Study of mixed virus infection in plants: Disease severity and management

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Masters of Science

in

Plant Biotechnology

Submitted by

Mansi Tiwari

Advisor

Dr. Rajesh Kumar

Dept. of Genetics and Plant Breeding
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi

DEPARTMENT OF GENETICS AND PLANT BREEDING
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI-221005
INDIA

ID. No. 19430PLB013 **2021** **Enrollment No. 419062**

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

कृषि विज्ञान संस्थान INSTITUTE OF AGRICULTURAL SCIENCES

DR. RAJESH KUMAR
Assistant Professor
E-mail rajitrc@gmail.com

Department of Genetics and Plant Breeding
Institute of Agricultural Sciences (B.H.U.)
Varanasi - 221005

Ref. No. ……. ***Date ……..***

CERTIFICATE

To,
The Registrar
Banaras Hindu University
Varanasi-221005, India

Through **The Head,** Department of Genetics and Plant Breeding
Institute of Agricultural Sciences, BHU, Varanasi-221005

Dear Sir,

I have great pleasure in forwarding the thesis entitled **"Study of mixed virus infection in plant: disease severity and management"** submitted by **Ms. Mansi Tiwari**, **Roll No. 19430PLB013**, in partial fulfilment of the requirements for the degree of **Master of Science** in **Plant Biotechnology**, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and placing on record that she has completed the requisite residential requirements as contained in the statue of the University.

I certify that the entire scheme of investigation presented herein was planned and carried solely by the candidate under my guidance and supervision. The data presented in the, thesis to the best of my knowledge and belief, are genuine and original.

Thanking you

Forwarded **Forwarded** **Yours faithfully,**

(Head) ***(Course Coordinator)*** **(Dr. Rajesh Kumar)**
Supervisor

Study of mixed virus infection in plant: Disease severity and management

By

Mansi Tiwari

Thesis submitted in partial fulfilment of the
requirements for the award to the degree of

Master of Science

in

Plant Biotechnology

DEPARTMENT OF GENETICS AND PLANT BREEDING
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI-221005

ID. No. 19430PLB013 **2021** **Enrolment No. 419062**

APPROVED BY ADVISORY COMMITTEE

Supervisor

Dr. Rajesh Kumar
Assistant Professor
Department of Genetics and Plant Breeding
Institute of Agricultural Sciences, BHU, Varanasi, India

Member

Dr. Ashok Kumar
Assistant Professor
Plant Biotechnology, Department of Genetics and Plant Breeding
Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi, India

Member

Dr. Vishal Srivashtav
Assistant Professor
Plant Biotechnology, Department of Genetics and Plant Breeding
Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi, India

External Examiner

ACKNOWLEDGMENT

"As with all commandments, gratitude is a description of a successful mode of living. The thankful heart opens our eyes to a multitude of blessings that continually surround us." – James E. Faust.

As it is said, each and every ordinary day has become a day of thanksgiving as I held immense gratitude in my heart for those who have helped this research work to success. Research is "creative and systematic work undertaken to increase the stock of knowledge". It involves the understanding, collection, organization and analysis of information to find a solution of the research problem.

I am blessed with Baba Vishwanath and Bharat Ratna Mahamana Pandit Madan Mohan Malviya and their blessings and grace which had furnished me with the power and strength to make me capable of performing my work with peace and efficiency.

My foremost and sincere regards to my supervisor Dr. Rajesh Kumar for his immense encouragement and enormous help that allowed my project to success. I am ever grateful to him for solving all my problems with utter patience and meticulous scrutiny. His timely advice and constant encouragement has been instrumental for the successful completion of my work.

Sincere thanks to Dr. Ravindra Prasad, Dr. Ashok Kumar, Dr. Abhinav Singh and Dr. Vishal Srivastav without whom this journey would be incomplete. They have always been encouraging and influenced me to aspire for more.

Special thanks to my friends for their help and support that added a lot to my wonderful experience. I offer my regards to Madan sir, non-teaching staff of M.Sc. Plant Biotechnology, RGSC, Department of Genetics and Plant Breeding for

his active co-operation. Thanks will be an understatement for my parents for being always by my side.

I shall ever, remain thankfully indebted to all those learned souls, my former teachers, known and unknown hands who directly and indirectly motivated me to achieve my goal and enlightened me with the touch of their knowledge and constant encouragement.

Date:
Place: Varanasi *(Mansi Tiwari)*

Curiginal

Document Information

Analyzed document	Study of mixed virus infection in plant without references.docx (D114828687)
Submitted	2021-10-11 07:42:00
Submitted by	
Submitter email	mansitiwari2501@gmail.com
Similarity	6%
Analysis address	cenlib2014.bhuni@analysis.urkund.com

Sources included in the report

W	URL: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7172858/ Fetched: 2021-10-11 07:44:00	7
W	URL: https://www.researchgate.net/publication/44690089_Host-dependent_differences_during_synergistic_infection_by_Potyviruses_with_potato_virus_X Fetched: 2021-10-11 07:44:00	4
W	URL: https://www.researchgate.net/publication/305750237_Synergies_and_antagonisms_in_virus_interactions Fetched: 2021-10-11 07:44:00	6
SA	**Saurabh Verma.docx** Document Saurabh Verma.docx (D37693405)	1
W	URL: https://digital.csic.es/bitstream/10261/220527/1/whenviruse.pdf Fetched: 2021-10-10 11:57:55	3

CONTENT

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Viruses are obligate parasites that are capable of synthesizing and assembling its nucleic acid, protective coat within the suitable host cell. Within such cells, virus replication is affected by factors such as: (1) dependent on the host's protein-synthesizing machinery, (2) organized from pools of the required materials rather than by binary fission, (3) take place in cytoplasm of host and (4) continuously mutating. (Mandhar, 1978).Viruses are considered among the most abundant biota and constitute a generous portion of the microbiota of earth. Viruses can infect bacteria, fungus, plants, and animals even in some special cases other viruses also. Virus infection requires carriers or vectors for successful infection in the host thus examples of vectors vary over a large range of the spectrum that includes aphids, leafhoppers, planthoppers, whiteflies, and multiple genera of insects. Some studies have also concluded transmission through the virus-encoded proteins (Ng and Falk, 2006; Hogenhout et al. 2008; Ammar et al. 2009; Blanc et al. 2011, 2014; Gray et al. 2014; Whitfield et al. 2015).

There are about 2000 species of viruses identified to cause plant virus diseases. It's quite common for plants to get multiple infections at a time by different virus genus or different strains of the same species (Chen et al. 2012).Viruses possess a serious threat to the agricultural world due to emerging diseases, a rapid increase in disease incidence, geographical range, and/or pathogenicity. The cure for viral diseases is not as easy as for a bacterial fungal disease that can easily be treated with the help of the anti-bacterial or fungicide respectively (Rubio et al. 2020).The three large groups of viruses are namely i.e., potyviruses, tospoviruses, and begomoviruses. The viral diseases cause major damages to plants which lead to the downfall of agro economics across the globe that has been estimated to around 30 billion dollars (Pimentel et al. 2000).

The disease management for the viral disease encompasses prevention of the viral infection to the host or development of the viral resistance in the host using various strategies that must include consideration of host and environment along with it must be specific for the virus we are focusing on that's too much of a hassle as viruses have high genetic variability due to their rapid replication and large population generation in a short span of infection (Rubio et al. 2020).

Reasons for the rapid increase in the virus infection in plant or crop can be tabulated as follow (Elena et al. 2014; Jones, 2009; Anderson et al. 2004):

- Agricultural practices focus on mono-crop cultivation which results in low genetic diversity and low plant diversity which gives an edge to the pathogens and pests over the vulnerable cultivation.
- Rapid evolution and adaptation
- Changing climatic conditions changes the pattern and infecting strategy of pathogen
- Exchange of germplasm that help in ascertaining dominance in new environment**.**

Mixed virus infection is common in the natural environment as the susceptibility of the host may lead to multiple attacks by various species of viruses which may or may not simultaneously infect the host. Presence of two or more viruses in a host cell is likely to generate conflicts of will and competition for the limited supply of nutrients will lead to rise of different level of interaction varies and each interaction may result in a mutual increase in strength of both the viruses, whereas some may result in the asymmetrical increase of one virus strength and no effect on another virus strength, or no effect on the virus but the asymmetrical decrease in the strength of co-infecting virus last but not the least mutual decrease in both the co-infecting virus due to mutual interaction (Chen et.al. 2012).The mixed virus infection strategy is a relatively novel approach in the production of transgenic plants with virus resistance as exploring the usage of co-infecting viruses to cross-

protect as an alternative solution to counter virulent plant viruses seems to be a viable alternative and when two unknown viruses interact in the same host, the outcome of the interaction is often unpredictable. Thus, the need to identify the dominant virus in co-infection is urgent (Chen et.al. 2012).

