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Review Article

BIOTECHNOLOGICAL APPROACHES FOR ENGINEERING RESISTANCE AGAINST VIRUSES IN PLANTS

Hussain Ara and Jawaid A. Khan*

Molecular Virology Lab, Department of Biosciences, Jamia Millia Islamia (Central University), Jamia Nagar, New Delhi -110025 India

Abstract

Plant viruses, generally cause diseases on wide varieties of agronomically important crop species and hence bring a serious threat to the food security and global economy as well. Introduction of crop immunity against viruses has been a major challenging task. The infection of viruses is difficult to control as they are strict intracellular pathogens. Moreover, their chemical control is not usually advised in practice for long as it not only affects the environment but also is supposed to lessen quality of the crops. Using biotechnological approaches for genetic engineering and molecular biology, induction of defence mechanism against viruses in crop plants is considered as one of the powerful alternative strategy. Over the past few decades tremendous progresses have been made to unravel our knowledge in the area of plant immunity against viruses. Present review describes existing strategies for developing resistance in plants against viruses. Role of protein- and nucleic acid- mediated resistance to generate pathogen-derived resistance is described. In addition, importance of the genes of host plant origin, plant's hormone and ribosome inactivating proteins for virus resistance is also discussed.

Key Words: Plant resistance, Pathogen-derived resistance, Transgenic plants.

Introduction

Demand of food production is increasing continuously with the rapid growth of global population. The world population growth rate is increasing rapidly and it is anticipated that the population is going to be doubled in next 5 decades. To meet the food necessities consequently, our production must be increased at least double by 2050 (Suweis *et al.*, 2015, Zaidi *et al.*, 2016). The agricultural crops are, however, threatened by various biotic and abiotic stresses worldwide. Plants are attacked by plethora of pathogens that create biotic stresses, among which the viruses alone shares 10–15% reduction in the global crop yields every year and ranks second after fungi for carrying disease pressure and economic losses (Khalid *et al.*, 2017). Ninth Report of International Committee on Taxonomy of Viruses elucidate that more than 6000 viruses have been identified so far among which 1300 are plant viruses (King *et al.*, 2012, Khalid *et al.*, 2017). Generally, viruses have extensive host range, for example, *Tomato spotted wilt virus* (TSWV a tospo virus) alone is efficient to infect more than 1000 species of plant kingdom belonging to 85 families (Prins *et al.*, 2008). *Cucumber mosaic virus* (CMV) has competence to infect more than 1200 species from 100 families consisting of ornamentals and vegetable plants (Zaidi *et al.*, 2016). The viruses like Potyviruses and Geminiviruses are considered dangerous for agricultural and horticultural crops as they brings drastic reduction in yield of the plants (Akmal *et al.*, 2017, Akhtar *et al.*, 2017). The crop plants are subjected to large number of different viruses that invade the plant cells systemically and inhibit normal growth of the plant. Once the plant is infected with viral pathogen, it shows stunted growth with decreased or total yield loss. The farmers rely on traditional cultural management practices to control insect vectors. These practices however, do not assure that the plants will be free of viruses in the field. Therefore, using resistant varieties remains the most favoured alternative for management of viral diseases. Conventional approaches to improve resistance are not only expensive but monotonous also (Wamiq and Khan, 2018). Thus, consciousness about enhancement of the host plant's

***Author for correspondence :** E-mail : jkhan1@jmi.ac.in

resistance and/or integration of resistance in host cells against viruses can be a noteworthy management to sustain productivity of the crops. Proper management of viral diseases requires enhanced knowledge about viral elicitors, their acceptance by the plant cells in support of infection, spreading of the disease signals as well as defense mechanisms of the plant against viral invasion that could help greatly in defeating the viral diseases.

Viruses are submicroscopic molecular pathogen consisting of a protein coat known as capsid and a nucleic acid core - either DNA or RNA. They exist as obligatory intracellular parasites and use host machinery for replication, transcription, and translation of the genome to produce its proteins and nucleic acid (Dijkstra and Khan, 2006). The viruses enter the plant cell passively through injuries caused by environmental factors or agents like insects, nematodes, fungi etc. The most favoured plant viral vectors are insects like aphids, leaf-hoppers, plant-hoppers and white-flies (Bragard *et al.*, 2013). The infectious cycle begins inside the host cells with decapsidation of capsids and then replication and translation of the viral genome. Descendant virus particles assemble, occupy the space inside host cells and pass through plasmodesmata systemically from cell to cell and finally, contaminate new hosts by the vectors. Viruses are found across the earth and are specific in selecting their host among all life forms. Viruses that infect vascular plants are highly diverse and have evolved unique genes that function to facilitate their entrance in their host's cells (Dietzgen *et al.*, 2016). Virus contamination often do not bring noticeable disease at once but affects physiological disorders which cause (i) reduction in growth and partial or complete crop failure, (ii) developmental abnormalities, (iii) drastic reduction in the yield (iv) altered vigor, (v) enhanced susceptibility to frost and drought, (vi) reduction in quality or market value, (vii) reduction in the storage quality, (viii) degradation in the consumer's choice unconstructively like changes in taste, texture, composition etc. of the plants.

Management of the population of vector organisms chemically or with pesticide applications is not very helpful as the viruses are strict intracellular pathogens. Moreover, controlling vectors by using chemicals is not supposed to be in practice for long as it not only negatively affects the environment but also degrades the quality of crops (Dietzgen *et al.*, 2016). Viral diseases cannot be managed without damaging the infected plants. In fact, management of viral diseases is a troublesome job due to efficient transmission, invasion and fast movement of the viruses in different parts of the plant as well as to other plants of the distant areas in an epidemic style (Dietzgen *et al.*, 2016). Extraction of diseased/ infected part(s) or complete plant is not promising as vector allows its transmission to the healthy plants or plant parts in systemic and epidemic form. Therefore developing virus tolerant or virus resistant varieties has been the most favoured strategy to manage virus elicitors so far. Conventional breeding is a time taking process and may not be always successful in achieving the goals. Hence, alternative strategies depending on molecular/ physiological mechanisms governing plant–virus interactions are mandatory to manage crop plants for viral diseases. Engineering resistance in plant species against viruses has been a powerful tool to achieve these goals professionally (Vanderschuren *et al.*, 2007, Zaman *et al.*, 2012, Wang *et al.*, 2017). Molecular information related to plant immunity for viruses are helpful in making strategies to fight with viral attacks in the fields. Currently, number of plant viruses which have been engineered for resistance in plants, and identification of new genes that are responsible for various diseases of the crops are increasing rapidly (Niscaise, 2014, Dietzgen *et al.*, 2016). Discovery of RNA interference pathways supported for efficient antiviral management scheme targeting infectious insect vectors. With the evolution of engineering technologies the non–conventional methods of generating virus resistance in the plant species have been successfully practiced (Niscaise, 2014, Dietzgen *et al.*, 2016, Khatoon *et al.*, 2016). Several methods are being used by researchers worldwide to develop plants conferring resistance against viruses (Lin *et al.*, 2007, Prins *et al.*, 2008, Collinge *et al.*, 2010, Niscaise, 2014, Dietzgen *et al.*, 2016, Calil and Fontes, 2017, Khalid *et al.*, 2017). Potential role of the host's miRNA in regulating genes of infecting viral pathogens and insect vectors has also been demonstrated in silico (Perez-Quintero *et al.*, 2010, Baig *et al.*, 2011, Baig and Khan, 2013, Shweta and Khan, 2014, Akmal *et al.*, 2017, Shweta *et al.*, 2018, Wamiq and Khan, 2018). This review is focused on biotechnological approaches for engineering resistance against viruses in plants. Application of transgenes from virus or host plant genome is also discussed.

