species of begomovirus naturally infecting soybean have been reported, wherever the crop is grown. Soybean chlorotic spot virus, a novel begomovirus infecting soybean from Brazil [21,22], Bean golden mosaic virus, Sida micrantha mosaic virus and Okra mottle virus from central Brazil [8], Soybean mild mottle virus (SbMMV) and Soybean chlorotic blotch virus (SbCBV) from Nigeria [23], Tomato severe rugose virus, a tomato-infecting Begomovirus, in soybean plants in Brazil [24], Mungbean yellow mosaic India virus (MYMIV), Mungbean yellow mosaic virus (MYMV), Dolichos yellow mosaic virus (DoYMV) (infecting *Lablab purpureus*) and Horsegram yellow mosaic virus (HgYMV) [25,6], Legume yellow mosaic viruses (LYMVs) [7], Sida mottle virus (SiMoV), Bean golden mosaic virus and a possible new viral species [isolate A, soybean blistering mosaic virus (SbBMV)] from Argentina [18] and Papaya leaf crumple virus from India [26], Rhynchosia golden mosaic virus (RhGMV) and Chino del tomate virus (CdTV) from Mexico [27], Tomato leaf curl Karnataka virus (ToLCKV) [28] etc. are some of the begomoviruses worth mentioning here.

MYMV, MYMIV, DoYMV, Horsegram yellow mosaic virus (HgYMV), Kudzu mosaic virus (KuMV), Rhynchosia yellow mosaic virus (RhYMV) and Rhynchosia yellow mosaic India virus (RhYMIV) are collectively called as Legume yellow mosaic viruses (LYMVs), which cause yellow mosaic disease [16.15,29]. These viruses have also been confirmed by sequencing of their genes also.

4. Symptomatology of begomoivirus infecting soybean

Plants naturally infected with begomoviruses showed various symptoms. These symptoms have been recorded wherever soybean is grown. The symptoms may vary based on the strain of virus infecting, variety of soybean plants and the weather conditions. The natural symptoms may be stunting, distorted growth, and leaf streaking and striations in monocots and leaf crumpling, curling, distortion, golden-light to green-yellow mosaic/mottle, interveinal yellowing, yellow spots, and vein swelling, purpling, and yellowing in dicot plants [20], chlorotic spots on the leaves [21], severe leaf curling, vein thickening and leaf yellowing, symptomless infection, severe yellow or golden mosaic, chlorotic mottling, blistering, leaf distortion and dwarfing [18], leaf yellowing and mosaic [6], stunting, distorted growth and blistering, chlorotic spots [20], chlorotic spots [21] and leaf crumple and yellow mosaic symptoms [26], interveinal chlorosis, stunting and mosaic leaf symptoms [30], severe yellowing, crumpling and distortion of leaves, dwarfing of plants with fewer flowers [31].

5. Detection of begomoviruses infecting soybean

Earlier, the identification of virus was primarily on the basis of symptoms and squash blot hybridization analysis [32] and PCR with degenerate begomovirus DNA-A and DNA-B primer pairs [33]. A PCR-based assay was used to establish the identity and genetic diversity of begomoviruses associated with bean and soybean crops in northwestern Argentina [18]. Universal begomovirus primers were used to direct the amplification of a fragment encompassing the 5' portion of the capsid protein gene.

An inexpensive protocol to detect genomic components of whitefly-transmitted begomoviruses in symptomatic legumes in the field was described by Rouhibakhsh *et al*. [34]. The method involved extraction with a modified CTAB buffer containing -mercaptoethanol upto 5% and sodium chloride concentration from 1.4 to 2.0 M. With this method DNA could be extracted from mature leaves of legume hosts rich in polyphenols, tannins and polysaccharides. The non-coding region and full-length DNA A, DNA B components of yellow mosaic viruses were consistently amplifiable employing primers specific for intergenic regions and full-length genome. The system was found to be robust and the protocol was useful for the detection and identification of begomoviruses infecting grain legumes. Detection of MYMIV infecting soybean was carried out using DNA-A (cp) and DNA-B specific molecular markers [19].