Not much is known about the mechanism of various interactions among viruses. This review addresses the host and viruses interactions, various virus- virus interactions, mechanism under which they operate in a host cell and utilization of mixed virus infection in generation of resistance in host for same as well as other infecting viral in specific plant species to generate resistance and a brief over view on the underlying molecular mechanism of these interactions.

Chapter 2

TYPES OF VIRUSES AND VECTOR RELATION

The transmission of the virus to plant requires vectors and their relationship may be of various types (**Figure 1**) and are discussed below with examples-

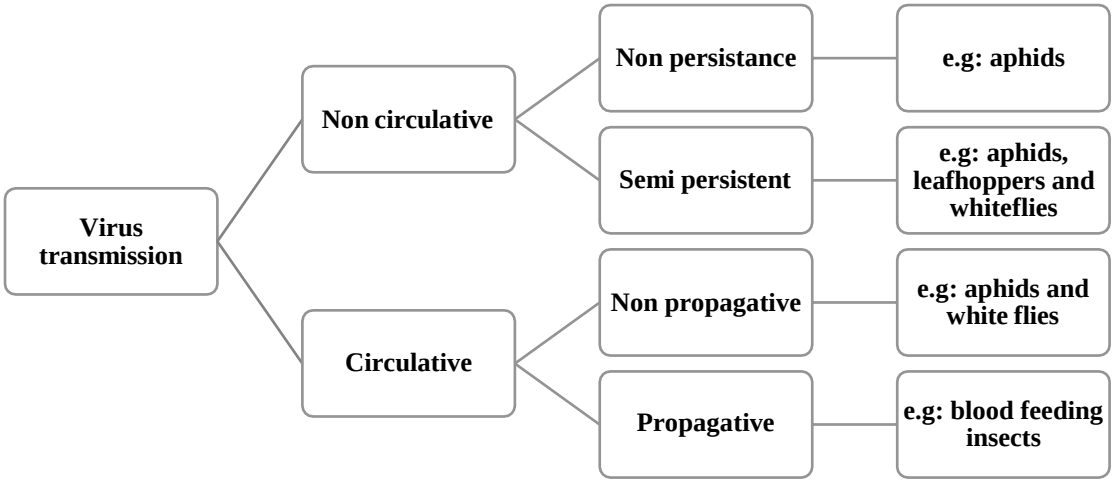

Figure 1: Relation between virus and transmitting vectors

Non-circulative- virus does not enter the insect body as the part of the transmission process but it gets retain in the foregut region or stylet. The sub-types of non-circulative interaction are discussed briefly below.

- Non-circulative non-persistence virus transmission- this type interaction is caused by aphids and this type of transmission mode is opted by *Potyvirus, Cucumovirus, Alfamovirus* and *Caulimovirus* taxas and the species example includes Potato *Y potyvirus* mediated by vector *Myzuspersicae* and transmission type includes capsid proteins and HC- Protein.

- Non-circulative semi persistence virus transmission type- this type interaction is observed in various virus taxas like *Crinivirus, Closterovirus, Waikavirus* and *Badnavirus* and their species example constitute *Lettuce infectious yellow crinivirus* that uses *Bemisiata baci* as transmitting vector whereas medium for transmission involves minor capsid protein and CPm. In this type of interaction, a virus can stay upto 2-7 days in stylet or foregut region of the vector.

Circulative- the infecting virus can either circulate or multiply within the vector body before transmitting to the host (Dietzgen et al. 2016; Whitfield et al. 2015; Shi et al. 2021).

- Circulative non-propagative virus transmission: this type interaction is observed following virus taxas *Polerovirus, Luteovirus, Enamovirus, Umbravirus, Begomovirus, Mastrevirus, Cutrovirus, Betacurtovirus, Eragrovirus, Turncurtovirus, Nanovirus, Babuvirus* etc. the Potato *leaf roll polero virus* by the mediating help of transmitting vector *Myzuspersicae* and transmission medium as Capsid protein and CP-RT (capsid protein read through).
- Circulative propagative type virus interaction: these are observed in the following virus taxas *Oryzavirus, Tenuivirus, Tospoviruse, Nucleorhabdovirus, Cytorhabdovirus, Phytoreovirus,* and *Fijivirus* while their preferred virus example includes *Rice dwarf phytoreovirus* which involve Virion protein P2 type transmission by vector *Nephotettix cinciticeps.*

Chapter 3

VIRUS - VIRUS INTERACTIONS

Mixed infections of viruses is an extraordinarily persistent phenomenon it is such that their occurrence can be considered as a rule rather than exception. In mixed virus infection there are various ways the virus may interact among themselves for the severity of the disease or symptoms. There are two major pathways for mixed viral infections: co infection and super infection (Miralles et al. 2001; Saldan˜a et al. 2003). The interaction can be defined as synergism, antagonism, neutralism and combination of both synergism and antagonism (Mascia and Gallitelli, 2016; Syller, 2012; Syller and Grupa, 2016).

Virus-virus interaction may also be described as-

- Direct interaction among gene product of two viruses.
- Indirect interaction or environmental intervening
- Immunological interactions.

Different types and sub types of virus virus interactions:

Direct type virus interaction includes helper type interaction, pseudo virus infection, superinfection exclusion, genomic recombination, embedded virus and heterologous transactivation.

Indirect type virus infection includes indirect transactivation of heterologous viral genes, altered host susceptibility due to breakdown of physical barriers, altered host susceptibility due to altered receptor expression and Heterologous activation of pro-drugs.

Immunological interactions involve altered immune cell activation, antibody-dependent enhancement of infection, heterologous immunity and VVI induced autoimmunity (DaPalma et al. 2010).

These interactions have been explained in details below (**Figure 2**):

A. Direct interaction among gene product of two viruses:

Direct interaction involves interaction among the gene or gene product of the two co-infecting viruses simultaneously at the same time in a host. This type of interaction can be further sub divided into 6 types that includes: helper viruses, pseudo type viruses, superinfection exclusion, genomic recombination, embedded viruses, and heterologous transactivation and are discussed below with suitable examples:

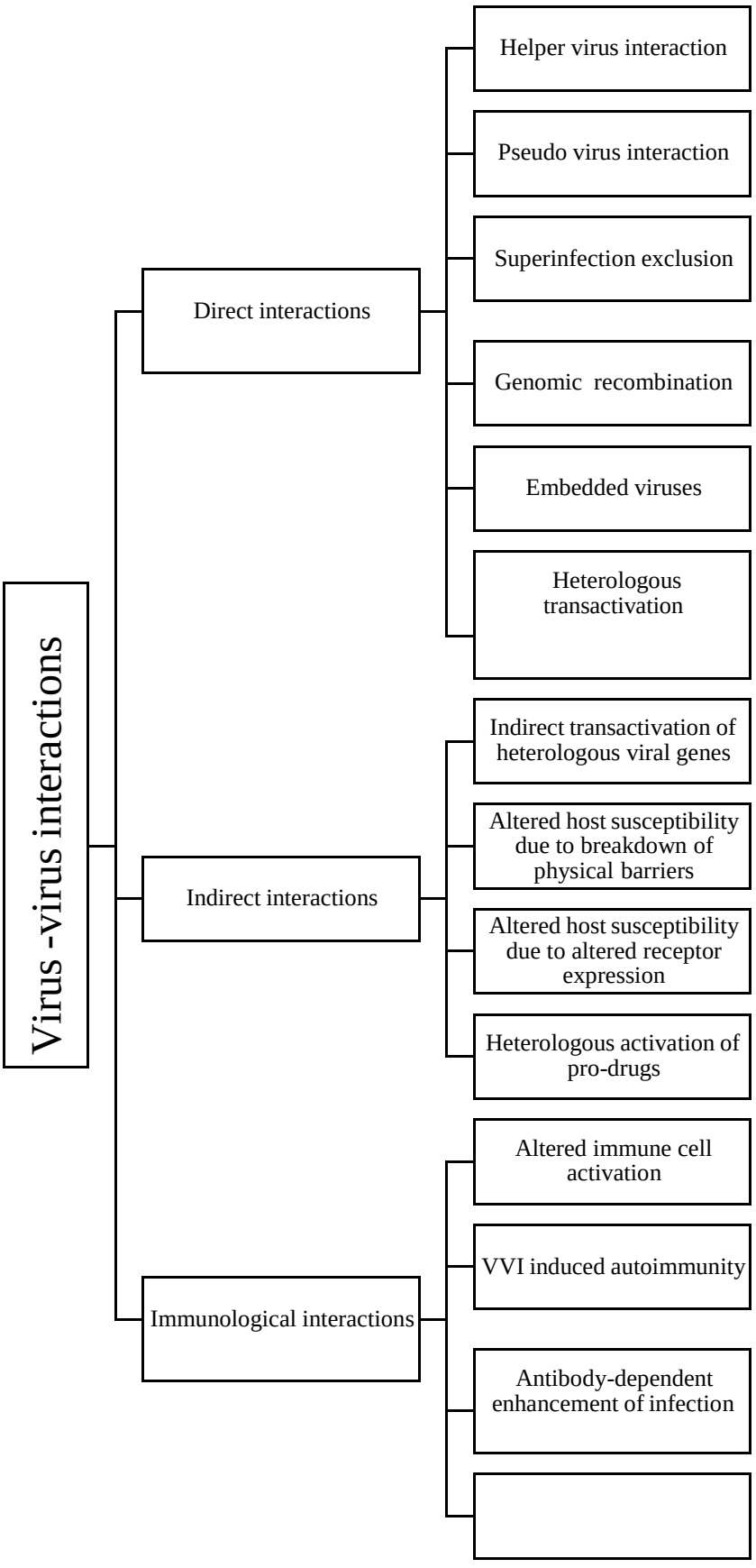
Virus -virus interactions
Direct interactions
Helper virus interaction
Pseudo virus interaction
Superinfection exclusion
Genomic recombination
Embedded viruses
Heterologous transactivation
Indirect interactions
Indirect transactivation of heterologous viral genes
Altered host susceptibility due to breakdown of physical barriers
Altered host susceptibility due to altered receptor expression
Heterologous activation of pro-drugs
Immunological interactions
Altered immune cell activation
VVI induced autoimmunity
Antibody-dependent enhancement of infection

Figure 2: Different types of virus-virus interactions.

A.1. Helper dependent viruses

This type of interaction take place when one of the simultaneously infecting viruses lacks a replicating component so it may require the other virus help in completing its replicating mechanism or may incorporate its genome in progeny of the second replicating virus. Bacteriophage P4 was described as first helper dependent virus which required bacteriophage P2 co infection to provide capsid component and cell lysis (Shore et al. 1978; Six and Klug, 1973).Waterhouse and Murant (2008) described carrot mottle virus dependence on viruses of *Luteoviridae* family for encapsidation and transmission along same vector i.e. aphids. The helper dependent mechanism is not unidirectional in any aspect in case of P4 bacteriophage, the bacteriophage significantly inhibits the replication of the P2 phage (Barrett et al. 1973; Timpe et al. 2006).

A.2. Pseudo typed virus

Zavada (1976) described this type interaction as phenotype mixing phenomenon that involves the production of phenotypically mixed progeny from the simultaneously co-infecting viruses. According to researchers this character of viruses can be utilised to create recombinant viral vectors to expand range of targets of hosts (Funke et al., 2009; Li et al., 2009; Wu et al., 2009).

A.3. Super infection exclusion

When two viruses co-infect a host the primarily infecting virus offers resistance against the secondarily infecting virus. It occurs between different strain of same virus and it does not confer an absolute form of resistance (Federico et al., 1996).The mechanism observed for exclusion is diverse in diverse species and depends upon the primary and secondary infecting strains along with the interaction of their gene product among each other's. For example, in plants, the interaction of heterologous viral messenger RNA molecules which contain sequence homology induces destruction of both messages by the RNA silencing mechanism and inhibition of replication (Saumet and Lecellier, 2006).

A.4. Genomic recombination

This type of interaction can raise pretty serious concerns for human as well as plant health, two or more type of virus interact with each other and due to possible genetic recombination, the progeny resulted have genomic mixture of parents. Ogawa et al. (2008) reported recombination driven altered virulence of viral pathogens of crop plants.

A.5. Embedded virus

Retrovirus fall under this category of this type of interaction the viral genome gets incorporated inside the large genome of host DNA virus. Friesen and Nissen (1990) discovered this type of interaction in genome of *Autographacslifornica* nuclear polyhedrosis virus where the genome of moth retroviral element was discovered to be embedded. Hertig et al. (1997) demonstrated the effects of embedded virus interaction in reticuloendotheliosis virus embeddement in fowlpox virus genome, it was concluded that due to the phenomenon of embedded virus interaction the virus gets an alternate pathway to enter the host cells as well as transmission method.

A.6. Heterologous transactivation

It does not have plant based applications but is utilized in animal cell lines to increase the gene expression of one the co-infecting viruses having a strong promoter.

B. Indirect interaction

As often noticed during dual infection in a host by multiple virus simultaneously, results in acceleration of disease, because of the compounded nature of the two viral cytopathic effects affecting the host in a negative manner. This kind of interaction results from variation in host environment due to simultaneous co-infection or pre-existing infection. These have been subdivided into five categories: indirect transactivation of genes, breakdown of host physical barriers against

infection, altered receptor expression, heterologous activation of antiviral pro-drugs, and modification of the interferon-induced antiviral state. These five types are discussed below briefly.

B1. Indirect transactivation of genes-

The unexplored knowledge about the transcription factors and there control inhibit us from completely understanding the mechanism of this kind in plants.

B2. Loss of host physical barriers after infection

Virus infection is characterized by cytopathic effect like tissue damage and loss of physical barrier within host, increasing susceptibility for secondary infection to generally protected tissue. This type of interaction is observed in zucchini squash (*Cucurbita pepo*), where some cucumber mosaic virus infect the zucchini plant but causes localized infection and coinfection with CMV and ZYMV systemic infection is observed at molecular analysis (Choi et al. 2002).

Altered receptor expression, Heterologous activation of pro- drug and Interferon induced antiviral state type of virus-virus interaction has only been observed in in vitro condition of animal cell line.

C. Immunological interaction

The immunological aspects of viral genome recognition even when more than one virus co-infect the hosts are strictly limited to the animal kingdom due to the presence of well-developed immune system, whereas the host limited to plant kingdom also do evolve in their own ways to prevent the viral infection or resist the infection at molecular level by various resisting mechanism such as PAMP (pathogen associated molecular pattern), UPS (ubiquitin- 26S proteasome system).

Chapter 4

TYPES OF INTERACTIONS

On the basis of the resultant of end product among the virus-virus interaction are following types (**Figure 3**):

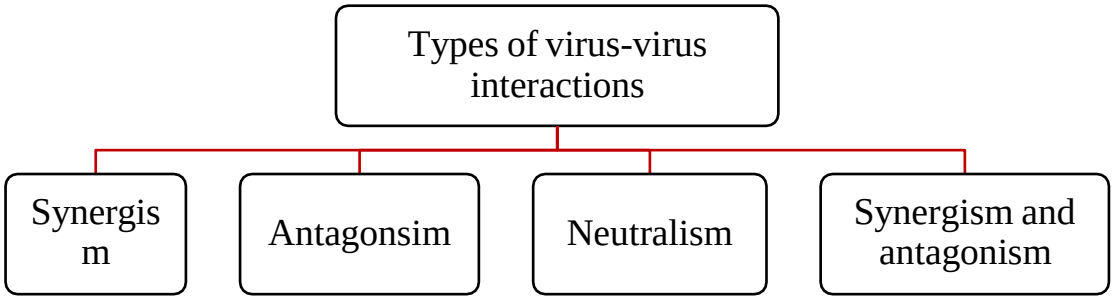

Figure 3: Types of interaction on basis of resultant at the end of interaction

4.1 Synergism

Synergism or synergistic interaction results in the elevation of the fitness and competitive abilities of the two viruses, by dramatically increasing the effects of the viral disease to more severe than expected by the cumulative addition (García -Cano et al. 2006; Tatineni et al. 2014; Zhang et al. 2001). Synergism interaction increases the chances of the replication of one or both virus machinery, may provide with the ability to invade new plant tissues, or support each other for the transmission and other essential functions. Viral suppressor proteins (VSPs) are also reported to play an important role in altering tissue movement patterns of viruses in mixed infection (García-Marcos et al. 2009; Gonalez-Jara et al. 2005).Examples of synergism can be observed in: (i) potyvirus infecting *Tobacco etch virus* (TEV) and *Plum pox virus* (PPV), despite the severe systemic necrosis of leaves and stems, leading to premature death of plants (Gonzalez-Jara et al. 2004, 2005), (ii) *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV), both belonging to the genus *Crinivirus*, family *Closteroviridae* (Wintermantel et al., 2008), (iii) *Pepper huasteco virus* (PHV)

and *Pepper golden mosaic virus* (PepGMV) (*Begomovirus, Geminiviridae*) (Me´ndez-Lozano et al., 2003), (iv) *Papaya ringspot virus* (PRSV; *Potyvirus, Potyviridae*) was inoculated into *Carica papaya* plants prior to, or simultaneously with, *Papaya mosaic virus* (PapMV; *Potexvirus, Alphaflexiviridae*) (Cha´vez-Calvillo et al., 2016).