Strategies for establishment of viral resistance in plants:

Viral infections in plants craft serious threats to agricultural production. Though viruses are naturally programmed with a restricted number of genes, interactions of their nucleic acids and proteins with the host factors have mystified the plant virologists for long. Disease management is habitually done by manipulating one or more of the components of disease triangle *i.e.*, host, pathogen and supporting atmosphere. Management

of viral diseases, however, is very difficult due to its style of transmission to healthy parts of the plant systemically or to other plant by vectors in epidemic form (Dietzgen *et al.*, 2016). Earlier, conventional strategies for controlling viruses focused on vector management with the use of pesticides or activating natural predators. Use of insecticides to kill the virus vectors is not only proved to be ecologically harmful in long run but also found to be a reason for reduction of the crop yield (Dietzgen *et al.*, 2016). For these reasons, conventional breeding methods have not been found to be satisfactory for management of viral diseases in plants. Hence developing resistant plant varieties against viruses or its vectors is the most suggested strategy for controlling viral diseases.

A resistant plant suppresses disease symptoms either by inhibiting replication or by blocking the viral gene expression after contamination with the viral pathogens. When a plant contaminated with the viral pathogen shows normal growth and produce normal or good yield, it is supposed to be a tolerant plant even mild symptoms of disease appear. The knowledge about the virus infections might be helpful in generating potential ideas for management of viruses in the plant species. However, rapid progression in the diversity of viruses and their ability of recombination is making the approaches complicated (Hanley-Bowdoin *et al.*, 2013). Biotechnologists have investigated and implemented numerous effective procedures for introduction of virus resistance in crop plants. Among these procedures engineering of the plants for cross protection, pathogen derived resistance, host derived resistance and more recently RNA interference comprise significance for resistance against viruses. To insert any foreign gene into a plant cell and to express that gene across the species, genus and family boundaries is now a common practice. Genetic engineering offers technologies for incorporation of new virus resistance into existing plant cultivars that are highly susceptible. Various steps involved in the strategy are specified in Fig. 1. Genetic engineering has been acknowledged as a key approach for improvement of the crop yield and lessening the losses due to abiotic and biotic stresses (Yuan *et al.*, 2011). The strategies so far used for genetic transformation in plants utilize different approaches to place DNA into the nucleus where it may integrate with chromosomes. These strategies are usually common to all pathogens although their efficacy for all pathogens varies (Zaman *et al.*, 2012, Wang *et al.*, 2017). Nevertheless, system for delivery of the molecule that could trigger the gene activity stably in the plants has been still a major challenge. Various methods including *Agrobacterium*-mediated transformation, microprojectile-bombardment, protoplast transformation, electroporation, pollen-tube pathway method, infiltration, microinjection, and silicon carbide-mediated transformation etc. have been evolved to fight these challenges. Among these *Agrobacterium*-mediated transformations is considered to be the most reliable and promising for stable transformation of the specific objectives. Micro-bombardment and virus induced gene silencing also considered useful for delivery of nucleic acid sequences during generation of resistance in plants.

Agroinfiltration is an influential means to enhance our knowledge about applications of RNAi for plants (Sharma *et al.*, 2013). This method involves insertion of *Agrobacterium* cloned with specific DNA constructs into the leaf cells/tissues where RNA silencing is activated for disease resistance in plants. Cytoplasmic RNAi can also be induced efficiently with this technology as in the same way to a strategy for transient expression of T-DNA vectors. The transiently expressed DNA generally encodes a ssRNA or dsRNA which are in general hairpin RNA (hpRNA) (Sharma *et al.*, 2013). It is evident from the literature that infiltration of hpRNA is specifically effective because dsRNA can be processed directly to siRNAs to facilitate silencing mechanism (Tenllado *et al.*, 2004, Dunoyer *et al.*, 2006, Mlotshwa *et al.*, 2008, Sharma *et al.*, 2013).

Micro-bombardment is a method used for transfer of linear or circular DNA template in the nucleus. Bombarding cells with dsRNA, siRNA or DNA usually encode hairpin construct as well as sense or antisense RNA (Sharma *et al.*, 2013, Puyam *et al.*, 2017). This method, generally, is applied to study gene expression as early as a day after bombardment in continuation up to few days or weeks that could direct systemic gene silencing in the system. These approaches have been exploited for resistance generation either by designing a gene to interfere directly or to induce the host resistance to interfere with the viruses. For induction of RNAi in plants a method popularly identified as virus induced gene silencing (VIGS) has also been applied (Lu *et al.*, 2003, Sharma *et al.*, 2013). Here, viral genes are modified to trigger RNA silencing in the plants. Cloning of the homologous gene fragments into viruses without compromising replication and movement of the viral genome is obligatory to silence endogenous plant genes. This was first demonstrated by Dallwitz and Zurcher in RNA viruses (Dallwitz *et al.*, 1998) and in DNA viruses by Kjemtrup and co-workers (Kjemtrup *et al.*, 1998). More recently, some new strategies came to revolutionize the scope of virus resistance generation in the plants. These are popularly called as genome editing system

(GES). The most practiced GES comprise transcription activators like effector-nucleases, short palindromic repeats like Case endo-nucleases as well as zinc finger nucleases (Romay and Bragard, 2017). However, these technologies have to be applied and evaluate for agriculturally important crop plants. The transgene used to confer resistance against viruses were initially based on the concept of pathogen derived resistance. Utilization of viral genes encoding structural and non-structural proteins has been shown to confer resistance in many plant species (Prins *et al.*, 2008, Mandadi and Scholthof, 2013). Later, use of non-coding viral RNA has been shown to be more potential transgene for virus resistance in plant species (Khatoon *et al.*, 2016). This led to the innovation of a novel resistance system in plants popularly known as RNA silencing. Based on transgene used for virus resistance in a plant species the strategy can be categorized into two broad categories (A) Pathogen Derived Resistance in which the transgenes used are taken from viral gene sequences, and (B) Non Pathogen-Derived Resistance in which transgenes are prepared with the genes from host plant or other sources that obstruct the target virus (fig.1).

A. Pathogen derived resistance:

A plant is said susceptible for a disease if the pathogen is allowed to invade plant cell / tissue and transmitted to other parts and subsequently disease symptoms appear (fig. 2). Modern biotechnology offers strategies for virus resistance in plants based on molecular mechanism of virus, vector and host interactions. Usually viruses enter plant cells through wounds caused by their vectors and then move from cell to cell via plasmodesmata in the form of viral ribo-nucleoprotein (vRNP) complexes (fig.2). Subsequently virus-encoding protein genes such as replicase (*Rep*), capsid protein (*CP*), and movement protein (*MP*) are supposed to be translated and actively support encapsidation, replication, translation and ultimately movement of the viruses within the cytoplasm of host cell. Cells of the plants that exhibit resistance against viruses have efficiency to counteract ETS (Effector- triggered susceptibility) of the viral pathogens as well as to activate ETI (Effector-triggered immunity) in response in the cell (fig. 3). So far, huge progresses have been made to understand plant's immunity against viruses at molecular level (Mandadi and Scholthof, 2013, Calil and Fontes, 2017). The strategies of genetic engineering have been emerged as a highly effective tool for management of viral diseases worldwide. This technique is in contrast to the conventional breeding where host plants were used to bring resistance. Currently small genes derived from host plant have also been identified, cloned and engineered to get resistance from viruses. The idea of resistance induction with genes of the pathogen by transformation was first proposed by Hamilton in 1980. Later it became a popular concept after the work of Sanford and Johnston in 1985 with coat protein (*CP*) gene of *Tobacco mosaic virus* (TMV). The first successful report of resistance engineering in tobacco plants with the *CP* gene of TMV came by the work of Powell and coworkers (Powell *et al.*, 1986). The concept further has been used up with the genes derived from plant viruses for example coat protein, replicase protein, movement protein, antisense RNA, satellite RNA etc. Up to now more than 30 virus groups have been utilized for engineering CP virus resistance (Mandadi and Scholthof, 2013). This idea of resistance generation in plants to oppose virus invasion is now accepted as pathogen-derived resistance (PDR). The strategy, based on transgene with viral CP has been the most studied application of PDR and has provided protection against several plant viruses since 1991 when Namba *et al.* (1991) expressed coat protein of *Cucumber mosaic virus* (CMV) in tobacco plants. The mechanisms for substantiate confirmation of PDR against viral infections explain expression of the genes encoding proteins of viruses in the host plant (Calil and Fontes, 2017). Acquaintance of these mechanisms can provide ways for protection of plants against viruses. PDR against viruses can be achieved by using protein sequences and/ or nucleic acid sequences of the viruses. These are described here separately.

