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Genetic Defense Approaches against Begomoviruses

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ABSTRACT

Concerns are increasing day by day as begomoviruses (Geminiviridea) are posing serious threat to large number of cultivated and non cultivated crops globally. Rapid emergence, diversity and spread of begomoviruses are mainly due to food trade and modernized agricultural practices. Strategies that can be adopted for defense against begomoviruses include several cultural, sanitary and chemical measures but all these are temporary, expensive, laborious and environmentally hazardous. So adopting genetic defense mechanisms against the begomoviruses can be a permanent and long lasting solution. These may include transgenic incorporated resistance in cultivars through biotechnological measures and pathogenic derived resistance via virus proteomic approaches. Similarly RNAi and Antisense RNA based technology can be utilized for virus disease management. The review converge its focus upon the modern day biotechnological approaches to cope the begomoviruses and sheds light upon various genetic defense approaches by summing up the recent documented research regarding the management of begomoviruses.

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Key Words: Begomoviruses; Defense; Coat protein; Genetic Alphasatellite; Betasatellite, Resistance;

Abbreviations: BGMV, Bean golden mosaic viruses; TYLCV, Tomato yellow leaf curl virus; CLCuD, Cotton leaf curl virus disease; PDR, Pathogen derived resistance;CP,Coat proteins;MP, Movement proteins; RG,Replicase gene; PTGS, Post transcriptional gene silencing;

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Introduction

Begomoviruses belonging to bean golden mosaic viruses (BGMV) are considered to be the most diverse and destructive group of viruses around the globe including tropical and subtropical zones[1]. Dicotyledonous crops are infected by begomoviruses in temperate and tropical climates[2-5] . The particular genus imbeds more than 288 species [6]. The viral genome is bifurcated in two strands i.e. DNA-A & DNA-B where DNA-B is dependent upon the “A” for its replication while both of these play equal role in causing an infection to the host (Figure, 1a). The size of each component is around 2.6-2.8 kb[7-8] . Reportingly, 133 species belong to monopertitie genome also known as old world strains that include the Indian, Asian, African and Japanese regions while bipartite or new world strains involves American regions [9-10]. Replication and encapsidation process is dependent upon DNA-A while systemic regulations and symptom production in the host is carried out by DNA-B [11-12]. DNA based replication takes place within the nucleus comprising of two steps that include ssDNA to dsDNA conversion and rolling circle amplification [13]. Overall both the DNA segments bear eight open reading frames (ORFs) out of which segment “A” includes six ORFs while segment “B” contains two ORFs. Segment “A” bears two positive oriented V1(R1) and AV2 coat and movement proteins as well as four negative oriented (AC1, AC2, AC3, AC4) proteins. Segment “B” includes one positively oriented V1 (R1) and one negatively oriented C1 (L1) protein (Table, 1). The size of both DNA-A and DNA-B of bipartite begomoviruses is same except common region[12]. The common region includes a loopy structure with TAATATTAC sequenced nucleotide and many other regulatory elements.

It also has multiple repeats of 6-12 nt sequences which act as binding sites[14]. Several associations of RNA satellites with plant viruses were reported [15] but in 1997 tomato yellow leaf curl virus (TYLCV) was found associated with a DNA satellite. The discovery was novel particularly for monopartite begomo--viruses[16] . After that new discoveries paved their way towards the association of two satellite molecule classes called Alpha and Beta satellites respectively (Figure 1c).

Begomoviruses imbed the most econo--mically important diseases that cause huge losses and famine in many countries of the world when hit their epidemics[17] . In China, some begomoviruses have caused significant yield losses to crops such as tobacco, squash, tomato and papaya in recent years[18-22]. Other examples include potato yellow mosaic virus (PYMV), first identified in the late 1980s that caused an infection in tomatoes resulting an estimated yield loss of