Causes of synergism interaction in plants

- **Helper dependence** - Helper dependence occurs when any of the viruses involved in mixed infection is faulty in one or more indispensable functions (dependent virus) which in turn can be enhanced by the other virus acting as the helper. Groundnut rosette disease, caused by interaction of anumbravirus *Groundnut rosette virus* (GRV), its satellite RNA and luteovirus *Groundnut rosette assistor virus* (GRAV), wherein GRV and its sat-RNA are entirely dependent on the coat protein of GRAV for encapsidation as well as transmission by aphid vector (Murant 1990; Singhal et.al 2021).
- **Heterologous complementation/trans complementation** – Is a form of facilitation for mixed virus infection in which some of the non-structural proteins called the helper components act as bridges between virus particles in turn facilitating vector transmission (bridge hypothesis). Heterologous complementation is observed in case of mixed infections of TICV (*Tomato infectious chlorosis virus*) and ToCV (*Tomato chlorosis virus*), which enables TICV transmission by *Trialeurodes abutilonea* (whitefly) from mixed infected plants, which otherwise is a non-vector of TICV alone (Wintermantel et al. 2008).
- **Overcoming tissue tropism**- It is the property of virus which enables to infect certain types of tissue and prevent them from infecting some specific types of tissues and cells such as phloem tissues (Mascia and Gallitelli 2016)., in case of mixed virus infection it is observed that the hindrance for virus can be overcome. *Tomato yellow spot virus* (TYSV) causes infection in mesophyll cells and *Tomato rugose mosaic virus* (ToRMV) infects in phloem in single

infections in *N. benthamiana* plants. But in mixed infection of tomato by both viruses, ToRMV acquires the ability to invade mesophyll cells too along with phloem (Alvez-Junior et al. 2009: Singhal et.al, 2021).

- **Altered host range** – It affects both viruses as well plant host as the plant become susceptible to the infection of other viruses which are unable to infect the host previously in case of single infections. in case of *Southern cowpea mosaic virus* (SCPMV) which infects only cowpea and *Southern bean mosaic virus* (SBMV) which infects only common bean in single infections. But in mixed infections, SCPMV also acquires the ability to infect common bean systemically in encapsulated form (Hacker and Fowler 2000).

MECHANISM OF SYNERGISM INTERACTION

Mechanism for synergism interaction plant is still an infant in the research area where a lot is unknown, recently a studied revealed that the pathogen infecting plant may undergo the interspecific signalling via quorum sensing signals among the present microbiota in the plant (Hosni et al.,2011).Study of synergism in case of potato virus X and potato virus Y conducted by (García-Marcosetal2009) suggest the increased changes in transcriptional expression and increased oxidative expression of an oxylipin gene which is responsible for positively regulated programmed cell death in synergistic mixed infection.

Table 1: Result comparison in co-infection with regard to dominance exerted by gene product of one virus over another

S.No.	Virus 1	Virus 2	Host	Dominance predicted	Dominance observed	Reference
1.	Maize chlorotic mottle machlovirus (MCMV)	Wheat streak mosaic rymovirus (WSMV)	N84Ht corn	MCMV	MCMV	Scheets (1998)
2.	Zucchini yellow mosaic virus (ZYMV)	Cucumber mosaic virus (CMV)	Zucchini	CMV	CMV	Fattouh (2003)
3.	Cucumber mosaic virus (CMV)	Pepper mottle virus (PepMoV)	*Capsicum annuum*	PepMoV	PepMoV	Murphy and Bowen (2006)
4.	Tomato chlorosis virus (ToCV)	Tomato infectious chlorosis virus (TICV)	*Nicotiana benthamiana*	ToCV	TiCV	Wintermantel et al (2008)
5.	Zucchini yellow mosaic virus (ZYMV-SD)	Cucumber mosaic virus (CMV-Fny)	Cucumber	ZYMV-SD	CMV-Fny	Zeng et al (2007)

4.2. Antagonism

Antagonism or antagonistic interaction are just opposite of the synergism as the one of the two infecting viral species are bound get more benefited in this interaction, and the benefited species have a detrimental effect on the other species in respect to the multiplication and accumulation. The antagonistic interaction is not much well researched and extensive knowledge is lacking in antagonistic reaction as compared to the synergistic interaction. The mechanism utilized in antagonistic is cross protection which is also referred to as homologous interference or super infection exclusion (SIE) (Bergua et al. 2014; Folimonova, 2012, 2013; Gutiérrez et al. 2012; Julve et al. 2013). Two primary interactions in antagonism are: (1) Cross protection (2) Mutual exclusion.

4.2.1. Cross protection

Cross protection is the phenomenon which occurs when a previous infection with one virus prevents or interferes with subsequent infection by a secondary virus (Gal-On and Shiboleth, 2006; González-Jara et al., 2009; Ziebell and Carr, 2010; DaPalma et al., 2010). Cross protection is also known as superinfection exclusion (SIE).

A study conducted for SIE mechanism by using *Citrus tristeza virus* (CTV) as infecting agent for host. Three hypothesis have been put forward by various researchers for the SIE mechanism and these hypothesis are: (a) entry of secondary virus in a cell preoccupied by primary virus, various sequence follow up in this hypothesis are: overexpression of coat protein of primary virus leading to degradation of secondary virus (Beachy et al., 1990; Lu et al., 1998); degradation of RNA of secondary virus by RISC factors of primary viruses; formation of double stranded RNA by hybridization between RNA of primary and secondary virus RNAs thus leading to its degradation by DICER. And other two hypothesis include degradation of secondary virus RNA by RISC factor due to generation of endogenous RNA dependent RNA polymerase (RdRp) leading to degradation of RNA of secondary virus and due to lack of presence of primary virus RNA in host cell of secondary virus.

Mechanism depicted or hypothesized for cross protection involve as mentioned below-

- **Depletion of host derived precursor or structure –**

Hypothesized by Barker and Harrison (1978) according to it an essential component required for multiplication of the competing strain is exhausted by other attacking strain to limit the growth or lessen the effect of competing strain.

- **Specific virus encoded inhibitors-**

Assumption of encoding of certain factors by primary infecting virus that reduces the chance of survival or competition by other related viruses, but case of unrelated virus not talked about.

- **Template substitution of competitive virus-**

Inducing virus may produce its harvest way more than the competing virus by replication (Ross 1974).

- **Coat protein sequestration-**

According to De Zoeten and Fulton (1975), Encapsulation of genome of the competing virus by primary inducing virus may results in antagonistic interaction.

- **Sequestering the challenging genome-**

This assumes that the (+) strands of the challenging RNA are reduced because the nascent (-) strand RNAs are hybridized by the more abundant (+) strands of the inducing strain (Palukaitis and Zaitlin, 1984).

- **Inhibition of systemic spread-**

Based on assumption that Primary Inducing strain constrict the movement of secondary competing strain. (Dodds et al., 1985).

Gene silencing-

Gene encoding for the coat protein or essential protein id silenced thus reducing the competition.

4.2.2. Mutual exclusion

First studies of mutual exclusion or spatial inclusion or spatial separation was described by Jedlinski and Brown (1965) when they inoculated oats with multiple virus to infect upon the initial observation revealed the mild symptoms, then after an interval of time complete recovery in the infected plant was observed and the genetic analysis of the plant for symptoms revealed no traces of infection.

Spatial separation can be explained as unwillingness of the virus particles to infect the already infected cell by another infecting party so that the first infecting party can maintain a monopoly over the resources and space available for its multiplication and division. It is observed in both the co infection and the super infection pathway of the infection but after events follow a different pathway in each.