1. Protein Coding Sequence Mediated Resistance:

Coat Protein (CP): Coat protein-mediated resistance (CPMR) is provided to the plants when transformed with the construct consisting of viral *CP* gene. The transformed plants with *CP* gene are supposed to convey mechanism to defend against infection by the viruses of the same group. Since the first successful implementation of PDR involving expression of TMV coat protein in tobacco plant by Powell *et al.* (1986), the technology of CPMR against viruses has been applied effectively in many plant species. Coat protein (*CP*) gene was then used to generate virus resistance in various transgenic plants (Beachy *et al.*, 1990, Lomonossoff, 1995). Beachy and co-workers have demonstrated interference and disassembly of TMV particles in the transgenic tobacco plants transformed with *CP*. Their findings opened new hope of protection of plants from viruses (Beachy *et al.*, 1990). Consequently the strategy, based on transgene with viral *CP* has been widely applied to study plant protection from viruses of various groups

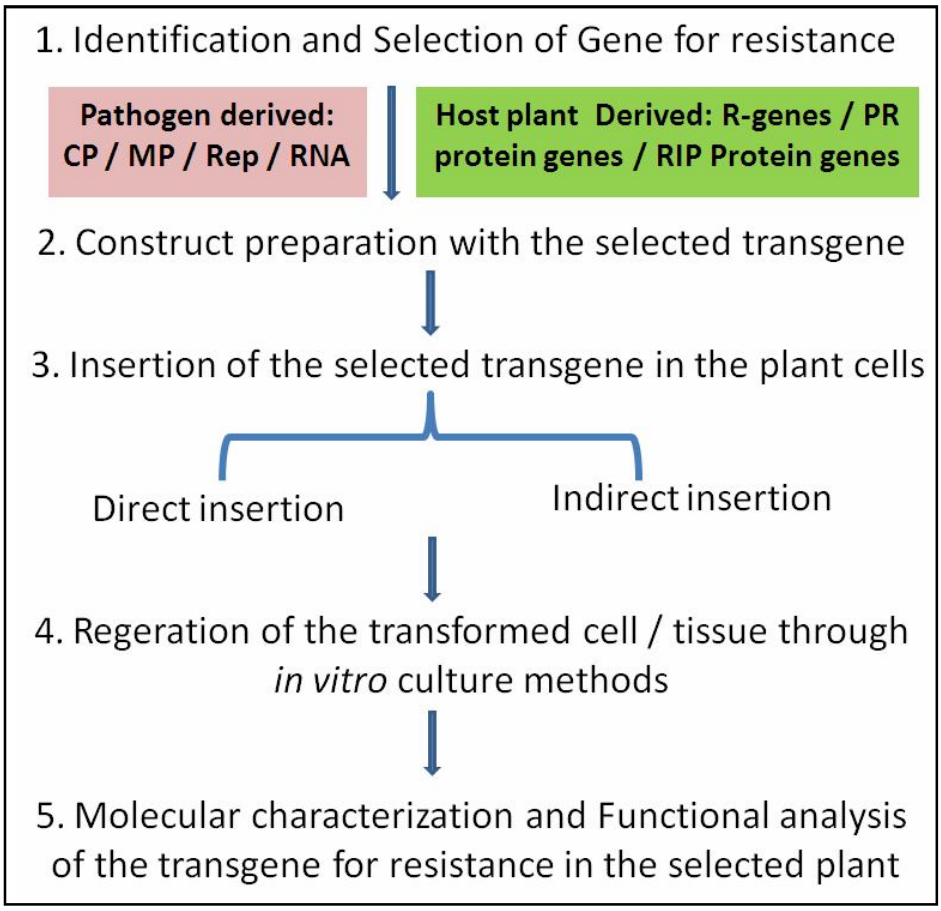

Fig.1: Steps for establishment of resistance in plants. This involves (1) Identification and selection of resistant gene from pathogen or host that to be used as transgene. The transgene from viral pathogen are used for implementation of pathogen-derived resistance,whereas, transgene should be taken from the plant for host-derived resistance. (2) Construct preparation with the selected transgene. The selected transgene is first required to clone in binary vectors and inserted in the *Agrobacterium tumefaciens* cells. These transformed *Agrobacterium* cells are ready for induction of stable transformation in plants(3) Insertion of the selected transgene in plant cells,(4) Regeneration of transformed cells/tissue to develop plant engineered with the specific transgene, (5) molecular characterization and functional analysis of the transgene for resistance in the desired plant.

and results provided various degrees of protection against numerous plant virus groups (Calil and Fontes, 2017). These work proved that the expression level of *CP* gene define efficiency of resistance against viruses in the transgenic lines. It has been reported that this strategy offers a range of degrees of protection like delayed development of the symptoms, complete or limited resistance against many plant virus groups (Lomonossoff, 1995). In some cases *CP* gene provides more protection against several strains of the viruses of the same group from which the *CP* gene is derived, or also for the closely related virus species (Prins *et al.*, 2008). CPMR has been reported for conferring resistance against TMV, ToMV (*Tomato mosaic virus*), PMMV (*Pepper mild mosaic virus*), TMGMV (*Tobacco mild green mosaic virus*), PVX (*Potato virus X*), PVY (*Potato virus Y*), AIMV (*Alfalfa mosaic virus*), CMV as well as TRV (*Tobacco rattle virus*) (Beachy *et al.*, 1990, Prins *et al.*, 2008, Calil and Fontes, 2017). CPMR, so far, has been described for more than 35 viruses belonging to tobamo-, potex-, cucumo-, tobra-, carla-, poty-, luteo-, and alfamo-viruses (Prins *et al.*, 2008, Calil and Fontes, 2017). Successes with CPMR lead to generation of the plants which are able to express viral CP sequences. Such plants confirm resistance to an extensive range of RNA viruses. CPMR also found to play no positive role in combating plant-infecting DNA viruses (Sudarshana *et al.*, 2007). However, successful CP-mediated protection has been established in the commercialization and squash lines resistance against CMV, *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), and papaya resistant to *Papaya ringspot virus* (Sudarshana *et al.*, 2007). Through further investigations it was demonstrated that transgenic lines with high virus