Fernandes *et al*. [8] detected and sequenced geminiviruses of the genus *Begomovirus* infecting soybean (*Glycine max*) in central Brazil. Infection was confirmed by PCR-based amplification of a DNA-A fragment with universal begomovirus primers. Total DNA from infected plants was then subjected to rolling-circle amplification (RCA), and 2.6-kb molecules were cloned into plasmid vectors and sequenced for further confirmation.

Molecular hybridization-based detection of the three begomoviruses [Bean golden mosaic virus (BGMV), Soybean blistering mosaic virus (SbBMV) and Tomato yellow spot virus (ToYSV)] was accomplished using a general probe

obtained by mixing full-length DNA-A clones of the three begomoviruses and specific probes comprising part of the common region of each viral genome [35].

Cloning of a particular gene of begomovirus and its sequencing is also another detection technique, which can be utilized to detect begomoviruses. Genomic components of the begomovirus causing yellow mosaic disease in soybean in Delhi were cloned, sequenced and infectivity was proved [36]. Begomovirus was detected by PCR using begomovirus coat protein gene-specific primers and total DNA isolated from infected leaf tissues. The PCR product was cloned, sequenced and it was found to be new begomoviruses [Tomato leaf curl Karnataka virus (ToLCKV)] and Cotton leaf curl Kokhran virus (CLCKV) [31,28].

Not only PCR, RFLP has also been proven a technique in detecting and confirming begomovirus infection in soybean. PCR and RFLP allowed the isolation and identification of complete A component sequences of two begomoviruses viz. Rhynchosia golden mosaic virus (RhGMV), while the second corresponded to the Chino del tomate virus (CdTV) from symptomatic soybean plants in the state of Sinaloa, Mexico [27]. Even the plant extracts of symptomatic soybean were analyzed by PCR using a pair of universal primers that allowed to amplify and confirmed the presence of the CdTV B component, therefore the second isolate identified in this work was proposed as a new CdTV strain called "Chino del tomate virus - Soybean" (Mexico: Sinaloa: 2005). The presence of this new CdTV strain isolated from soybean is important because to date virus infection was naturally restricted to plants of the Solanaceae family [27].

To differentiate the detection of MYMIV and MYMV simultaneously, Ramesh *et al*. [7] used total DNA extracted from infected soybean leaves and it was used as template for PCR amplification of MYMIV and MYMV specific coat protein (AV-1) region using forward primers (MYMIV F 5'GCATCAAGTCCGTGTACATTAC 3'and MYMV F 5'GTGTTAAGTCTATCTGGG3'), respectively. However, a common reverse primer (YMV R 5'CACAGGATTTGATG-CATGAG 3' was used for detection of YMV species. Rolling circle amplification was performed. The RCA derived DNA was digested with selected restriction endonucleases individually to release unit-length viral genomes. The resultant fragments of expected size were used for further downstream process for confirmation.

Total DNA from symptomatic leaves of soybean was isolated followed by PCR detection of begomovirus by degenerate primers. RCA product of viral genome was digested by restriction enzyme, cloned in pBluescript (pKS+) cloning vector and sequenced. Partial tandem repeats were constructed in pCAMBIA2300 plant binary vector for demonstration of koch`s postulate [6].

But recently, the genomics and sequencing have emerged to detect and identify the virus at genomic level. Viral metagenomics (viromics) is a powerful tool for viral diversity exploration in a wide range of environments [37]. When a metagenomic study is linked to a host, it is called ecogenomics [38]. In particular, rolling circle amplification (RCA) [37] as a viral genome enrichment technique, coupled with next-generation sequencing (NGS), has been used to identify geminiviruses, especially begomoviruses and their satellites in different crops [39,40,41,42,43].

Circomics, the combination of RCA-restriction fragment length polymorphism (RCA-RFLP) and pyro-sequencing, has shed light on begomovirus species identification [44,45). Another similar methodology is vector-enabled metagenomic (VEM), which surveys begomovirus using whiteflies [46,47]. VEM involves the purification of virus particles obtained from the insect vector and metagenomic sequencing. Deep sequencing of virion-associated nucleic acids (VANA), small-interfering RNAs (siRNA), and total RNA libraries were also applied for begomovirus identification [48,49,50,51]. The application of deep sequencing strategies in virology offers the opportunity to detect mutations, as well as to understand evolutionary strategies and viral population dynamics [52].