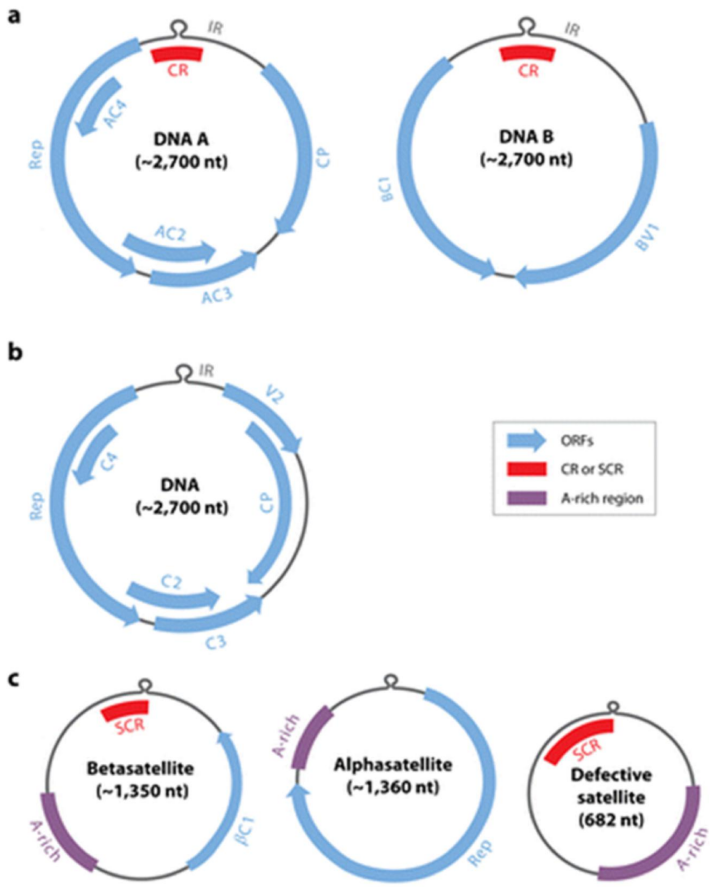

Figure 1: Begomovirus and its associated satellites (Zhou et al, 2013)

Table 1: Begomovirus genes

Proteins	Orientation	Type	Size (kDa)
Segment-A			
V1 (R1)	Positive	Coat	29.7
V2	Positive	Movement	12.8
C1(L1)	Negative	Replication initiation	40.2
C2(L2)	Negative	Transcription activator protein	19.6
C3(L3)	Negative	Replication enhancer	15.6
C4	Negative	Symptom expression determiner	12.0
Segment-B			
V1 (R1)	Positive	Nuclear shuttle	33.1
C1(L1)	Negative	Movement	29.6

50-60% in Trinidad and has become threatening in the Caribbean.Similarly cotton leaf curl disease has resulted in epidemics two times in Pakistan wiping out almost all the crop[23]. The cotton crop accounts 60% of the foreign exchange earnings for Pakistan, so disease results in huge economic loss[24]. Another example is cassava mosaic disease as cassava is mostly grown as a food source in Africa and is the third largest source of carbohydrates in the world[25]. Annual economic losses in east and central Africa are estimated to be between US$1.9 billion and $2.7 billion [26]. Moreover tomato yellow leaf curl disease results in 90-100 % losses as estimated and millions of hectares under tomato crop are affected annually worldwide leading towards its infection to other crops like pepper and beans[27].

Genetic management of begomoviruses is very important to reduce the viral diseases and their impact upon the economy because viruses have tendency to recombine, re-organize and rapidly emerge again such as (a) Some non-cultivated plants are hosts to crop infecting begomoviruses e.g. Datura stramonium and Malva parviflora harbor the TYLCV when the original host is not available. (b) Some crop infecting begomo--viruses may arise from non hosts or weed infecting begomoviruses. e.g. Ludwigia hyssopifolia , Emilia sonchifolia , Vinca alba, Vernnia cinereaI and Xanthium strumarium which are common weeds in China are the hosts of begomoviruses and in future the particular virus can shift to the economically important crops [28-31]. (c) Recombination between some non cultivated plant infecting viruses and crop infecting begomoviruses may result in emergence of novel begomoviruses which may infect crops and can have increased pathogencity. Keeping an eye upon the importance of begomoviruses, modern technology must be used to manage the target viruses. In this review, we have focused upon the efficient genetic defense strategies that can be adopted against the begomovirusees by using the latest biotechnological and molecular approaches.