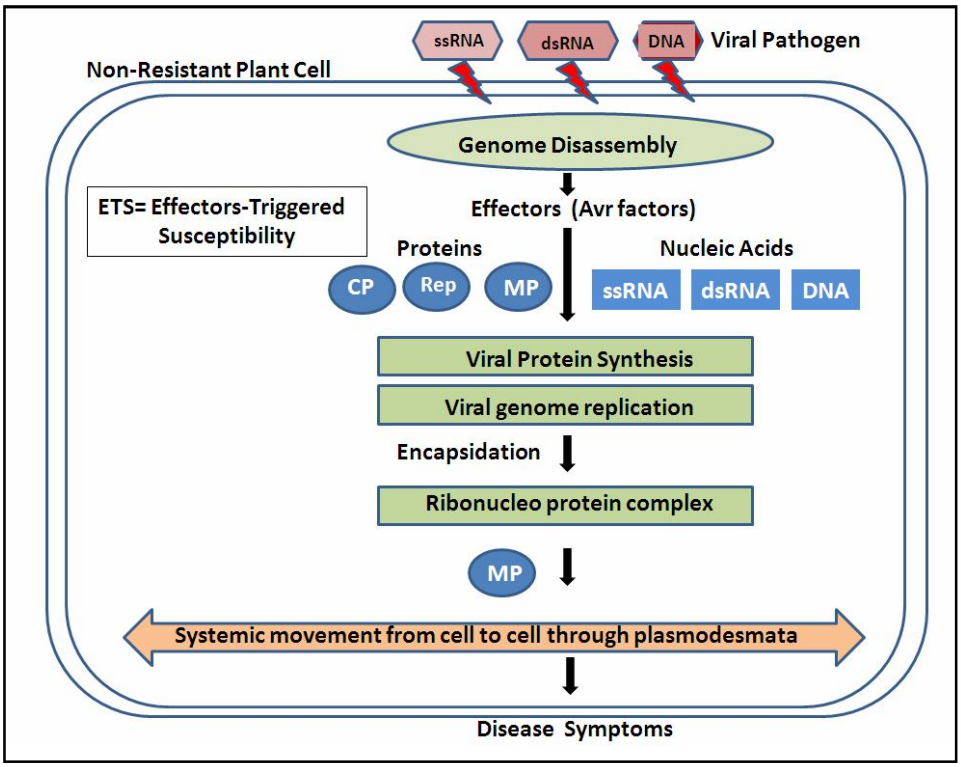

Fig. 2: Illustration of entry and invasion of viral pathogen in a susceptible plant cell: When the viral pathogen (with ssRNA / dsRNA / DNA) enters into plant cell through cellular damage (shown by red lightning bolt), ETS (Effector triggered susceptibility) of the cell activates in response to the effectors released from the viral pathogens. This in turn facilitates translation of virus encoded proteins like coat protein (CP), replicase protein (Rep) and movement protein (MP). Products of CP and Rep encapsidate to produce viral ribonucleo protein complex (vRNP). With the co-operation of MP this complex finally move to next cells through plasmodesmata. In this way the viral pathogens invade plant cells and move systemically to other parts also and the plant shows disease symptoms. Such plant is known as susceptible or non resistant plant.

resistance levels did not express any viral CP (Prins *et al.*, 2008). Instead, the CP RNA level became low in these resistant plants (Prins *et al.*, 2008). Consequent works explained the phenomenon of non-expressing *CP* gene in the transgenic plants that resistance is occurred because of the expression of CP mRNA activates post-transcriptional gene silencing (PTGS) and provide RNA-mediated resistance for viruses by the siRNA pathway (Prins *et al.*, 2008, Calil and Fontes, 2017). Still, the actual mechanism of CPMR has not been completely understood, various plant species has been transformed with CP gene of viruses and high level of resistance observed in comparison to non transformed plants (Kamo *et al.*, 2010, Dubey *et al.*, 2015).

Replicase Protein: *Replicase* gene of virus is the second most widely used transgene that confers resistance against plant viruses, and the strategy is known as Replicase-mediated resistance (Rep-MR). It provides highly specific resistance for the virus from which transgene is isolated (Varma *et al.*, 2002). Rep-MR to TMV was first demonstrated in the plant species by Golemboski *et al.* in 1990. They reported a sequence containing 54 kDa fragment of replicase protein translated to produce replicase protein. Production of replicase protein has been reported to be a requirement to confer resistance against different sub groups of the same virus (Calil and Fontes, 2017). However, a mutant of replicase derived from CMV sub group I virus has been found to confer high levels of resistance to all strains of the same group of CMV in tobacco plants, but not to the other sub groups of CMV or even to any other virus (Zaitlin *et al.*, 1994). Since the first report of the transformation with replicase protein gene from TMV (Golemboski *et al.*, 1990), the technique has been effectively exploited in 16 RNA/DNA viruses representing 11 plant virus groups (Snehi *et al.*, 2015). Rep-MR has also been established successfully in *Lilium* which conferred resistance to CMV (Azadi *et al.*, 2011). Recently, genes of replicase protein of two geminiviruses - *African cassava mosaic virus* and *Tomato yellow leaf curl virus* have also been reported to induce resistance (Zaidi *et al.*, 2016). It has been reported that the plants transformed with *Rep* gene

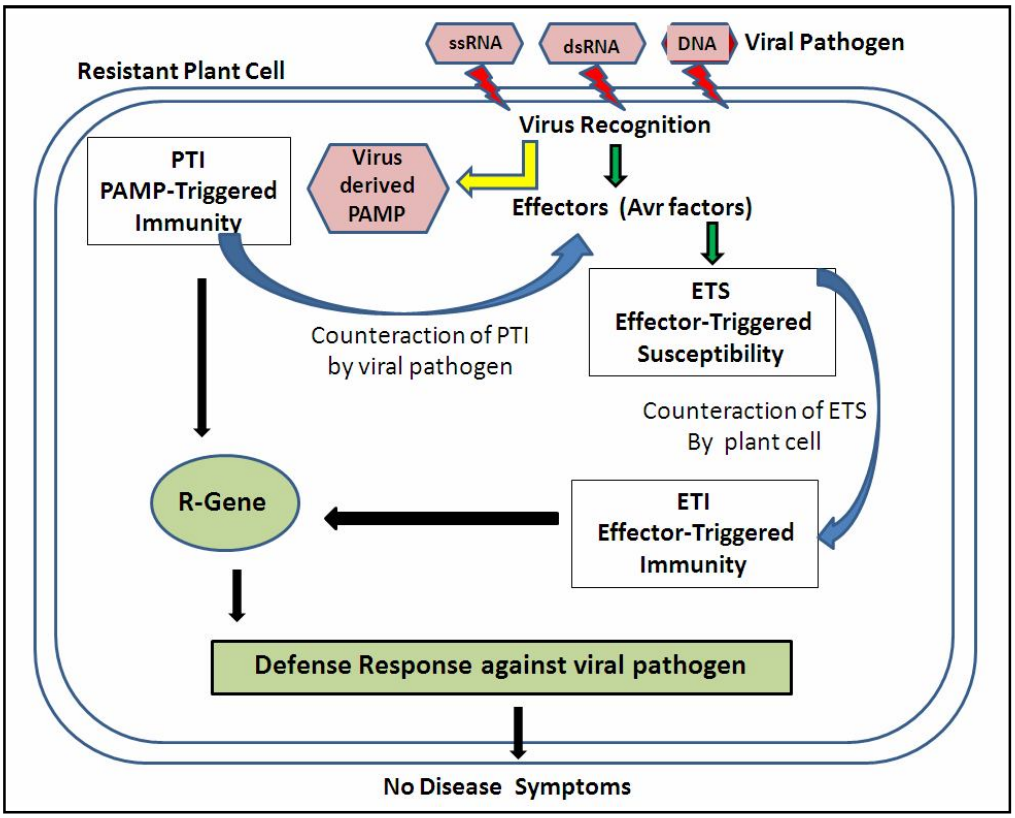

Fig. 3: Illustration of activation of plant's innate immunity against viral pathogen: when vial pathogen comes in contact with the cell, its conserved motifs (known as PAMP: Pathogen Associated Molecular Patterns) are recognized and PAMP triggered immunity (PTI) activates in response. To overcome this first line of defense, the pathogen starts releasing effectors in the plant cell which inhibit PTI and tries to activate ETS (Effector-Triggered Susceptibility). However, when the plant is not susceptible it counteracts the virulence strategy of the pathogen. To counteract ETS plant cell synthesize intracellular resistance (R) protein which specifically recognize pathogen effector and activate ETI (Effector-Triggered Immunity). The plant showing activation of such immunity against pathogen is known as resistant plant.