The potential mechanism as purposed by Elena et al (2011) in case of infection *N. benthamiana* upon infection with *Plum pox potyvirus* (PPV), *Tobaco vein molting virus* (TVMV), *Clover yellow vein virus* (CIYVV) complementary DNA along with GFP tagged marker revealed that the compartmentalisation of the viral DNA along the cell lining of the inoculated cells which is also known as spatial separation.

The same phenomenon of spatial separation was also reported by Takahashi et al. (2007) in case of *N. benthamiana* leaves inoculated by with *Bean yellow mosaic virus* (BYMV) differently labelled with Yellow Fluorescent Protein and Cyan Fluorescent Protein.

Antagonistic interactions have been observed in (a) Different strains of **Pepino mosaic virus** (PepMV), (b) *Cucurbit yellow stunting disorder virus* and

Zucchini yellow mosaic virus (CYSDV-ZYMV) (c) *Cucurbit vein yellowing virus* and *Zucchini yellow mosaic virus* (CVYV-ZYMV).

Superinfection exclusion in plant viruses has primarily been recognized as the induction of RNA silencing by the primary virus. However, it cannot be excluded that different mechanisms are involved in this phenomenon, which depends on the virus, viral strain or its isolate (Folimonova, 2012; Zhou and Zhou, 2012; Ziebell and Carr, 2010). Proposed mechanisms of superinfection exclusion fails to explain the intact process of and many molecular details remain obscure.

4.3. Neutral interactions

In case of neutral interactions, no effect of any of the coexisting viral species have been so far observed. Neutral interactions are also considered as special type of the synergism with respect to the virus concentration not virulence.

Neutral interaction has been observed, between (a) *Potato virus Y* and *Potato virus X* (PVY-PVX), (b) *Tobacco etch virus* and *Potato virus X* (TEV-PVX), and between (c) *Plum pox virus* and *Potato virus X* (PPV-PVX) *in Nicotiana benthamiana* plants (Gonza´lez-Jara et al., 2004, 2005).

Synergism and antagonism interaction

Synergism and antagonism occur simultaneously in a host sometimes performing their respective roles, as synergism alleviating the expression of the co-host whereas the antagonism detreating the same co-host. These interactions may lead to recombination, complementation and ressortment leading to evolution of the virus some time which may result in the formation of the new species of virus. For example, because of the recombination and ressortment in EACMV and ACMV (*African cassava mosaic virus*), due to the exchange in the coat protein a highly virulent recombinant *East african cassava mosaic virus* Uganda-2 (EACMV) which lead to the disease outbreak.

Chapter 5

MIXED VIRUS INFECTIONS IN PLANTS ACROSS WORLD

Papaya (*Carica papaya*) is an important fruit crop in India and world faces many viral and other pathogen infection. Mixed virus infection of *Papaya ringspot virus* (PRSV) from the *Potyviridae* family and *Papaya mosaic virus* (PapMV) from *Alphaflexiviridae* family are + single stranded RNA virus with genome of 10.33 kb size along with a genome linked viral protein (VPg) at the 5'end and a genome of 6.66 kb size (+) ssRNA genome that includes a 5'- UTR-bound m7GpppN structure respectively (Chung et al. 2008; Vargas-Mejía et al. 2020). PRSV is characterized by mottling and malformation of leaves, ring spots and streaking on fruits, stem and petioles whereas PapMV is characterized by mosaic formation, curling, crinkling and leave deformation, vein clearing and vascular yellowing leaves. Mixed virus infection of PRSV & PapMV shows synergistic interaction as well as antagonism interaction is which causes accumulation of one of the viral genome and dominant symptoms of one of the virus strains. Detection of the mixed virus is done by the help of the RT-PCR or PCR technique (Noa-Carrazana et.al, 2006).

Timing of infection is a determinate step in deciding the interaction and symptoms dominating and molecular status of the infected host. When PRSV infection was followed by PapMV infection then antagonism interaction was observed, when the order of infection was reversed the interaction switched to synergism type further it was also observed that there was not much changes observed in the molecular or RNA level in case of synergism but the symptoms were severely dramatic in appearance whereas the antagonism showed slight decrease in the level of PapMV coat protein level (García-Viera et.al, 2006; Chávez-Calvilloet. al, 2016).

It was concluded that during antagonism, elevated pathogenesis-related (PR-1) gene expression resulted in increased reactive oxygen species production results in

development of resistance against PRSV. It was concluded that if primary infection occurs of PapMV it activates a defence response against PRSV and establishes a protective or shielding with papaya host (García-Viera et.al, 2006; Chávez-Calvillo et. al, 2016).

Mixed virus infection studies in various plants and for various viral species are discussed in the table below:

Table-2: Mixed virus infection in various plants.

S.No.	Virus infecting in collaboration	Host plant	Symptoms	Type of interaction	References
1.	*Abutilon mosaic virus* (AbMV) and *Cucumber mosaic virus* (CMV)	*Nicotiana benthamiana* & *Solanum lycopersicum*	AbMV is phloem nuclei restricted and moderate level of viral DNA, on coinfection with CMV increased level of DNA.	Synergism	Wege and Siegmund, 2007
2.	*African cassava mosaic virus (ACMV)* and *East African cassava mosaic camneroon virus (EACMCV)*	*Nicotiana benthamiana* & *Solanum lycopersicum*	High severity symptoms and higher accumulation of viral DNA on coinfection.	Synergism	Vanitharani et al. 2004
3.	*Potato leafroll virus* (PLRV) and *Groundnut rosette virus* (GRV)	*N. clevelandii* & *N. benthamiana*	Co infection induced necrotic symptoms like line patterning and vein yellowing.	Synergism	Mayo et al. 2000
4.	*Cowpea chlorotic mottle virus (CCMV)* and *Soyabean mosaic virus* or *tobacco ring spot virus (TRSV)*	*G. max* (soyabean)	Reduction of height and yield.	No synergism	Demski and jellu 1975
5.	*Lettuce infectious yellow virus (LIYV*) and *Turnip mosaic virus (TuMV)*	*N. benthamiana*	Enhanced accumulation of *LIYV*.	Synergism	Wang et al. 2009
6.	*Papaya ring spot virus (PRSV)* and *Papaya mosaic virus (PapMV)*	*Cracica papaya*	First infection by *PapMV* causes antagonism whereas first *PRSV* infection causes synergism.	Synergism and antagonism	Chávez-Calvillo et al. 2016
7.	*Soyabean mosaic virus (SMV)* and *Tobacco ring spot virus (TRSV)*	*G. max* (Soyabean)	Reduction of height and yield.	No synergism	Demski and Jellu 1975
8.	*Sweet potato chlorotic stunt virus (SPCSV)* and *Sweet potato feathery mottle virus (SPFMV)*	*Ipomoea batatas* (Sweet potato)	Individually not much infection symptoms or DNA accumulation but on coinfection DNA accumulation increased by 600 folds of SPFMV and severe symptoms.	Synergism	Karyeijia et al. 2000
9.	*Tobacco mosaic virus (TMV)* and *Hibiscus latent Singapore virus (HLSV)*	*N. benthamiana*	*TMV* was able to dominate the infection in long run.	-	Chen et al. 2012
10.	*Tomato leaf curl new delhi virus (ToLCNDV)* and *Pepper leaf curl*(PLV)	*Cucurbita moschata* (Pumpkin)	Pumpkin plants showing yellow vein mosaic symptoms.	Synergism	Namrata et.al 2010.

Chapter 6

GENES INDUCING SYNERGISM AND ANTAGONISM

Genes involved in mechanism of synergism and antagonism are known as viral suppressors of RNA silencing (VSRs). These genes block the RNA silencing cascade of the plant defence mechanism VSRs are protein encoded by the viral factors that target the effector of RNAi mechanism. Some identified VSRs play a crucial role in the virus life cycle such as replicases, helper component in the virus transmission. VSR are diverse in nature and hence they can block or target multiple steps involved in the RNAi mechanism (Csorba, 2015).