Genetic Defense Approaches

Management of begomovirus infected plants cannot be dealt by usage of chemicals. However efforts can be done for the prevention of viral diseases by utilizing some cultural practices that may include inter-cropping, hygienic implem--ents, resistant varieties, crop residual burning or some other avoidance measures along with some vector management via chemicals or other means [32-37]. But all these practices limit us at some stage or have some adverse effects upon environment or human beings[36-38] so finding the better genetic solution against the begomoviruses gets higher importance for the researchers to ensure better food security in future. Here we

have tried to describe some possible genetic management strategies against begomo--viruses.

1.Genetic resistance in cultivars against begomoviruses

Breeding genetic resistance in the host is a reliable method for efficient and long lasting management against any disease or pathogen as it can benefit quantitatively as well as qualitatively[6,39]. There are a few success stories in introgression of resistance against the target viruses. For example, tomato infecting begomoviruses has been neutralized to some extent via breeding host resistance by incorporation of genes from Solanum species (Solanum peruvianum, Solanum habrochaites, Solanum pimpine--llifolium and Solanum chilense) [40]. Molecular mapping and characterization of resistance genes via use of molecular markers have been done[40].

TY-1 which is a major and partial dominating resistant gene was identified from Solanum chilense line # LA1969 and was introgressed, mapped towards the shorter arm of choromosome 6 [41]. Similarly from Solanum pimpinellifolium, another major resistance QTL was exhibited and was mapped at same chromosome 6 (TG153--CT83) but conferring a different position[42]. Another dominant gene (Ty-2) introgressed from Solanum habrochaites accession H24 was mapped to shorter arm of chromosome 11[43]. Correspondingly, mapp--ing of TY-3 which is categorized as partially dominant major gene extracted out from Solanum chilense accessions LA1932 and LA2779 was done at chromosome 6 [44]. The particular gene derived from LA2779 was considered to be greater in length and its linkage with TY-1exhibited that both of these (Ty-3 and TY-1) are code specific and are allelic towards RNA dependent polymerase[45].

Further mapping revealed the exhibition of TY-4 mapping to chromo--some 3 at its longer arm. In relation to the development of symptoms in the host, TY-4 gene encounters 16% variation as compared to TY-3 which accounts 60% major effects[46]. Alternatively, upon chromosome 4, a resistant but recessive gene TY-5 was introgressed from a genotype called Tyking[47]. The particular gene has similarities with TY-5 loci exhibiting 40% symptomatic variation [48]. All these resistant genes encourage towards acquiring resistance against the begomoviruses by contributing lower levels of viral particle accumulation in these genotypes. The tomato genotypes having TY-1 or Ty-3 genes exhibited 10% less virus symptoms than the susceptible ones[45]. Similarly tomato accessions carrying TY-2 genes showed least virus particle accumulation[49]. The other successful examples in which resistance has been tried to achieve through pyramiding of virus genes via crossing or back crossing[50] include glycine max-

-soybean mosaic virus (SMV)[51], Capsicum annuum-pepper veinal mottle virus (PVMV) [52], barley yellow mosaic virus (BaYMV), Hordeum vulgare-barley mild mosaic virus (BaMMV) [53], Phaseolus vulgaris-bean common mosaic virus (BCMV) [54] and tomato leaf curl disease (ToLCD) [55]. Resistant accessions via pyramiding have been developed by introgession of TY-2 and TY-3 genes extracted from Solanum habrochaites and Solanum chinense respectively [56].

Begomoviruses re-organize themselves and go under recombination leading towards their spread towards the cultivars which are thought to be immune against them[37] . For example, tomato cultivars i.e. Roma and Moneymaker which were famous for their resistant characters against begomoviruses and better yields became susceptible to ToLCD[57-58]. To manage this problem, new tomato cultivars have been adopted widely worldwide which are tolerant to begmo--viruses infections and gives better yield even after being infected by viruses [59-60]. Recently, 41 tomotato genotypes were screened in Senegal for their resistance against TYLCD[58] out of which 12 were found to have durable resistance against the disease. But when these 12 genotypes were infected by other RNA viruses, they lost their considerable resistance to TYLCD [60]. Similar experiments in Nigeria revealed resistant pepper and tomato cultivars against begomoviruses[61-64]. In Asian regions, cotton is the most important crop which is under constant threat to cotton leaf curl virus disease (CLCuD). Researchers evaluated that Gossypium gossypioides still have durable resistance against CLCuD[65]. Furthermore, considerable resistance have been achieved through transgenics showing repression genes via utilization of Agrobacterium tumefaciens mediated transformations[69].

