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Risk Assessment of The Virus Resistant Transgenic Plants To Develop The Policy For Commercialization of Transgenic Plants

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Abstract- Continuous increasing population and decreasing agricultural land has developed a pressure on the agricultural fields to increase the production. Pressure on the fields has been further increased by the plant viruses which cause loss of millions of dollars every year all over world. Development of virus resistant transgenic plants (VRTPs) is the hope for the agricultural benefits. However, in last few decades several questions have been raised concerning potential ecological impact on VRTPs production. Various studies have suggested the possible strategies to eliminate the potential risk to develop the VRTPs. Comparison of risks associated with the VRTPs and nontransgenic plants will lead to the overall analysis of advantages and disadvantages of VRTPs and nontransgenic plants. Risk assessment of VRTPs can provide a way to make the strategy to release the transgenic plants for commercial use.
Index Terms- Virus resistant transgenic plants, Coat Protein, Transgenic Plants

1. INTRODUCTION

Plant viruses cause huge crop loss every year worldwide (Varma and Malathi 2003). Viral infection on different crop plants contribute major obstacle of food security in developing countries. Various conventional approaches such as breeding, application of insecticides, cross protection and heat treatment are available to fight against viruses. Breeding to develop the resistant plant against viruses faces some difficulties such as availability of virus resistance gene in the wild relative. Another challenge during breeding is to develop a dominant molecular marker associated with the virus resistance and quick introgression of resistance gene into the susceptible plant variety. Current advances in understanding the molecular mechanism of virus resistance has promoted the development of new strategies against viruses. The new idea of getting nonconventional resistance is the transformation of plants with DNA sequence which interferes with the virus life cycle. Development of genetically modified plants possess improved resistance against viral disease is the prime objective of plant biotechnology. During mid-1980s Beachy's group has demonstrated that resistance against virus can be obtained in the transgenic plants expressing coat protein (CP) gene of virus (Abel et al, 1986). This research has provided the strategy to develop VRTPs. Further, most of the VRTPs developed were based on the pathogen-derived resistance (Sanford and Johnston 1985) and CP was used for the development of resistant plant. CP of viruses was found a potential candidate for the development VRTPs because CP plays key role on completing the life cycle of the viruses and interact with several host proteins. CP is primary choice for VRTP development because it does not produce any abnormality in the transgenic plant. Similar to the CP, replication associated protein (Rep) and βC1 genes were also used to develop VRTPs against geminiviruses (Yang et al, 2004; Soharab et al, 2016). Other than viral genes few host genes has also been used for the generation of VRTPs but they don't show higher degree of resistance (Robaglia and Tepfer 1996). However, VRTPs will increase the productivity but the commercialization of VRTPs always have biosafety concern. Risk assessment of VRTPs includes the analysis of advantages and disadvantages of VRTPs and nontransgenic plants (Herman et al, 2019). Study of risks associated with VRTPs provide the answers of questions associated with VRTPs production and facilitate the path of commercialization of transgenic plants.

2. RISKS ARISE DUE TO THE OVEREXPRESSION OF DIFFERENT VIRAL PROTEINS

2.1. Risk arise by the coat protein

CP of plant viruses encapsidate the genome and plays a key role on virus transmission through insect vector. Specificity of the insect vector for a virus is determined by the CP of the virus. Several studies have demonstrated the role of virus encoded CP for the essential function of virus life cycle such as cell to cell movement and long distance movement (Rojas et al, 2001; Callaway et al, 2001). First time improved resistance against any virus by expressing viral protein was reported in the transgenic tobacco overexpressing coat protein (CP) of tobacco mosaic virus (TMV) (Abel et al, 1986). Resistance attained by the transgenic tobacco is due to the interference with the uncoating of the incoming TMV (Powell et al, 1990; Beachy 1999). Following the CP mediated resistance strategy, several plants have been engineered to obtain resistance against RNA and DNA viruses. Cotton plants expressing antisense coat protein gene shows resistance against *Cotton leaf curl virus* (Amudha et al, 2011). Gene encoding CP of *Tomato mosaic virus* (ToMV), *Potato virus*

X (PVX) and *Cucumber mosaic virus* (CMV) has been used to develop transgenic plants (tomato, potato and tobacco) against ToMV, PVX and CMV respectively. Transgenic plants overexpressing coat protein achieve resistance either through post transcriptional gene silencing (PTGS) or protein mediated resistance.

First risk due to the CP overexpression is the complementation of the virus movement, virus host range and tissue specificity of the viruses. Transgenic plants expressing CP could complement a virus strain in which CP gene is inactive or mutated. CP of a virus determines the insect vector and insects spread the viruses on different hosts according to their choice and availability thus CP influences the host range of the virus. Sometime CP also involved in the virus movement. Transgenic plant overexpressing CP are surrounded by the questions whether CP gene expression could lead to the enhanced disease symptoms, alteration of tissue specificity and adaptation for the new host infection. Hitherto, these fears have proved baseless while this possibility was existing earlier.