Two most studied VSRs are: potyviral HC-pro and cucumoviral 2b, potyviral HC-pro actively block the RNAi silencing mechanism and promotes the infection of cucumber mosaic virus (CMV) in multiple infection between CMV and PVY whereas cucumoviral 2b aid PVY in infecting the plant by preventing the systemic spread of silencing signal (Mascia, 2010)

Other similar viral proteins playing a part in the silencing of the RNAi mechanism and promoting the synergism/ antagonism or viral infecting proteins are:

1. *Sweet potato chlorotic stunt virus* (SPCSV) encodes dsRNA- specific class 1 RNA endoribonuclease III (RNase3) that suppresses RNAi. RNase3 acts by cleaving the 21–24 virus-specific small interfering RNAs, obtained by Dicer-like enzymes, to shorter fragments of 14 bp, making them inactive and hence inhibiting the RNAi machinery (Untiveros, 2007).

2. *Beet curly top virus* (BCTV) a curtovirus encodes for a protein C2 protein, restores the competency of DNA replication in differentiated cells that Geminiviruses use for their replication. But in case of mixed virus infection of *Tomato yellow leaf curl sardinia virus* (TYLCSV) and *Beet curly top virus*

(BCTV) in *N. benthamiana*, increased TYLCSV/DNA accumulation with severe symptoms was reported (Caracuel, 2012).

3. P3N-PIPO protein encoded by the potyvirus white *Clover yellow vein virus* (WClYVV) synergistically interacts with the potexvirus *White clover mosaic virus* (WClMV) in pea (*Pisum sativum*) and facilitates its systemic spread, without enhancing its accumulation per cell (Yusuke Hisa, 2014).

Chapter 7

PLANT VIRAL DISEASE MANAGEMENT

The virus diseases are difficult to control and eradicate completely due to complex and high evolving rate of virus genome. So, to damage the control inflicted by the viral diseases specific, fast and reliable diagnostic tools. Disease management depends on: (1) Immunization that includes genetic resistance obtained by plant breeding, plant transformation, cross-protection, and other methods and (2) Prophylactic measures to restrain virus dispersion including quarantine, certification, removal of infected plants, control of natural vectors etc. (Rubio et al. 2020).

7.1. Immunization measures for virus resistance

This involve plant breeding techniques of introgression of resistance gene selected from wild and cultivated species of cultivars into susceptible crops for the back cross, this is the most widely used immunization method. Immunization is of two types: (1) Active immunization (2) Passive immunization.

7.1.1. Active immunization

Active immunization involves resistance proteins encoded by dominant genes, that are capable of recognizing a specific sequence or conformational protein or structure of a virus gene most probably avirulence determining gene, after the recognition of the specific protein it induces apoptosis of the infected cell thus stopping the virus from infecting the adjacent cells or systemic infection by virus (Peiró et al., 2014; DeRondeet al. 2014).

7.1.2. Passive immunization

Passive immunization is conferrers by recessive resistance genes, that encodes for the host factors critical for viral infection that mostly involves eukaryotic

translation initiation factors (EIF) 4E & 4G and their isoforms (Hashimoto et al., 2016;Truniger and Aranda, 2009).The basic set goal for immunization which is set is complete resistance which is a bit difficult to reach in case of viruses as they have high rate of mutations although immune assays as ELIZA and molecular hybridization are used for the purpose of screening of resistance germ plasm and analysis simultaneously (Soler et al., 2015).

7.1.3. RNA silencing for virus resistance

Another resistance conferring technique involves RNA silencing which is a natural anti-viral defence mechanism and a regulatory gene expression in eukaryotic cell. The host RNA silencing targets the dsRNA that are formed due to replicative viral intermediates or secondary structures of genomic RNA formed due to internal complementarity, these dsRNA structures are recognised by RNases and cleaved into small RNA duplexes known as siRNA or miRNA of 21-24 nucleotides. One strand of the small RNAs is assigned to RISC complex that cleaves the similar viral RNA in a specific manner. The cleaved viral RNA is amplified by the RNA dependent RNA polymerase which result in effective virus inhibition at a local and systemic level (Guo, 2019).The viruses also have a counter defence mechanism for supressing the RNA silencing mechanism, many viruses encode for RNA silencing suppressors which interferes at different steps of the silencing pathway thus rendering it ineffective.

7.1.4. Cross resistance

Cross protection is also a type of virus resistance strategy which is generally utilized in mixed virus infection of antagonistic types cross protection involves inoculating mild or attenuated viral strains to protect plants against severe strains of the same virus, this technique has been applied to several viruses and crops, such as *Pepino mosaic virus* (PepMV) in tomato and Citrus tristeza virus (CTV) in citrus crops (Pechinger et al., 2019). Mechanism of cross protection is not clear or is poorly understood and several models have been purposed such as: prevention of virus entry

into cells, competition of host factors for replication, interference with disassembly in the host, translation of replication, and induction of RNA silencing leading to sequence- specific degradation of the superinfecting virus in plant hosts (Folimonova, 2013).Cross protection strategy can backfire easily as the mild inoculation can result in the full-blown disease with high severity that can be difficult to control and the assays of cross protection can be detected using the real time qPCR and SSCP analysis.

7.1.5. Resistance breakage

The infection by single or multiple virus can be due to the loop hole in the resistance mechanism of the plant as well as due to some external injury, but the infection by multiple virus takes a toll on the plant defence system and make it susceptible to the further infection by other pathogens.

Garcia-Cano et al. 2006 observed resistance breakage in case of tomato plants when co infected by T*omato chlorosis virus* (ToCV) and *Tomato spotted wilt virus* (TSWV), in case of cucumber by Wang et al. 2004 when co infected with CMV and ZYMV and also in case of sweet potato by Karyeija et al. 2000; Mukasa et al. 2006; Untiveros et al. 2007 when simultaneously infected by several different viruses and sweet potato chlorotic stunt virus (SPCSV).

7.1.6. Posttranscriptional gene silencing (PTGS)

Posttranscriptional gene silencing (PTGS) involves degradation of viral and cellular mRNA in a homology dependent manner in plants PTGS act as natural antiviral defence because plant viruses act as initiators and targets of the phenomenon. Though mechanism of PTGS is not clearly understood but dsRNA act as strong inducer of PTGS, which are produced during replication of an RNA virus or conversion of aberrant ssRNA into dsRNA in the cell by host encoded RNA directed RNA polymerase. These dsRNA act as guide after begin processed by DICER enzyme, which act on homologous RNA molecule. In plants, gene silencing is known

to trigger PTGS in distant tissues and graft by an unknown mobile signal (Vanitharani et al. 2004).

7.2 Prophylactic measures

These involve various physical methods such as phytosanitory certificates, quarantine or control of borders and control of vectors spreading the disease by means of insecticides and antiviral agents etc. are the methods employed for virus management.

As complete resistance is not possible so the attainable goal is to induce partial resistance or tolerance. The resistance induced can be measured by real-time qpcr. Degree of resistance in any plant can be predicted as the probability/possibility of no infection or no symptoms, respectively, by survival analyses (Kaplan and Meier, 1958).

Virus resistance in plants can be detected by real time qPCR however keeping track of the resistance is a tough task because generally the resistance is conferred by mutation in single nucleotide hence its detection is a tough task. Di Rienzo et al. (2018) reported the development of real time qPCR with Taqman probes to detect single nucleotide polymorphism associated with resistance breakdown *for beet necrotic yellow vein virus* (BNYVV) and tomato spotted wilt virus (TSWV). There is no absolute guarantee that this technique is 100% successful.

Chapter 8

CONCLUSION AND FUTURE PROSPECTIVE

Mixed viruses infection has a relevance for plant pathology along with virus ecology, virus epidemiology and these mixed infections also play an important role in evolution of the plant defence mechanism along with evolution of components in virus suppressing the defence shield of plant. Virus ecology generally deals with the interaction of virus population with environment on the other hand virus epidemiology deals with complexities of the infection caused and interaction of virus with plant as well as vectors.

The mixed virus infection study can provide us with the rich source of information that can be used to create specific tools and measures in defence of plants. Cross protection an antagonism interaction defence mechanism has been tested on economically beneficial crops by inoculating the plants by mild strains of viruses. While designing mixed virus infection for defence their impact on nature can't be out looked. One such loss of resistance in designed resistance was observed in case of *Turnip mosaic virus* (TuMV) in *Arabidopsis thaliana* transgenic plants where expression of artificial microRNA targeting the viral RNA silencing suppressor was introduced, high resistance against single virus infection was noted but if the plant has been infected previously by *Tobacco rattle virus*, *Cauliflower mosaic virus*, or CMV there was break of resistance observed.