But in spite of being the best way, there are several limitation such as, (a) the resistance exploited by the breeders is mostly conferred by a single dominant gene[70] which do not prove long lasting in the field and gets hammered after a couple of years in the field by the pathogen[71]. (b) unavailability of desired genetic resistance in wild type relatives. (c) linkage of non desired traits with the resistance conferring gene. (d) desired resistance may be multigenic which may possess difficulties in gene knock down and transfer of genetic traits. (e) larger genomic size with higher representative DNA. (f) difficulties in cloning the resistance encoded genes because of in-sufficient mapping of various plant species. (g) difficulty in tagging for identification and isolation of resistant genes against viruses due to lack of knowledge about available resistance in most plant species against begomo--viruses[6].

2.Defense through selective proteins

Durable resistance in genotypes against the begomoviruses has not been developed successfully by breeders in most of the countries. Alternatively genetic engineering tools can be considered as best management strategy against the viruses. Similarly, involvement of latest modern breeding techniques, hybrid seeds, mechanized farming and pest management can improve the situation[72]. Use of A. tumefaciens as vector for the production of a transgenic plant introgressed by resistant genes led towards the management of begomoviruses. Firstly, tobacco plants were successfully engineered by incorporating resistant genes against viruses[73]. Utilization of protoplasts for receiving transformational changes was common in early experiments[74]. But now the developments in transformation techni--ques has lead towards the utilization of leaves, shoots and roots for genetic transformation in dicotyledons [72]. Some proteomic approaches to cope the begomoviruses have been tried to include subsequently.

2.1 Resistance through pathogen genes / pathogen derived resistance (PDR)

The particular concept of induction of resistance against the begomoviruses was first introduced in 1980 by Hamilton when he proposed the idea of transformation of genes derived from pathogen itself [75]. In 1985, the hypothetical concept was taken to next levels by Sanford and Johnson[76] .

The concept is basically evolved from cross protection phenomenon in which host is inoculated by a non symptomatic virus strain that provide protection to host against aggressive virus strains. Inserted pathogenic strain excites by disturbing the pathogen genetic expression in host via causing disruption in normal life cycle of pathogen through friendly invasion. For invasion or interference at various stages of virus life cycle, alternative or native non aggressive genes can be used that may play their role in replication, uncoating, within or between the cell movement and vector misguiding. All these interferences at important stages result in development of resistance in host[77]. In the early 90s, the above mentioned phenomenon was tested by many researchers upon various begomoviruses through transformation and expression of several least aggressive viral particles[78-82]. The pathogen derived viral particles used in this concept are CP genes, movement protein (MP), Replicase gene(R), Antisense RNA (As-RNA), satellite RNA (S-RNA) and defective interfering genes (DI).

2.1.1 PDR through coat proteins

Achieving resistance through CP gene involves creation of transgenic plants via expression of virus coat protein genes (Figure,2)[78].Initially, CP gene introgress--ed from tobacco mosaic virus (TMV) was inserted in tobacco plants for