6. Gene sequences of begomoviruses infecting soybean

By going through the gene sequences available in GenBank data base, it was found that various genes of begomoviruses infecting soybean have been sequenced and these gene sequences are available for the scientists. It is found that DNA-A component and coat protein gene has been sequenced for most of the begomoviruses infecting soybean. These gene sequences available in GenBank database are helpful for comparison, confirmation of virus, to study the homology (similarity), DNA mapping, identification of species and to establish phylogeny of viruses using the gene sequences obtained during the study.

Two new 'legumoviruses' (genus *Begomovirus*; family Geminiviridae) naturally infecting soybean (*Glycine max* L. Merr.) in Nigeria were molecularly characterized. Based on characteristic symptoms in soybean, the two viruses were provisionally designated as Soybean mild mottle virus (SbMMV) and Soybean chlorotic blotch virus (SbCBV). SbCBV has a bipartite genome, whereas SbMMV has only a DNA A component. The DNA A component of SbMMV is 2,768

nucleotides (nt) long and the DNA A and DNA B components of SbCBV are 2,708 and 2,647 nt long, respectively. In pairwise comparisons, the DNA A component of SbMMV and SbCBV showed 62% nt sequence identity, indicating that these two viruses are distinct. Whereas the DNA A of SbMMV contains two virion- and four complementary-sense open reading frames that of SbCBV lacks the virus-sense AV2, a signature gene present in 'Old World' begomoviruses. A pairwise comparison with the corresponding nucleotide sequence of other begomoviruses in the databases indicated that SbCBV had a maximum of 74% identity with Cowpea golden mosaic virus and SbMMV had a maximum of 65% identity with MYMIV and Kudzu mosaic virus. Phylogenetic analysis of the DNA A component of both SbCBV and SbMMV together with those of other begomoviruses available in the databases showed clustering of the two viruses within the 'legumovirus' clade of the begomovirus phylogenetic tree. In addition, the DNA A and B components of SbCBV from *Centrosema pubescens* Benth were found to be identical to those from soybean, indicating that leguminous wild species are a potential alternative host for the virus [23].

A begomovirus causing yellow mosaic disease in soybean in Delhi was cloned and sequenced. Nucleotide sequence analysis of the virus isolate revealed more than 89% identity with MYMIV and therefore, it was designated as soybean isolate of MYMIV (MYMIV-[Sb]). Total nucleotide and predicted amino acid sequence analysis of MYMIV-[Sb] with other yellow mosaic virus isolates infecting legumes established dichotomy of the isolates into two species namely MYMIV and MYMV [36].

A leaf crumple disease of soybean was observed. The PCR amplified coat protein gene product was cloned, sequenced for 771 bp. Analysis of nucleotide sequence data by Blast search revealed the highest nucleotide sequence identity (96-98%) with Tomato leaf curl Karnataka virus (ToLCKV) and named it as soybean isolate of ToLCKV [31]. In another study Raj *et al*. [28] reported Cotton leaf curl kokhran virus (CLCKV) infecting soybean. The virus was confirmed by sequencing of PCR products amplified by using coat protein gene specific primers. The gene sequence homology data indicated that the virus associated with yellow mosaic disease of soybean was an isolate of CLCKV rather than MYMIV-Sb (1) reported earlier on soybean from northern India [28].

Two new begomovirus viz. Rhynchosia golden mosaic virus (RhGMV), while the second corresponded to the Chino del tomate virus (CdTV) symptomatic infection on soybean, were also confirmed by sequencing and BLAST search in the state of Sinaloa, Mexico [27]. Both genomes were analyzed by comparison of their nucleotide sequences with those available in the NCBI database. The first isolate corresponded to RhGMV, while the second corresponded to the CdTV, which showed a 92.3% overall identity with its closest relatives, CdTV-[8] and CdTV-[RK].

Evolutionary study of begomovirus using the gene sequences of DNA amplified by PCR using degenerate primers have also been analyzed by constructing phylogenetic tree [6]. Amplified DNA using specific coat protein (AV-1) region specific primers were used for differentiation of two viruses viz. MYMIV and MYMV [7]. The eluted DNA fragments were ligated with vector and recombinant clones were generated. Virus genome sequence information was obtained through primer walking strategy and complete genome sequences were submitted to GenBank.