Mixed virus infection can also be used as a tool in biotechnological procedures of delivering a gene, expressing an important protein or valuable by-products in plant that can be induced by infection of mixed virus infection, it can also be used to study stress management, strong defence mechanism of plants in nature and further genomics study.

REFERENCES

Acosta-Leal, R., Duffy, S., Xiong, Z., Hammond, R. W., & Elena, S. F. (2011). Advances In Plant Virus Evolution: Translating Evolutionary Insights Into Better Disease Management. *Phytopathology, 101*(10), 1136-1148.

Alcaide, C., Rabadán, M. P., Moreno-Perez, M. G., & Gómez, P. (2020). Implications of Mixed Viral Infections on Plant Disease Ecology and Evolution. *Advances In Virus Research, 106*, 145-169.

Alves-Júnior, M., Alfenas-Zerbini, P., Andrade, E. C., Esposito, D. A., Silva, F. N., Da Cruz, A. C. F., & Zerbini, F. M. (2009). Synergism And Negative Interference During Co-Infection Of Tomato And NicotianaBenthamiana With Two Bipartite Begomoviruses. *Virology, 387*(2), 257-266.

Ammar, E. D., Tsai, C. W., Whitfield, A. E., Redinbaugh, M. G., & Hogenhout, S. A. (2009). Cellular And Molecular Aspects Of Rhabdovirus Interactions With Insect And Plant Hosts. *Annual Review of Entomology, 54*, 447-468.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging Infectious Diseases Of Plants: Pathogen Pollution, Climate Change And Agrotechnology Drivers. *Trends In Ecology & Evolution, 19*(10), 535-544.

Balogun, O. S., XU, L., Teraoka, T., & Hosokawa, D. (2002). Effects Of Single And Double Infections With Potato Virus X And Tobacco Mosaic Virus On Disease Development, Plant Growth, And Virus Accumulation In Tomato. *Fitopatologia Brasileira, 27*(3), 241-248.

Beachy, R. N., Loesch-Fries, S., & Tumer, N. E. (1990). Coat Protein-Mediated Resistance against Virus Infection. *Annual Review of Phytopathology, 28*(1), 451-472.

Bergua, M., Kang, S. H., & Folimonova, S. Y. (2016). Understanding Superinfection Exclusion by Complex Populations of Citrus Tristeza Virus. *Virology*, *499*, 331-339.

Bergua, M., Zwart, M. P., El-Mohtar, C., Shilts, T., Elena, S. F., & Folimonova, S. Y. (2014). A Viral Protein Mediates Superinfection Exclusion at the Whole-Organism Level But Is Not Required For Exclusion at the Cellular Level. *Journal of Virology*, *88*(19), 11327-11338.

Blanc, S., Drucker, M., & Uzest, M. (2014). Localizing Viruses In Their Insect Vectors. *Annual Review of Phytopathology*, *52*, 403-425.

Blanc, S., Uzest, M., & Drucker, M. (2011). New Research Horizons In Vector-Transmission Of Plant Viruses. *Current Opinion In Microbiology*, *14*(4), 483-491.

Boukari, W., Mollov, D., Wei, C., Tang, L., Grinstead, S., Tahir, M. N., ... & Rott, P. (2021). Screening For Sugarcane Yellow Leaf Virus In Sorghum In Florida Revealed Its Occurrence In Mixed Infections With Sugarcane Mosaic Virus And A New Marafivirus. *Crop Protection*, *139*, 105373.

Chávez-Calvillo, G., Contreras-Paredes, C. A., Mora-Macias, J., Noa-Carrazana, J. C., Serrano-Rubio, A. A., Dinkova, T. D. & Silva-Rosales, L. (2016). Antagonism Or Synergism Between Papaya Ringspot Virus And Papaya Mosaic Virus In Carica Papaya Is Determined By Their Order Of Infection. *Virology*, *489*, 179-191.

Chen, Z., Tan, J. Y., Wen, Y., Niu, S., & Wong, S. M. (2012). A Game-Theoretic Model Of Interactions Between Hibiscus Latent Singapore Virus And Tobacco Mosaic Virus. *Plos One*, *7*(5), E37007.

Choi, S. K., Yoon, J. Y., Ryu, K. H., Choi, J. K., Palukaitis, P., & Park, W. M. (2002). Systemic Movement Of A Movement-Deficient Strain Of Cucumber Mosaic

Virus In Zucchini Squash Is Facilitated By A Cucurbit-Infecting Potyvirus. *Journal Of General Virology, 83*(12), 3173-3178.

Chung, B. Y. W., Miller, W. A., Atkins, J. F., & Firth, A. E. (2008). An overlapping essential gene in the Potyviridae. Proceedings of the National Academy of Sciences, 105(15), 5897-5902.

Csorba, T., Kontra, L., & Burgyán, J. (2015). Viral Silencing Suppressors: Tools Forged To Fine-Tune Host-Pathogen Coexistence. *Virology*, *479*, 85-103.

Dapalma, T., Doonan, B. P., Trager, N. M., & Kasman, L. M. (2010). A Systematic Approach To Virus–Virus Interactions. *Virus Research*, *149*(1), 1-9.

De Ronde, D., Butterbach, P., & Kormelink, R. (2014). Dominant Resistance Against Plant Viruses. *Frontiers In Plant Science*, *5*, 307.

Demski, J. W., & Jellum, M. D. (1975). Single and double virus infection of soybean: plant characteristics and chemical composition. *Phytopathology*, *65*(10), 1154 -1156.

Di Rienzo, V., Bubici, G., Montemurro, C., & Cillo, F. (2018). Rapid Identification Of Tomato Sw-5 Resistance-Breaking Isolates Of Tomato Spotted Wilt Virus Using High Resolution Melting And Taqman SNP Genotyping Assays As Allelic Discrimination Techniques. *Plos One*, *13*(4), E0196738.

Dietzgen, R. G., Mann, K. S., & Johnson, K. N. (2016). Plant Virus-Insect Vector Interactions: Current And Potential Future Research Directions. *Viruses*, *8*(11), 303.

Fattouh, F. A. (2003). Double Infection Of A Cucurbit Host By Zucchini Yellow Mosaic Virus And Cucumber Mosaic Virus. *Plant Pathology Journal*.

Folimonova, S. Y. (2013). Developing An Understanding Of Cross-Protection By Citrus Tristeza Virus. *Frontiers In Microbiology*, *4*, 76.

García-Marcos, A., Pacheco, R., Martiáñez, J., González-Jara, P., Díaz-Ruíz, J. R., & Tenllado, F. (2009). Transcriptional Changes And Oxidative Stress Associated With The Synergistic Interaction Between Potato Virus X And Potato Virus Y And Their Relationship With Symptom Expression. *Molecular Plant-Microbe Interactions*, *22*(11), 1431-1444.

García-Cano, E., Resende, R. O., Fernández-Muñoz, R., & Moriones, E. (2006). Synergistic interaction between Tomato chlorosis virus and Tomato spotted wilt virus results in breakdown of resistance in tomato. *Phyto-pathology*, *96*(11), 1263-1269.

Gómez, P., Sempere, R. N., Elena, S. F., & Aranda, M. A. (2009). Mixed Infections Of Pepino Mosaic Virus Strains Modulate The Evolutionary Dynamics Of This Emergent Virus. *Journal Of Virology*, *83*(23), 12378-12387.

Gómez-Aix, C., Alcaide, C., Gómez, P., Aranda, M. A., & Sánchez-Pina, M. A. (2019). In Situ Hybridization For The Localization Of Two Pepino Mosaic Virus Isolates In Mixed Infections. *Journal OfVirological Methods*, *267*, 42-47.

González-Jara, P., Atencio, F. A., Martínez-García, B., Barajas, D., Tenllado, F., & Díaz-Ruíz, J. R. (2005). A Single Amino Acid Mutation In The Plum Pox Virus Helper Component-Proteinase Gene Abolishes Both Synergistic And RNA Silencing Suppression Activities. *Phytopathology*, *95*(8), 894-901.

González-Jara, P., Fraile, A., Canto, T., & García-Arenal, F. (2009). The Multiplicity Of Infection Of A Plant Virus Varies During Colonization Of Its Eukaryotic Host. *Journal Of Virology*, *83*(15), 7487-7494.

González–Jara, P., Tenllado, F., Martínez-García, B., Atencio, F. A., Barajas, D., Vargas, M., & Díaz–Ruíz, J. R. (2004). Host–Dependent Differences during Synergistic Infection ByPotyvirusesWith Potato Virus X. *Molecular Plant Pathology*, *5*(1), 29-35.