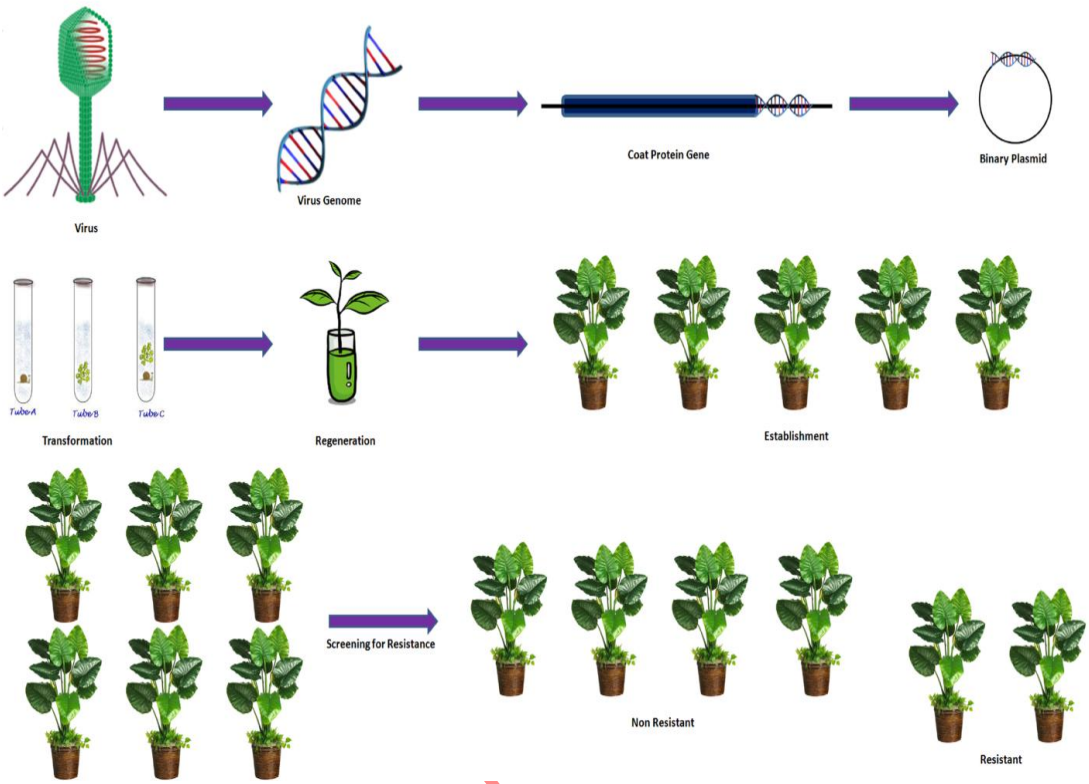

Figure 2: Schematic Diagram of Genetic defense approaches against Begomoviruses (Beachy et al, 1993)

resistance and confirmation of transgenic plants was done regarding evidence of foreign DNA sequences in primary as well as secondary transformants [73].

Further experimentation upon these transgenic plants revealed the expression of CP gene, thus exhibiting resistance against TMV as compared to control [83]. The phenomena involving CP genes is considered to be effective in minimization of begomovirus infections resulted by phenotypic resistance. Disease resistance genetically closely related viruses[72]. Some viruses against which the particular CP resistance has been coined effective are potato leaf roll virus[84] , alfaflfa mosaic virus[75], potato virus[86], tomato mosaic virus [87], cucumber mosaic virus [88], potato virus[89], tobacco mosaic virus[90] and tomato yellow leaf curl virus[91].

CP gene expression in transgenics can lead towards the differentiation in

2.1.2 PDR through ribose nucleic acid

assessments involve inoculation of transgenics which provide expression of CP (+) genes. Preference is normally given to the resistant progeny (RP1) as they show similarities in size, growth rate and age. Induced gene can be segregated in coming progeny by utilization of CP respondent antibodies[78]. Researchers found that there is difference in transgenics regarding expression of protection mechanism in virus coat protein host combination[83]. The differentiation is dependent upon viral transgenes or virus groups [82]. Resistance induced by CP gene is protein mediated particularly when insertion of a single copy of transgene is done. The transgene further undergoes transcription and translation mechanism, thus enhancing the protein levels[92]. Resistance achieved by such mechanism is of moderate level covering a wider range of similar viruses.

Initial assumptions revealed that the particular mechanism is somehow similar to the cross protection phenomena involving the interference of CP towards un-coating the virions thus inhibiting the virus spread from cell to cell leading to minimization of infection[93]. For example, In case of potato virus, tobacco mosaic virus, rice stripe virus and alfalffa mosaic virus, resistance level was directly proportional to the accumulation of transgenic CP in transgenics[73,89,94,95] .