inhibit replication strongly and in many cases exhibit resistance significantly to its susceptible viruses. It is assumed that the protein formed by the *Rep* gene interferes with the replicase of viruses by binding the host factors or virus proteins which in turn regulate replication as well as expression of the viral gene (Zaidi *et al.*, 2016). Hellwald and Palukaitis (1995) have reported inhibition of both virus replication as well as systemic infection against CMV by Rep-MR. Later, Palukaitis and Zaitlin (1997) have suggested two mechanisms for Rep-MR. According to them, the resistance occurs directly with the expression of transgene protein in transformed plants or it is correlated inversely with accumulation of transgenic RNA as in case of PVX and CymRSV.

Movement Protein: Transport of viruses between adjacent cells of plants is supported by viral-encoded movement protein (MP). MP has also been manipulated to produce transgene for generating viral resistance in plants. These proteins are supposed to interact with the plasmodesmata and swell the macromolecular exclusion limit which then facilitates movement of the virus to neighboring cells (Prins *et al.*, 2008). MP also helps in formation of tubules so as to allow intercellular trafficking of ribonucleo-protein complexes consisting of viral RNA and capsid proteins (Calil and Fontes, 2017). In the beginning, when tobacco plants were transformed with a construct consisting of MP from TMV mutant, and with the MP from *Brome mosaic virus* separately, the transgenic tobacco plants occurred with both the MPs exhibited resistance against infection by TMV (Malyshenko *et al.*, 1993). It seems that, presence of the MP in the plants before infection with the viral inoculum confines the capacity of the incoming viral MP to interact with the plasmodesmata successfully. Long-distance movement of six different groups of plant viruses has been found to be restricted with the use of defective movement proteins as transgene (Varma *et al.*, 2002). In another report, Cooper *et al.* (1995) described broad spectrum resistance in the transgenic tobacco plants with MP of TMV. They have reported the transgenic tobacco plants showed resistance against a number of viruses

like *Alfalfa mosaic virus* (AlMV), CMV, *Tobacco ringspot virus* (TRV), *Peanut chlorotic streak virus* (PCSV) and *Tobacco ringspot virus* (TRSV). Similarly, Tacke *et al.* (1996) raised transgenic potato with MP of *Potato leaf roll virus* (PLRV) and observed resistance against PVY and PVX. On the other hand, the plants transformed with MP of *Tomato spotted wilt virus* (TSWV) showed resistance against TSWV strains only (Varma *et al.*, 2002). Tobacco transgenic plants engineered with the movement proteins - BV1 or BC1 of *Tomato Mottle Virus* (TMoV) showed a significant delay in infection to TMoV (Duan *et al.*, 1997). Over the years, other full-length or condensed genes from viral origin have been widely used to engineer plants to exhibit virus resistance (Khalid *et al.*, 2017).

2. Nucleic Acid Sequence Mediated Resistance:

RNA Silencing: Pathogen-derived resistance in plants has also been found through the use of RNAs of viruses. This resistance is attained by inactivating a gene by homology-dependent silencing. RNA silencing is a technique of making a specific gene inactive by inserting a small sequence of RNA that match partially or completely the target gene sequence and no proteins are formed (Duan *et al.*, 2012). Generally, double-stranded secondary structures are formed from the RNA genome of plant viruses. These secondary structures form complex with the action of RNA polymerases during replication steps. These complexes then activate RNA silencing machinery to produce virus derived sRNAs (vsRNAs). Later, it assembles to produce a specific protein complex that is subjected to degrade the viral nucleic acids. Mobile silencing signals of resistance are generated then and spread between cells through plasmodesmata and also to long distance to other parts of the plant through phloem. This process activates RNA silencing in healthy cells and stimulate plant recovery phenomenon (Duan *et al.*, 2012, Nicaise, 2014). Nucleic acids of viruses (RNA and DNA) replicate when associated with the virus-derived small RNAs. During accumulation of nucleic acid the host plant recognizes and helps in the amplification of exogenous sequences by its own RNA polymerase. As a result dsRNAs are formed which activate RNA silencing by targeting the homologous pathogen genome for degradation. This phenomenon of RNA-silencing for resistance against viruses was first reported by Lindbo *et al.* (1993). Previously, it was expected that silencing could be related with the co-suppression mechanism of Napoli *et al.* (1990). Since then engineering of the plants to insert single-stranded sense sequences or antisense viral sequences became a regular policy for pre-activation of the silencing machinery and to obtain resistance against viruses (Ritzenthaler, 2005, Khatoon *et al.*, 2016, Akhtar *et al.*, 2017).

RNA silencing-based resistance has been an influential way for engineering resistance in a variety of crops for the last two decades and recognized as an antiviral device to protect diverse plant species against RNA viruses (Tenllado *et al.*, 2004, Prins *et al.*, 2008, Rodrigues *et al.*, 2009, Bologna and Voinnet, 2014). Plants usually combat viral infection by degrading viral RNAs specifically through RNA interference (RNAi) resulting in silencing of the target gene and inactivate invading nucleic acids (Muthamilarasan and Prasad, 2013, Khalid *et al.*, 2017). During this silencing practice the dicer allows cleavage of dsRNA precursor into 21-26nt long nucleotides which are designated as short interfering RNAs (siRNA) and microRNAs (miRNAs). These interfering RNAs help in the formation of an RNA-induced silencing complex (RISC) that degrades single-strand RNA. RISC complex is composed of Argonaute (AGO) protein. When Small RNA molecules arrive to RISC, the AGO along with the other related proteins allows cleavage of the target RNA. Ultimately, translation of the target mRNA is supposed to be suppressed. siRNAs have also been shown to cause methylation of the homologous DNA to express transcriptional gene silencing (Muthamilarasan and Prasad, 2013). Silencing pathways in plants are diverse and overlapping, however three basic processes are involved: (i) Cytoplasmic RNA silencing or PTGS mediated siRNAs, (ii) Silencing mechanism by miRNAs of plant, and (iii) Transcriptional gene silencing occurred by methylation of DNA and histone proteins directed by siRNAs. All these pathways have been revealed to have significant defensive role against viral pathogens (Baig *et al.*, 2011, Baig and Khan, 2013, Bologna and Voinnet, 2014, Shweta and Khan, 2014, Dietzgen *et al.*, 2016, Calil and Fontes, 2017, Shweta *et al.*, 2018).