7. Options for Begomovirus Disease Management in Soybean

Management of geminiviruses is a worldwide challenge because of the widespread distribution of economically important diseases caused by these viruses. Regardless of the type of agriculture, management is most effective with an integrated pest management (IPM) approach that involves measures before, during, and after the growing season. These include starting with resistant cultivars and virus- and vector-free transplants and propagative plants. Measures that should be used for crops in open fields include rouging infected plants and insect vector management. Application of insecticide to manage vectors (whiteflies and leafhoppers) is the most widely used measure but can cause undesirable environmental and human health issues. For annual crops, these measures can be more effective, when combined with host-free periods of two to three months. Finally, given the great diversity of the viruses, their insect vectors, and the crops affected, IPM approaches need to be based on the biology and ecology of the virus and vector and the crop production system [53].

The weeds not only reduce the crop yield through competition with crops but they also act as reservoir of begomoviruses and create the great problem to the farmers. The weeds like *Croton*, *Acalypha*, *Malvastrum*, *Eclipta*, *Ageratum*, J*atropha*, *Parthenium*, *Sida*, and *Sonchus* have been found to be a potent begomovirus inoculum [54,55]. More than 18 different weed species have been found as reservoir of Tobacco leaf curl virus in southern India [56]. Thirteen weed species were identified as an alternative host of Tomato leaf curl virus in India [57]. *Sida* sp. and *Abutilon indicum* were also identified as alternate host of Cotton leaf curl virus in Punjab and *Croton bonplandianum* of Tomato leaf curl New Delhi virus (ToLCNDV) and *Parthenium hysterophorus* was found as alternate host of ToLCKV [58] and Ageratum enation virus. *Nicotiana plumbaginifolia*, *Physalis minima*, *Coccinia grandis*, *Solanum nigrum*, *Momordica charantia*

(wild) and *Luffa* sp. have been found to be the hosts of geminivirus [59]. Therefore, the incidence of begomoviruses can be minimized by elimination of such weeds from and nearby cultivated fields and weed management can also be integrated with other measures.

In some regions of Madhya Pradesh, incidence of geminivirus in soybean has been minimized significantly through sowing of resistant/tolerant varieties (Mosaic PK 262, SL 96, Shivalik, SL 295, MACS 450, PK 1092, SK 744, Co 3, PK 1024, PK 1029, PK 1042, SL 525, Birsa soy 1, Pusa 97-12, MACS 57, KB 79, SK 744, Pusa 98-14, PS 1347, PS 1225) after 2-3 months of host free period because Zaid crops are not possible to grow in these regions due to less availability of irrigation water.

Genes introgressed from *Solanum peruvianum*, *S. chilense*, *S. pimpinellifolium* and *S. habrochaites* are one of the best examples of breeding-mediated resistance against begomovirus infection in tomato [60]. A recessive resistance gene (Ty-5) was identified on chromosome 4 in the lines derived from cultivar Tyking [61], and a dominant resistance gene, Ty-2, in *S. habrochaites*-derived line H24 has also been identified and mapped [62]. Plants developed by mediated resistance gene (Ty-2, Ty-1, Ty-3 and so on) against begomoviruses are limited only at laboratory level. However, these methods of resistance are not applicable at farmers' field.

Development of begomovirus-resistant transgenic plants through recombinant DNA technologies has emerged as one of the reliable strategies. The use of genetically modified resistant plants is one of the most efficient, sustainable, and frequently employed strategies for management of virus infections in fields. The stable expression of gene of interest from organism of different species or kingdom into desired plants represents one of the most significant developments in a series of advances in bio-agriculture and biotechnology that includes modern plant breeding, hybrid seed production, farm mechanization. The establishment of *Agrobacterium tumefaciens* as foreign gene carrier to develop transgenic plants has made major breakthrough in the management of viral diseases. Along with engineered herbicide resistance and insect resistance using the *Bacillus thuringiensis* toxin gene, virus resistance was one of the first successes in the genetic engineering of a useful trait into plants [63]. Resistance to begomoviruses has been achieved in various plants either by the use of begomoviral genes (known as the pathogen-derived resistance or PDR) or through the expression of non-begomoviral genes from different organisms.