Insertion of multiple copies of transgenes for the induction of CP gene is dependent upon RNA and resistance acquired by these means is highly strain specific RNA mediated resistance[82]. The expression of transgene is only up to mRNA level and shows very low level of protein accumulation. But when the accumulation levels of mRNA exceed a designated threshold level, gene silencing mechanism gets initiated which directly shows its affects upon viral multiplication and transgenic expression[92].

For imitation of viral suppressing phenomenon, identical virus genome sequence to transgene is necessary[96]. The particular phenomenon is named as post transcriptional gene silencing (PTGS)[97]. Such kind of resistance is referred as homology dependent as it reflects the homology dependent silencing relationships[98]. This defense mechanism involves mRNA degradation by the invading viruses as well as the transgene[99]. The examples include potato leaf roll virus (PLRV), ToMoV, TSWV, luteoviruses, nepo-luteoviruses, carla--luteoviruses and PV-Y[100-102] .Resistance in all these virus groups doesn’t show correlation with CP accumulations in transgenics[100]. Several transgenic resistant accessions which shown moderate level of resistance against

several begomoviruses did not exhibited any presence of CP, thus the resistance was correlated with pathogen derived RNA[102]. For example, (a) Development of transgenic cassava against african cassava mosaic virus (ACMV) expressed small interfering RNA (siRNA)[103]. (b) Transgenic resistant Phaseolus vulgaris development via RNAi AC1 virus gene silencing [104]. (c) resistant tobacco plants (TLCV-AU)[105] .

2.1.3 PDR through replicase gene

As discussed earlier, CP is most widely exploited worldwide in transgenics to attain the pathogen mediated resistance. Secondly expression of RG is considered valuable for management of begomoviruses in host plants through functional or alternated RG [72]. Resistance through RG has been successfully exploited against sixteen DNA/RNA viruses[92]. For this purpose, Altered, truncated as well as full length RG read throughs are commonly used. The examples include tomato yellow leaf curl virus and African cassava mosaic virus[106]. Resistance acquired through RG expression involves inoculation of the plants with higher virus concentration levels and is considered to be strain specific[107].

Although RG mediated pathogenic derived resistance has been considered successful in the above mentioned examples but in contrast Palukaitis and Zaitlin explained about the breakdown of such resistance against PT-X, PT-Y, CMV and potato mild mottle virus (PMMV)[108-112].

2.1.4 PDR through movement proteins

Movement proteins are associated with viral encoded intracellular movement of plant viruses[113]. Interaction of MPs with plasmodesmeta correlates the modification and facilitation of intracellu--lar movements of plant viruses [114]. Six different virus genera including begomo--viruses have displayed longer intracellular movements through MP interference[92]. Resistance expressed by transgenic tobacco via defective TMV-MP against various viruses (CMV, TRSV, TRV, AIMV and PCSV) was explained by Cooper[114]. In a similar context Tacke [115] generated transgenic potato which expressed seventeen PLRV-MP and was found to be resistant against PVX and PVY. Further efforts in this line of action of production of transgenics lead towards the production of TSWV mutants which shown resistance towards various TSWV strains[92] .

2.1.5 PDR through RNA silencing.

The mechanism of RNA silencing is characterized by suppression of genetic expression by sequence specified mRNA degradation (Figure, 3). Initially the mechanism was termed as post transcriptional gene silencing (PTGS) in plants[116], RNA interference in animals[117] and quelling in mycoflora[118] . Some of the basic ingredient molecules which take part in this mechanism are ribonuclease dicer (RNA-D) and

argonaute (AGO). Overall there are three specific pathways in this mechanism such as endogenous mRNA silencing through miRNAs, cytoplasmic interfering [119] and DNA methylation[120]. The mechanism performs better in plants rather than mycoflora and animals. For example, the model plant *Arabidopsis thaliana* encodes ten argonaute proteins, six RNA dependent RNA polymerses and four dicer enzymes.

2.1.6 PDR through beta-satellite suppression

Begomoviruses are invaders as well as are the direct target of post transcriptional gene silencing (PTGS). After the invasion of virus in the hosts, small RNAs can be easily located inside the infected cells[121].