Second risk is the heterologous encapsidation and virus transmission. Sometimes, infection of two related viruses to the single plants leads to the heterologous encapsidation (Bourdin and Lecoq 1991; Robinson et al, 1999), i.e., genome of one virus can be encapsidated within the CP of other virus. During mixed infection, heterologous encapsidation may lead to the change in vector specificity thus change in host range. Experimental evidences have proved that the phenomenon of heterologous encapsidation is rare (on the order of 10^{-7}) (Candelier-Harvey and Hull 1993).

Third risk is the synergistic interaction of viruses during mixed infection. Mixed infection of related or non-related viruses may lead to the synergistic interaction of viruses (Vance 1991; Singh et al, 2016). Synergistic interaction of viruses causes severe symptoms and higher virus accumulation in the infecting plant. Synergistic interaction of viruses following infection of two different viral genomes to a single plant is known in the nature but synergistic interaction of one viral genome and single gene of other virus is unknown in nature.

Potential risks raised due to the CP expression in the plants can be easily eliminated through mutation or modification of CP gene according to the need. Expression of modified or mutated CP gene include expression of nontranslatable CP gene, expression of CP gene which cannot make assembly with the viral genome but can induce PTGS and expression of truncated CP gene.

2.2. Risk arise by other proteins used for the generation of virus resistant transgenic plants

Replication associated protein (Rep) of geminiviruses is a multi-functional protein and primery controller of interaction with plant cell cycle regulatory proteins (Kong and Hanley-Bowdoin, 2002; Aragão and Faria 2009). Interaction with multiple host protein and critical function in the virus life cycle makes Rep an exceptional target for developing broad spectrum virus resistant transgenic plant. Transgenic tomato plants expressing part of the Rep gene showed resistance against geminivirus under field condition (Yang et al, 2004). Satellite DNA of geminiviruses encodes a protein βC1 which is involved in the pathogenesis. βC1 gene has also been targeted to develop geminivirus resistant transgenic plant (Sohrab et al, 2016; Sohrab 2018). βC1 protein is toxic to plant cell but different strategies has been used to develop βC1 expressing transgenic plants.

Rep and βC1 protein of the geminivirus interact with several host and viral proteins this property make them key target for the transgenic preparation. Expression of either Rep or βC1 has the risk of recombination, gene flow and synergistic interaction. Recombination of Rep or βC1 gene expressed in the transgenic plants with the virus infecting the transgenic plant can develop severe symptom. Severity of symptom developed by infecting virus may also increase due to the synergistic interaction of virus and viral protein expressed by plant. Another risk associated with the transgenic plants is the gene flow of the transgenes to the wild type plants (Ellstrand et al, 1999). These risks can be avoided by expressing mutated/modified gene, nontranslatable viral protein or fragment of viral protein in the transgenic plants.

2.3. Risk associated with the CaMV 35S promoter

Most of the transgenes are being overexpressed in the transgenic plants under the control of 35S promoter of CaMV (*Cauliflower mosaic virus*). Ho (et al,1999) have explained that 35S promoter sequence is mobile likely to be mobile element in the plant genome and it can also be inserted into the genome of organism who will consume the transgenic plant. If, 35S promoter sequence is mobile in the plant genome then it can change the expression of other plant proteins. Apart from this research it is a fact that people take considerable amount of 35S promoter DNA through CaMV infected plants without any ill effect. Effect of 35S promoter DNA has not been found in effecting animal health.

3. CONCLUSION

In nature different kind of plant viruses are present which differ in type of genome, genome size and particle morphology. Plant viruses are classified according to the most important characteristic property which is type of genome. In broad range, plant viruses classified as double stranded DNA (dsDNA) viruses, single stranded DNA (ssDNA) viruses, double stranded RNA (dsRNA) viruses, single stranded RNA viruses with positive sense or negative sense genome. Plant viruses are spread worldwide and cause considerable losses every year. New approach to control the viruses is transformation of plants with DNA/gene sequence which interfere with the life cycle of viruses. Latest non-conventional strategies to develop the VTRPs are to use CP gene of viruses, antisense nucleic acids sequences, sequences of satellite molecules, defective interfering molecules and nonstructural genes of viruses. CP is present in all the viruses infecting plants. CP of viruses was found a potential candidate for the

transformation of plants because CP plays key role on completing the life cycle of viruses and interact with several host proteins, ultimately, interference on the function of CP will reduce the virus infection in the plants. CP is primary choice for VRTP development because it does not produce any abnormality in the transgenic plant. Rep and βC1 genes of geminiviruses are the second choice for the plant transformation because even they provide considerable resistance to the plants but proteins are toxic for the plant cells. Wild type Rep and βC1proteins can produce some abnormalities on the transformed plants but transformation with the mutated or antisense gene will be ecologically safe and virus resistant event. Even 35S promoter has not been found mobile in the plant genome but plant specific constitutive promoter. Finally, expression of mutated/truncated gene of virus producing antisense nucleic acid under control of host specific promoter in the plant will be ecologically safe and virus resistant. Ecologically safe and virus resistant plants will be answer of all concerns raised against commercialization of VRTPs.

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