The silencing mechanism is categorized into two groups- TGS (transcriptional gene silencing) and PTGS (post transcriptional gene silencing). All the RNA-mediated resistance engineering in plants is considered as example of PTGS (Prins *et al.*, 2008). PTGS was first demonstrated by Napoli and coworkers in *Petunia hybrida* using *chalcone synthase* gene (Napoli *et al.*, 1990). They noticed that in the transgenic plants the host gene and the transgene encoding the same RNA became inactivated. This inactivation, later described as RNA silencing or RNA interference and found in a variety of eukaryotic organisms (Mlotshwa *et al.*, 2008, Rodrigues *et al.*, 2009). Since then RNA silencing has been the

natural strategy to control gene expression during fundamental progression such as development, gene regulation and /or defence against pathogens. The strategies based on RNA silencing have been extensively used to produce virus resistant transgenic plants (Rodrigues *et al.*, 2009, Wamiq and Khan, 2018). PPRV resistant 'SunUp' papaya and *Potato leaf roll virus* (PLRV) resistant potato varieties "NewLeaf Plus" and "NewLeaf Y" is the example (Duan *et al.*, 2012). Major factors for stability of RNA silencing include structure, copy number, or expression level of the transgene, environmental conditions or developmental stages of the plant (Majewski *et al.*, 2009). However, induction of RNA silencing can be destabilized during cell proliferation and appears to be re-initiated in next generation (Furutani *et al.*, 2007, Kasai and Kanazawa, 2012). Nevertheless, transgene-mediated RNA silencing can induce a strong, tissue-specific or ubiquitous silencing and is suitable for producing plants in which one or more genes are stably silenced in presence of the transgene (Shweta *et al.*, 2018, Wamiq and Khan, 2018).

Antisense RNA: The antisense technology has been considered as a potential therapy to treat many genetic and metabolic disorders, for identifying gene functions and in crop development. Antisense RNA which is supposed to be complementary to part of the viral genome restrains expression of viral mRNA. During this inhibition process a complementary dsRNA is formed that is further recognized and degraded by the host silencing machinery. Regulation of gene expression by antisense RNA was discovered in bacteria also as a natural phenomenon (Simons, 1988). This technology of antisense is based on blocking the signal transduction in the central dogma from DNA to protein via RNA by the introduction of an RNA strand complementary to the sequence of the target mRNA. It was hypothesized that the antisense RNA base-pair with its target mRNA thereby forming dsRNA duplex causing the blockage of mRNA maturation and/or translation (Duan *et al.*, 2012). Through this technique the targeted mRNA is not permitted for its translation into a viable protein that is required for the virus invasion. Antisense RNAs have been used to introduce antiviral resistance in several species of plants. The technique has been demonstrated for inhibition of the expression of the *polygalacturonase* gene in tomato (Smith *et al.*, 1988). Duan *et al.* have recorded effective reduction in the chalconesynthase expression and ethylene synthesis also (Duan *et al.*, 2012). Significant resistance against *Potato leaf roll virus* has been reported very early in 1989 in potato plants by Kawchuk *et al*. Later, Waterhouse and co-workers have observed induction of immunity in potato plants against PVY (Waterhouse *et al.*, 2001). Day and coworkers (Day *et al.*, 1998) used antisense AL1 transcripts of *Tomato golden mosaic virus* (TGMV) to engineer geminivirus resistance in tobacco plants. Resistance against *Cotton leaf curl virus* by using anti-sense constructs of *Rep*, *REn* and *TrAP* genes have also been reported to be engineered (Asad *et al.*, 2003, Ahmad *et al.*, 2009). Antisense sequence of âC1 was found to advance resistance in symptom development (Akhtar *et al.*, 2017).

Satellite-RNA: Satellite RNAs provide an additional approach to confer protection against viruses. Usually, these are dependent relatively upon helper virus for their replication and invasion in the infected plants. Some viruses have specific satellite RNA molecules which may intensify the disease caused by virus or may ameliorate the disease. The satellite RNAs have been used successfully for developing virus resistant transgenic plants for resistance against cucumo- and napoviruses (Varma *et al.*, 2002). Transgenic tobacco expressing satellite RNAs of CMV or TRSV exhibited attenuation of disease symptoms (Harrison *et al.*, 1987). Nevertheless, this strategy has not gained much acceptance for two reasons- first the resistance is incomplete and second is the chance of minor mutations in satellite RNAs. There is however, a possibility of combining satellite RNA-mediated resistance with CPMR for developing stable resistance (Yie *et al.*, 1992).

Initially it was believed that plant's inherent immune response to virus invasion activates resistance by producing a gene product that is executed to establish local cell death and systemic acquired resistance (Witham *et al.*, 2006). However, in subsequent studies it was proved that the plants also communicate resistance to some degree by expressing the truncated viral protein sense sequence or the non-coding viral sequences such as the satellite RNA sequences. Report of involvement of satellite RNAs (satRNAs) of CMV in lethal necrosis in tomato brought the attention of researches towards satRNAs for providing resistance in plants against viruses. The use of satRNAs for attenuation of CMV symptoms has been reported by Simon *et al.* in 2004. The plants engineered with CMV satRNA developed attenuated symptoms in tobacco, petunia, pepper and tomato (Harrison *et al.* 1987, Yie *et al.* 1992, Kim *et al.* 1995 and 1997, Stommel *et al.* 1998). However, all the satellite sequences are not supposed to provide symptom attenuation (Simon *et al.* in 2004). They can even cause necrosis hence it is believed that there is a threat in amplification of satellite RNA in transgenic plants as it may result in necrotic signals when it is naturally infected

by spacific virus. For this reason, use of satellite RNA is not considered as safe for generating virus resistance in plants unless further studies reveal high level of stability in the system. However, Akhtar *et al*. (2017) have reported expression of intron hairpin (ihp) construct against *βC1* gene of CLCuMB (*Cotton leaf curl Multan Beta satellite*) in transgenic plants of *N. tabacum* that showed resistance towards disease. Earlier, Khatoon *et al.* (2016) have discussed resistance in cotton against CLCuRV (*Cotton leaf curl Rajasthan virus*) employing intron hairpin construct. Use of ihpRNA constructs targeting AC1 of *Cotton leaf curl Kokhran virus Burewala* (CLCuKoV-Bu) and *Cl* of CLCuMB has conferred resistance towards viral expression in *Gossypium hirsutum* (Ahmad *et al.*, 2017*,* Akhtar *et al.*, 2017).

Artificial miRNA: Artificial microRNA (amiRNA) technology is based on designing miRNA or engineering miRNA artificially that has efficiency to imitate the intact structures of the endogenous miRNA precursors which utilize the natural silencing pathway to target desired transcripts (Duan *et al.*, 2012). Variation in some nucleotides within sense and antisense strands of miRNA has no consequence on its biogenesis and maturation if the endogenous secondary structure of miRNA remains unaltered. The amiRNA acts as a specific and influential means for study of metabolic pathways, gene functions as well as for improving traits in plant species. The amiRNA machinery was first applied for gene knock-down in human cell lines by Zeng *et al.* in 2002. Later it was applied in a plant system – *Arabidopsis* (Parizotto *et al.*, 2004). Generally, amiRNA sequences are arranged in a way that the determinants of plant miRNA be able to aim specifically to silence its intended target genes. They resemble the natural miRNAs in containing a Uracil residue at their 5' end, having an Adenine/ Uracil residue as their tenth nucleotide and displaying 5' instability (Reynolds *et al.*, 2004). Then in 2006 it was demonstrated that amiRNAs expression under constitutive or tissue specific promoters can down-regulate a number of endogenous genes without disturbing the expression of any other genes (Schwab *et al.*, 2006). Very recently, plant host-encoded miRNAs (ghr-miR398 and ghr-miR2950) over-expressed in *G. hirsutum* showed noticeable resistance against *Cotton leaf curl Multan virus* and *Cotton leaf curl Multan beta-satellite* (Akmal *et al.*, 2017).