At this moment, siRNAs levels are negatively correlated with the level of infection severity [122]. Thus to deactivate or defeat the host defenses, viruses have developed RNA silencing suppressors[123]. Here the βC1 proteins are likely to play their part as suppressors. These proteins are CLCuMuB-βC1[124], TYLCCNB-βC1 [125], BYVMV-βC1 (Bhendi yellow vein mosaic virus) [126], ToLCCNV-βC1 (Tomato leaf curl China virus) [127] and ToLCJAV-βC1 (Tomato leaf curl Java virus)[128].

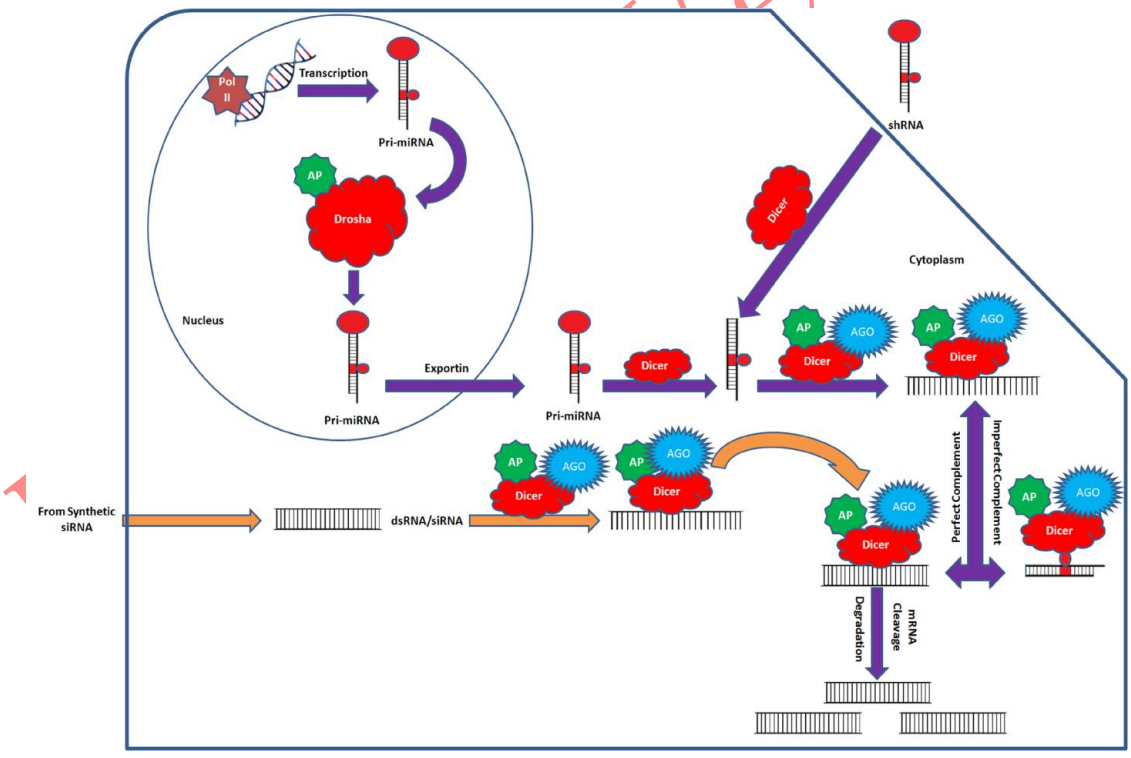

Figure 3: Model for RNA Silencing Mechanism

The TYLCCNB-βC1 proteins have the ability to bind both ssDNA and dsDNA in a non specific manner and with the help of βC1 nuclear motif, it can perform suppression mechanism[124]. Localization of ToLCCNV-βC1 proteins is specific as it requires amino acids 44-74 for specific silencing suppression [127]. Similarly, substantial dsRNA as well as DNA binding activities are exhibited by CLCuMuB--βC1[129]. It is further thought that CLCuMuB-βC1 can block PTGS signals through dsRNA or siRNA sequestering thus preventing insertion in RNA silencing complex[130-131].