Kunik *et al*. [64] first time demonstrated tomato cultivars transformed with coat protein gene of Tomato yellow leaf curl virus (TYLCV) and found resistant to the virus, and transgenic plants remained asymptomatic or delayed disease symptoms. Another gene often used to obtain PDR to begomoviruses is the replication-associated protein (Rep) gene, usually expressed by C1. Noris *et al*. [65] gave the first evidence of the high levels of resistance in *N. benthamiana* plants by the expression of a truncated Tomato yellow leaf curl Sardinia virus (TYLCSV) Rep (encoding the first 210 amino acids of the Rep protein and potentially co-expressing the C4 protein). Most of the T1 transgenic lines show delayed and mild symptoms as compared to non-transgenic control lines. Recently, a dominant resistance gene, Ty-1 resistance gene from tomato against tomato yellow leaf curl virus (TYLCV) encoding an RNA-dependent RNA polymerase (RdRp), is proposed to confer resistance to TYLCV by amplifying the RNAi signal. In future such technologies with the help of biotechnology and breeding may be helpful in developing geminivirus resistant leguminous crops and especially soybeans.

Small RNA (siRNA) based genetic engineering (SRGE) technology had also been explored for crop protection against viruses e.g., *Jatropha curcas* is susceptible to a number of begomoviruses such as Jatropha mosaic India virus, Indian cassava mosaic virus (ICMV), etc. and very often viral disease outbreaks. In a study by Ye *et al*. [66], the transgenic *J. curcas* plants expressing hairpin, double stranded (ds) RNA with sequences homologous to five key genes of ICMV-Dha strain DNA-A were generated which silenced the viral genes expression, thereby conferring ICMV resistance. MicroRNAs (miRNAs) have also been employed to control begomovirus diseases. The *in silico* analysis of MYMIV and MYMV reveals the micro-RNA (miRNA) target [67]. The MYMV genome was targeted by 70 miRNAs. The miRNAs derived from soybeans (*Glycine max*), wild soybean (*Glycine soja*), and chickpea (*Cicer arietinum*) display 63, 18, and 8 potential target sites on the begomovirus genomes, respectively. The resistance against many begomovirus infection can be developed bymiRNAs engineered to alter their target specificity and such artificial miRNAs (amiRNAs). The amiRNA approach can deliver efficient resistance in plants against a monopartite begomoviruses, and multiple target site incorporation can make it the potential broad-spectrum, virus resistance method. The incidence of cotton leaf curl disease (a begomoviral disease) can be minimized through whitefly-resistant transgenic cotton lines expressing Tma12 resistant to whitefly, with no detectable yield reduction. Therefore, Tma12 may be used for deployment in GM crops to control whitefly and the viruses it carries [68]. Proteins, which can control whiteflies effectively in transgenic crops, would be valuable that these proteins do not affect host plant biology and are safe to humans and other nontarget organisms.

Recently genome engineering strategies have emerged as promising tools to introduce desirable traits in many plants e.g., the clustered regularly interspaced palindromic repeats (CRISPR) or CRISPR-associated 9 (CRISPR/Cas9) because of its simplicity, efficiency, and reproducibility. Resistance against many begomoviruses such as Tomato yellow leaf curl virus (TYLCV), Bean yellow dwarf virus (BeYDV), and Cotton leaf curl Kokhran virus (CLKCoV) has been developed by using CRISPR/Cas9 technique. These resistant plants were developed by targeting the virus genome using this technique. Efforts to engineer resistance against the genus Begomovirus were focused mainly on silencing of complementary-sense virus genes involved in virus replication. They targeted a complementary-sense gene (ACI) encoding replication initiation protein (Rep) to develop resistance against soybean isolate of Mungbean yellow mosaic India virus -[India:New Delhi:Soybean 2:1999]. It was found that the legume host plants coagroinoculated with infectious constructs of soybean isolate of MYMIV [India:New Delhi:Soybean 2:1999] along with this antisense Rep gene construct showed resistance to the virus [69].