B. Host derived resistance:

1. Host Genes-Mediated Resistance: Besides PDR strategy for engineering virus resistance the alternate approach for generation of virus resistant plants include the use of transgene originated from plant's gene having features of expressing virus-resistance in plants. There are genes present in the plant genome which provides resistance to the plant itself against various pathogens. These genes are known as resistance genes. Resistance to the host plant can be achieved by two routes: first by using dominant Resistance genes (*R*-genes) and second with the use of recessive resistance gene that are supposed to be critical for plant viral infection (Hashimoto *et al.*, 2016). Most of the dominant *R*-genes code for the proteins with nucleotide-binding sites and leucine-rich repeats (NB-LRR) that are able to recognize the viral avirulence gene (*avr*) particularly through gene-for-gene interaction (Nicaise, 2014). *R*-genes have been found to be active for providing resistance to the host against other pathogens (bacterial and fungal) also (Padmanabhan and Kumar, 2014). So far many NB-LRR proteins have been identified for conferring resistance against viruses (de Ronde *et al.*, 2014). *Rx1* gene is the best example of *R*-gene for resistance against PVX (Nicaise, 2014). Earlier, Tobacco *N* gene product has been described to interact directly with the replicase of TMV (Ueda et al., 2006).

The second route provides resistance to the host plant against viruses with recessive *r*-genes. This is widely exploited in many crops with the use of biotechnology for generating viral resistance (Wang and Krishnaswamy, 2012). There are eukaryotic translation initiation factors like - eIF4E and eIF4G or their isoforms that have been identified as recessive resistance genes (Hashimoto *et al.*, 2016). To establish broad spectrum virus resistance using recessive resistance gene it is essential to enhance our deep knowledge about genetic resources for the recessive resistance. Absence of transcription factor - eIF4Es is not helpful in inducing resistance in a plant system. Hence, introduction of a mutation is often recommended for a potential recessive resistance (Wang and Krishnaswamy, 2012). Recessive resistance is fairly prominent in the plant species. This resistance has been reported to occur as a result of incompatibility between the host proteins and the pathogen derived factors, with which they need to interact to establish an infection (Robaglia and Caranta, 2006). The recessive resistance is supposed to be based on the molecular interactions between viruses and host plant species. Plant viruses capture the host cellular proteins and try to disseminate their genomes in the plant cells, and ultimately move to the adjacent cells and tissues which are healthy (Wang, 2015). However, any mutations in the plant genes which are known for expression of the factors required for viral infection and their invasion after infection. Another

promising system for recessive resistance against viruses in plants relies on the self activation of the plant defense responses (Hashimoto *et al.*, 2016).

Expression of recessive resistance controlled by eIF4Es was first observed in the mutants of *Arabidopsis thaliana* against a potyvirus - *Tobacco etch virus* (Lellis *et al.*, 2002). Subsequently, eIF4Es-mediated recessive resistance against various potyviruses has been recognized in a number of plant species like pepper, lettuce, and wild tomato (Hashimoto *et al.*, 2016). The recessive resistance controlled by eIF4Es has also been detected for other viruses like *Cucumber mosaic virus* in *Arabidopsis* (Yoshii *et al.*, 2004), *Turnip crinkle virus* in *Arabidopsis* (Yoshii *et al.*, 1998), *Melon necrotic spot virus* in melon (Nieto *et al.*, 2006), *Barley mild mosaic virus* and *Barley yellow mosaic virus* in barley (Kanyuka *et al.*, 2005, Stein *et al.*, 2005) as well as *Rice yellow mottle virus* (genus *Sobemovirus*) in rice (Albar *et al.*, 2006). Besides effectiveness of the eIF4Es for expression of recessive resistance genes, identification and characterization of additional gene targets for such resistance against a wider range of viruses especially harmful for production of economically important crop species, is required (Hyodo and Okuno, 2014, Wang, 2015). Genome-wide screening using the heterologus yeast system for *Brome mosaic virus* as well as for *Tomato bushy stunt virus* has proved that viral infections are controlled by more than 100 host genes. These genes instruct distinct sets of the host transcription factors responsible for stoppage of invasion of the viruses (Gancarz *et al.*, 2011, Nagy, 2016). Furthermore, other host proteins identified from naturally occurring resistant cultivars could also be used as the important genetic resources for induction of recessive resistance. However, molecular analyses are always obligatory to reveal efficiency of the positive regulators for execution of recessive resistance against viruses in plant species.

It is clear from the literature that most of the successful resistance against RNA viruses is mediated by RNA silencing in general. In contrast, successful resistance against DNA viruses is seldom obtained as compared to the RNA viruses. The viruses belonging to *Geminiviridae,* a family of plant DNA viruses appeared to be less susceptible to RNA silencing (Duan *et al.*, 2012). It is reported that over-expression of the gene driven by a geminiviral promoter could be silenced if infected with a homologous geminivirus (Khan *et al.*, 2015). When this process is correlated with the other RNA silencing phenomenon, it is concluded that the geminivirus genome may possibly be targeted by the RNA silencing mechanism. In one report of Duan *et al.*, it is demonstrated that transformation of black gram (*Vigna mungo*) leaves with a hpRNA construct containing the promoter sequence of geminivirus *Vigna mungo yellow mosaic virus* (VMYMV) under the control of the 35 S promoter resulted in recovery of the plants successfully after VMYMV infection (Duan *et al.*, 2012). Their results suggest that the strategy of RNA silencing could be effective for engineering resistance against DNA viruses also. Similarly, *Bean golden mosaic virus* (a begomovirus) is also found to be suppressed by the expression of hpRNA gene derived from a replicase coding sequence (AC1) (Aragao and Faria, 2009). Thus it is anticipated from these reports that geminiviruses can be targeted by both PTGS and TGS mechanisms (Aragao and Faria, 2009, Duan *et al.*, 2012).

2. Plant Hormone-Mediated Resistance: The plant hormones including abscisic acid (ABA), ethylene (ET), Jasmonic acid (JA) as well as brassino-steroids play important role in signal transduction involved in plant defenses. These hormones also been used to modulate antiviral and other biotic resistance mechanisms in various plant species (Robert-Seilaniantz *et al.*, 2011). Generally, endogenous accumulation of the hormones occurs during viral infection in the plants. These accumulations play an important role in controlling transcriptional reprogramming of the genes encoding pathogenesis related (PR) proteins (Tsuda *et al.*, 2008, Yi *et al.*, 2014). The signal are transferred to the uninfected tissue of the plant as an heterocomplex probably in association with methyl-SA and lipid-transport proteins so as to facilitate their movement through the phloem to other parts of the plant. Participation of methyl-SA in perpetuation of SAR defense has been reported in TMV infected tobacco plants (Park *et al.*, 2007, Dempsey and Klessig, 2012). It is suggested from the reports that composition of immune signals in SAR rely on a complex network of cross-interacting signals that vary with the plant species as well as type of the plant–pathogen interactions (Spoel and Dong, 2012). It is described that JA also involved in establishment of defense against viruses in plants initiated by Avr-R protein interactions. However, a balance between endogenous level of JA and SA determine degrees of resistance (Thaler *et al.*, 2012). Most of the viruses have evolved mechanism to target hormone pathways of the plant although details about their function in plant–virus interactions yet not understood completely (Kazan and Lyons, 2014). Various reports however, indicate that the plant hormones including abscisic acid, ethylene, and brassino steroids modulate antiviral resistance mechanisms (Chen *et al.*, 2013, Ali *et al.*, 2014, Seo *et al.*, 2014).