2.2 Limitations of PDR and their possible solutions

Even though PDR has been successfully implemented to produce tolerant cultivars against begomoviruses, however there are certain limitations. For example PRD exhibits suppression of targeted gene reducing its ability to express. But still enough amounts of functional proteins can be generated from that particular reduced transcript. This may not be enough to produce any phenotypic variation in silenced plant. Thus PDR do not guarantee complete functionality of the silenced genes.

Similarly in the next stages, when random screening trails are conducted, the masked phenotype will affect the proper selection of plants for the next generations. This occurs in closely relative plants mostly so the problem can be solved through maintaining the sequence records of these plants before conducting the experiments. Another limitation of PDR is its non uniformity. Variation is levels of gene silencing are observed in infected plants. This leads towards the complication in interpretation of expected results which can be more worst if the phenotypic changes in plants are not visible. The solution to this problem is induction of positive controls for virus induced gene silencing vectors. It will help in marking out the silenced regions though visible phenotypes.

Furthermore, PDR in begomoviruses is affected by plant-virus-vector interactions. For example, if the plants are artificially inoculated by viruses, the viruses will induce certain symptoms though altering the leaf morphology, plant height reduction and variation in phytohormones. So when the experiments for achieving PDR are conducted through gene silencing, the virus symptoms show dominant effects thus masking the desired phenotypic changes. This is especially observed in TMV and PV-X. The problem can be solved by using tobacco rattle virus mediated gene silencing mechanism. Lastly, most frequent concern for PDR is that gene silencing mechanism can show off target activity by silencing the non target genes. Off target activity is very difficult to rule out especially when experimenting with the plants lacking the

sequenced genomes. However these few limitations should not over shadow the successful implementations of PDR mechanism to achieve the tolerant cultivars against begomoviruses because several studies have credited precession and accuracy while using the virus induced gene silencing [35,72,132-134].

Conclusion and Future prospects

Genetic resistance in cultivars have been incorporated via insertion of genes from wild types against several begomoviruses but such type of resistance gets compromised within a few years of field plantation of these cultivars because the resistance is mostly conferred by single dominant gene. Furthermore the evolution in pathogen results in breakdown of resistance in such cultivars. Now a day, engineered resistance against begomoviruses through proteomic approaches has achieved valuable importance and has been proven successful against begomovirus management world--wide.

It involves various interesting mechanism such as CP protection, MP gene cross protection, suppression of RG, RNA silencing and post transcriptional gene suppression of betasatellites which have been found effective against several begomoviruses. But as we know, all the viruses continuously undergo evolutionary phases and lead to development of new

As a counter defense, plants have strains so more and more efforts are required to find resistant wild type plant species against begmoviruses. These wild type resistant traits should be characterized to incorporate into the economically important crop plants. Similarly the interaction of begomoviruses and its insect vector (whitefly) should be widely studied and integrated management approaches must be utilized to minimize the vector populations. These vectors are harbored by thousands of different weed species all around the world. So control of these weeds is also a necessary step for the management of viruses.

In the last decade, begmoviruses have grouped with betasatellites and posed serious threat to crops in various parts of the world. As betasatellites have the ability to trans replicate with monopartite begomoviruses, so in the near future they can emerge as new disease complex which will may affect economically important crops.

Betasatellites play active role in determination of hosts for begomovirus infection and β C1 is multifunctional proteins which take part in symptom production, suppression of post transcriptional gene silencing (PTGS), bypassing plant defense mechanisms to alteration in phytohormones and viral movement from cell to cell in plants.

evolved the mechanism of phosphorylation of β C1 protein thus reducing its function as a pathogenesis determinant and degrada--tion of the β C1 by the ubiquitin/26S proteasome system to attenuate the virus symptoms. Special efforts are required to investigate the β C1-plants interaction so that we can better understand the begomovius offensive mechanism and plant defense strategies.

Similarly alphasatellites have been found associated with begomoviruses but nothing is known about their roles in virus infections yet. So biotechnological approaches like vector enabled metagenomics (VEM), next generation sequencing (NGS), Zinc finger mechanism (ZFM) and Crisper-Cas9 are needed to be tested to stay ahead and for development of begomovirus free crops.

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