Evaluation of soybean genotypes for yellow mosaic disease caused by MYMIV resistance involves field screening at disease hot spots or in a protected environment using infectious clones or viruliferous whiteflies as sources of virus inocula. Development of efficient virus inoculation and quantification protocols to screen soybean genetic stocks against YMD is imperative for breeding resistant varieties. Binary plasmids harbouring complete, tandem dimeric genomic components DNA A and DNA B of MYMIV-soybean isolate were engineered. The infectivity of the clones was shown in soybean genotypes JS335 and UPSM534 that display contrasting YMD resistance. As a follow-up, soybean germplasm lines, breeding lines and representative cultivars, which were initially screened at an YMD hot-spot were then subjected to *Agrobacterium*-based infection with MYMIV. Quantitative real time polymerase chain reaction (qRT-PCR) based copy number analysis of MYMIV genomic components allowed soybean genotypes to be classified into three discrete categories; resistant, moderately resistant and susceptible to the viral infection. Thus, a soybean germplasm disease screening system based on agro-infection and qRT-PCR based quantification of MYMIV was developed to facilitate breeding YMD resistant soybean [70].

Early seed sowing, crop rotation, avoidance by growing plants in isolated areas and installing physical barriers like screens or cages toward off insect vectors can minimize the incidence of begomoviruses [71]. These methods can be integrated with other management practices.

Blocking virus transmission by altering the biology of vector species, such as the whitefly, can also be a potential approach to manage these devastating diseases. Virus transmission by insect vectors to plant hosts often involves bacterial endosymbionts. Molecular chaperonins of bacterial endosymbionts bind with virus particles and have a key role in the transmission of Geminiviruses. Hence, devising new approaches to obstruct virus transmission by manipulating bacterial endosymbionts before infection has opened new avenues for viral disease control. The exploitation of bacterial endosymbiont within the insect vector would disrupt interactions among viruses, insects and their bacterial endosymbionts [72].

Because geminiviruses are seed transmissible up to 46.6% [73] therefore, it can be managed by the use of healthy seeds procured from healthy crop. At farmers level it is not easy to identify that a particular healthy crop of soybean is free from virus infection.

Use of balanced fertilizers, early sowing and sparse crop can reduce the incidence of virus infection. Spray of neem oil and natural pyrethroid as botanical insecticides and *Verticillium lacanii* as a bioinsecticide can minimize vector population, which are ecofriendly, inexpensive and naturally available. Spray of insecticides [Imidacloprid 17.8% SL, Acetamiprid 20 SP, Indoxacarb14.5% + Acetamiprid 7.7% SC, Spinosad 45 SC, Afidopyropen (Inscalis™) 50 g/L DC] from crop emergence to full vegetative growth are helpful in reducing insect vectors, which carry the virus from virus infected host to healthy soybean crop. However, it is universal truth that viral diseases can not be completely controlled by any control method but the losses caused by them can be minimized by any effective management practice or by the integration of more than one management practices.

8. Viral disease resistant soybean

With the fast increase in area under soybean in India, diseases and insect infestation had also started increasing. The major diseases affecting soybean include bacterial pustule, pod blight, yellow mosaic virus, rhizoctonia, aerial blight, myrothecium and bud blight. To tackle the virus diseases, resistant/tolerant varieties have been developed by the soybean research system but these could not be performed better on the farmers' fields. However, breeding work for resistance taken up from 1980s onwards led to the release of varieties that are tolerant/resistant to major diseases and pests affecting soybean [74]. The promising variety JS 335 (released in 1994) substantially contributed to the increase in yield of soybean in the country, and is still ruling the soybean seed chain.

Improved production technology for soybean have been developed and being popularized through training of trainers, demonstrations at farmers' field, participation of farmers in farmers' fair, as well as through print and electronic media [75].

In general, strategies for controlling begomovirus infection in soybean include the following: planting resistant or tolerant varieties, insect vector management, managing with alternative weed or crop hosts of viruses and changing crop cultural practices to those less favorable for disease development.

9. Conclusion

Soybean is an important crop which provide oil and protein both. Inspite of food importance it is also important for soil fertility. Madhya Pradesh is the leading state in area and production of soybean in India but due to mosaic disease both area and production has decreased drastically for last few years. Farmers used to grow soybean in large area as a sole crop in kharif season due to high price and low cost of production by using all capital resources but year after year they got yield loss and sank in debt due to innocence about cause and management of this disease, therefore, many farmers have attempted suicide due to crop failure. A number of plant virologists/plant pathologists have detected begomoviruses from soybean as well as from other leguminous crops and sequenced the genome of the virus for solving the problem but they could not succeed. This review article will help to the farmers society for knowing about the actual cause, vector and possible management practices to minimize the losses.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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