3. Ribosome Inactivating Proteins for Virus Resistance: Sometimes plant cells produce toxic proteins in response to infection with the pathogen. These proteins provide defense mechanism to fight with the pathogenic invaders. These anti-pathogenic protein toxins popularly known as Ribosome Inactivating Proteins (RIPs), are a group of plant enzyme that functions for inactivation of ribosomes by modifying its rRNA molecules which cause inhibition of protein synthesis (Peumans *et al.*, 2001, Kaur *et al.*, 2011). RIPs removes adenine residues from 28S rRNA by the activity of N-glycosidase which makes ribosomes inactive (Sharma *et al.*, 2004). In addition to its N-glycosidase activity some other RIPs are having DNAse, DNA Glycosylase as well as apurinic pyrimidinic lyase activities (Sharma *et al.*, 2004, Domashevskiy and Goss, 2015). PAP (pokeweed antiviral protein) and Saporin are identified as the RIP proteins obtained from pokeweed (*Phytolacca americana*) and soapwart (*Saponaria officinalis*). These RIPs have been reported to show increased antifungal and antiviral activities. Once RIPs are synthesized they are exported out of the cell and localized within the cell wall matrix. It is hypothesized that the RIPs gain entry into the cytoplasm as soon as the pathogen comes in contact with the cell and later promote their activity by damaging the host ribosomes. This leads to inhibition of protein synthesis required for virus invasion (Sharma *et al.*, 2004, Domashevskiy and Goss, 2015). RIPs exhibit broad spectrum antiviral properties against different viruses and it inhibits replication of RNA as well as DNA viruses. For example, RIP from *Mirabilis jalapa* has aniviral activity against TMV, PVX, PVY and viroids, such as, *Potato spindle tuber viroid* (Sharma *et al.*, 2004). Recently, the RIP protein isolated from the extracts of pokeweed leaves showed various antiviral activities. Moreover, PAP acts as strong antioxidant and does not allow initiation and accumulation of the harmful ROS in response to virus infection (Zhu *et al.*, 2016).

Stability and Risk Associated with transgenic Virus Resistance:

Plant viruses have evolved as a major threat for agricultural crops worldwide because conventional measurements are not well efficient to control viruses directly in the field. Generating resistance in the plants against viruses remains the only effective way to control plant viruses. No doubt the knowledge of *Agrobacterium* -mediated genetic transformation has opened prospects for engineering resistance against viruses in plants. These methods proved to be the efficient and safe approach of controlling pathogens and plant diseases (Fuchs and Gonsalves, 2007). The existing procedures of resistance generation against viruses in plants are not only difficult but also rare because of the high rate of mutation of the viral genomes and at the same time, every plant species does not possess same response to the technology. Hence the technology which is efficient for one species may not be efficient for other plant species or even for its close relatives. This leads to open the options of investigation for a full-proof technology that might be responsive and functional to all the plant species. Major attention should be given to the threats associated with the plants transformed for virus resistance. Potential food safety issues have been reviewed on a regular basis however (Hammond and Cockburn, 2008, Alderborn *et al.*, 2010, Breyer *et al.*, 2012). Another limitation is presence of the heterologous viruses that are efficient to infect plants in the field. Due to this the resistance of transgenic plants engineered for a specific virus could be broken by any other viruses. It is expected that the heterologous viruses hold back the RNA silencing machinery of the plant partially or completely in association with its silencing suppressor protein(s) that could be the reason for stoppage of initially incorporated resistance. Furthermore, the transgenic plants will also have to withstand all the biotic and abiotic stresses during its life cycle. These conditions avail the chances of recombination between the transgene mRNA and RNA of the infecting virus in the transgenic plant cells when replication of the RNA of viruses occur. Accordingly, chances of genotype alteration exist with the chimeric RNAs that are highly efficient to modify prospects of the transgenic plants. Occurrence of recombination in the transgenic plants, between mRNA produced from viral transgene as well as RNAs of the infecting virus has also been a subject for regular analysis (Turturo *et al.*, 2008, Morroni *et al.*, 2009 and 2013, Tepfer *et al.*, 2015). Thus recombination has elevated the issues of creation of viral genomes that might be efficient in bringing new diseases. Vigne *et al.* (2004) studied about occurrence of the recombination events in transgenic plants of grapevine with *CP* gene of *Grapevine fanleaf virus* (GFLV). It was not apparent from their results however, that in the transformed plants of grapevine viable GFLV recombinants is formed or not. But they have discussed that molecular diversity of GFLV populations was affected. Later, Turturo *et al.* (2008) observed recombinant viruses in the transgenic and non transgenic plants both. They further reported that the populations of the recombinant viruses in the transgenic plants expressing a CMV *CP* transgene as well as in the non transgenic plants were equivalent.

Besides, there are chances of hetero-encapsidation in plants infected with multiple viruses where one virus

encapsidate with the coat protein of a closely related virus. This problem could also arise in virus resistant transgenics expressing a coat protein where its subunits encapsidate with genomic RNA of a related virus. During this happening, the infecting virus is transmitted to a transgenic plant where progeny of the challenging virus will encapsidate with the homologous coat proteins and heterologous coat protein sub units encoded by the transgene. The coat protein is characterized for pathogenesis or insect-vector specificity, hetero-encapsidation might change properties of infecting viruses in the transgenic plants. In this way it is anticipated that new virus epidemics could result from hetero-encapsidation. In transgenic plants hetero-encapsidation in TMV, CMV, PVY, ZYMV has been studied extensively (Mawassi and Gera, 2012). Nevertheless, the RNA technology is considered as an eco-friendly and biologically safe technology as it reduce risks related with transgenic plants carrying first generation viral constructs.

Conclusions and prospects of the existing approaches:

Disease caused by the viruses is considered as one among the most limiting factors for crop production. The problem of long-lasting encounter of plant and viruses has been realized. It is essential to converse about induction of stable disease resistance against viral pathogens in plants to meet the global challenges of increased demand for agricultural production in the present scenario. It is highly desirable to generate resistant plants with the efficacy to execute resistance against wide spectrum of viruses to meet current demand of food. To manage off set of crop losses due to viral pathogens attempts have been made all over the world for the last few decades. These practices resulted in the expansion of a number of new and innovative approaches to produce virus-resistant plants exhibiting a regulatory framework of the genes useful for crop production.

In order to achieve stability of the resistance in transgenics, it is imperative to target regions of the viral genome which are able to show minimum sequence variability. To protect the economically important crop plants, a fresh approach to engineer virus resistance is needed. There are strategies based on pathogen-derived transgenes consisting of coat protein of viruses, the replicase proteins and post-transcriptional gene silencing as well as the strategies based on non-pathogen-derived transgenes that offer broader resistance. However, detailed studies on the factors controlling interactions between transgene associated with viruses and the host derived transgene are still obligatory to clarify the mechanisms of resistance and pathogenicity. Researchers have developed new and novel ideas of designing constructs to target a broad range of the plant viruses. Among these construction of oligo-adenylate synthetase pathways in host plants has been characterized for a broad range and durable resistance against RNA viruses. This technique has assured to provide a stable resistance in various economically important crops. Alternatively, complete resistance against DNA viruses has been induced with the constructs consisting of TALEN, ZFN, and CRISPR-Cas9 genes. Among these, CRISPR-Cas9 is easiest to design and have provided the best results. For this reason, CRISPR-Cas9 is being exploited rapidly to engineer resistance against DNA viruses. CRISPR-Cas9 has additional advantages over TALEN and ZFN because it provides resistance against many viruses simultaneously. The techniques of TALEN, ZFN, and CRISPR-Cas9 can also be used to restrain host susceptibility to provide resistance against a broad range of viruses. Virologists are using these technologies to offer encouraging approach to avoid labour intensive methods of the conventional genetic engineering and traditional breeding techniques.

The strategies evolved so far are efficient to protect plants against viruses and other vectors either partly or entirely. However, most of the strategies are required further investigations to make them applicable for economically important plants as earlier the technologies have been set for the model plant systems. More researches are needed towards explanations of the related molecular pathways leading to virus-host interactions. A number of questions pertaining to plant viruses and their interactions within the host cells still have to be answered. Furthermore, the knowledge of virus genes and protein functions as well as the native immune system which protects plants against viruses, will permit us to develop unique tools to expand our current capacity to stabilize crop production in virus epidemic zones.

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