Gray, S., Cilia, M., & Ghanim, M. (2014). Circulative,"Nonpropagative" Virus Transmission: An Orchestra Of Virus-, Insect-, And Plant-Derived Instruments. *Advances In Virus Research*, *89*, 141-199.

García-Viera, M. A., Sánchez-Segura, L., Chavez-Calvillo, G., Jarquín-Rosales, D., & Silva-Rosales, L. (2018). Changes in leaf tissue of Carica papaya during single and mixed infections with Papaya ringspot virus and Papaya mosaic virus. *Biologia plantarum*, 62(1), 173-180.

Guerret, M. G., Barbetti, M. J., You, M. P., & Jones, R. A. (2016). Effects of Temperature On Disease Severity In Plants Of Subterranean Clover Infected Singly Or In Mixed Infection With Bean Yellow Mosaic Virus And Kabatiella Caulivora. *Journal Of Phytopathology*, *164*(9), 608-619.

Guo, Z., Li, Y., & Ding, S. W. (2019). Small RNA-Based Antimicrobial Immunity. *Nature Reviews Immunology*, *19*(1), 31-44.

Hacker, D. L., & Fowler, B. C. (2000). Complementation Of The Host Range Restriction Of Southern Cowpea Mosaic Virus In Bean By Southern Bean Mosaic Virus. *Virology*, *266*(1), 140-149.

Hammond, J., Lecoq, H., & Raccah, B. (1999). Epidemiological Risks From Mixed Virus Infections And Transgenic Plants Expressing Viral Genes. *Advances In Virus Research*, *54*, 189-314.

Hashimoto, M., Neriya, Y., Yamaji, Y., & Namba, S. (2016). Recessive Resistance To Plant Viruses: Potential Resistance Genes Beyond Translation Initiation Factors. *Frontiers In Microbiology*, *7*, 1695.

Hogenhout, S. A., Ammar, E. D., Whitfield, A. E., & Redinbaugh, M. G. (2008). Insect Vector Interactions With Persistently Transmitted Viruses. *Annu. Rev. Phytopathol.*, *46*, 327-359.

Karyeija, R. F., Kreuze, J. F., Gibson, R. W., & Valkonen, J. P. T. (2000). Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology*, *269*(1), 26-36.

Lu, B., Stubbs, G., & Culver, J. N. (1998). Coat Protein Interactions Involved In Tobacco Mosaic Tobamovirus Cross-Protection. *Virology*, *248*(2), 188-198.

Mandahar, C. L. (1978). Introduction to Plant Viruses.

Mascia, T., & Gallitelli, D. (2014). Synergism In Plant–Virus Interactions: A Case Study Of CMV And PVY In Mixed Infection In Tomato. In *Plant Virus–Host Interaction* (Pp. 195-206). Academic Press.

Mascia, T., & Gallitelli, D. (2016). Synergies And Antagonisms In Virus Interactions. *Plant Science*, *252*, 176-192.

Mascia, T., Cillo, F., Fanelli, V., Finetti-Sialer, M. M., De Stradis, A., Palukaitis, P., & Gallitelli, D. (2010). Characterization Of The Interactions Between Cucumber Mosaic Virus And Potato Virus Y In Mixed Infections In Tomato. *Molecular Plant-Microbe Interactions*, *23*(11), 1514-1524.

Mayo, M., Ryabov, E., Fraser, G., & Taliansky, M. (2000). Mechanical transmission of Potato leafroll virus. *Journal of General Virology*, *81*(11), 2791-2795.

Mendez-Lozano, J., Torres-Pacheco, I., Fauquet, C. M., & Rivera-Bustamante, R. F. (2003). Interactions Between Geminiviruses In A Naturally Occurring Mixture: Pepper Huasteco Virus And Pepper Golden Mosaic Virus. *Phytopathology*, *93*(3), 270-277.

Moreno, A. B., & López-Moya, J. J. (2020). When Viruses Play Team Sports: Mixed Infections In Plants. *Phytopathology*, *110*(1), 29-48.

Murant, A. F. (1990). Dependence Of Groundnut Rosette Virus On Its Satellite RNA As Well As On Groundnut Rosette Assistor Luteovirus For Transmission By Aphis Craccivora. *Journal Of General Virology*, *71*(9), 2163-2166.

Murphy, J. F., & Bowen, K. L. (2006). Synergistic Disease In Pepper Caused By The Mixed Infection Of Cucumber Mosaic Virus And Pepper Mottle Virus. *Phytopathology*, *96*(3), 240-247.

Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. T. (2006). Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweetpotato plants. *Plant pathology*, *55*(3), 458-467.

Namrata, J., Saritha, R. K., Datta, D., Singh, M., Dubey, R. S., Rai, A. B., & Rai, M. (2010). Molecular characterization of tomato leaf curl Palampur virus and pepper leaf curl betasatellite naturally infecting pumpkin (Cucurbita moschata) in India. *Indian Journal of Virology*, *21*(2), 128-132.

Ng, J. C., & Falk, B. W. (2006). Virus-Vector Interactions Mediating Nonpersistent and Semipersistent Transmission Of Plant Viruses. *Annu. Rev. Phytopathol.*, *44*, 183-212.

Noa-Carrazana, J. C., González-de-León, D., Ruiz-Castro, B. S., Piñero, D., & Silva-Rosales, L. (2006). Distribution of Papaya ringspot virus and Papaya mosaic virus in papaya plants (Carica papaya) in Mexico. Plant disease, 90(8), 1004-1011.

Panno, S., Davino, S., Rubio, L., Rangel, E., Davino, M., García-Hernández, J., & Olmos, A. (2012). Simultaneous Detection Of The Seven Main Tomato-Infecting RNA Viruses By Two Multiplex Reverse Transcription Polymerase Chain Reactions. *Journal Of Virological Methods*, *186*(1-2), 152-156.

Pechinger, K., Chooi, K. M., Macdiarmid, R. M., Harper, S. J., & Ziebell, H. (2019). A New Era For Mild Strain Cross-Protection. *Viruses*, *11*(7), 670.

Peiró, A., Cañizares, M. C., Rubio, L., López, C., Moriones, E., Aramburu, J., & Sánchez-Navarro, J. (2014). The Movement Protein (Nsm) Of T Omato Spotted Wilt Virus Is The Avirulence Determinant In The Tomato Sw-5 Gene-Based Resistance. *Molecular Plant Pathology*, *15*(8), 802-813.

Pimentel D. Lach L. Zuniga R. Morrison D. (2000). Environmental and economic costs of nonindigenous species in the United States. BioScience50: 53–65.

Rentería-Canett, I., Xoconostle-Cázares, B., Ruiz-Medrano, R., & Rivera-Bustamante, R. F. (2011). Geminivirus Mixed Infection on Pepper Plants: Synergistic Interaction between PHYVV and Pepgmv. *Virology Journal*, *8*(1), 1-14.

Roossinck, M. J. (2011). The Good Viruses: Viral Mutualistic Symbioses. *Nature Reviews Microbiology*, *9*(2), 99-108.

Rubio, L., Galipienso, L., & Ferriol, I. (2020). Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity And Evolution. *Frontiers in Plant Science*, *11*, 1092.

Sastry, K. S., Mandal, B., Hammond, J., Scott, S. W., & Briddon, R. W. (2019). *Encyclopedia Of Plant Viruses And Viroids* (Pp. 2382-2393). New Delhi, India:: Springer.

Scheets, K. (1998). Maize Chlorotic Mottle MachlomovirusAnd Wheat Streak Mosaic Rymovirus Concentrations Increase In the Synergistic Disease Corn Lethal Necrosis. *Virology*, *242*(1), 28-38.

Shi, X., Zhang, Z., Zhang, C., Zhou, X., Zhang, D., & Liu, Y. (2021). The Molecular Mechanism Of Efficient Transmission Of Plant Viruses In Variable Virus-Vector-Plant Interactions. *Horticultural Plant Journal*.

Silva-Rosales, L., Vargas, P., Alcalá-Briseño, R., & Casarrubias, K. (2017, October). Revisiting Cross-Protection In The Form Of Antagonism: The Case Of Papaya

Mosaic Virus Protecting Papaya Plants Against Papaya Ringspot Virus. In *V International Symposium On Papaya 1250* (Pp. 55-62).

Singhal, P., Nabi, S. U., Yadav, M. K., & Dubey, A. (2021). Mixed Infection Of Plant Viruses: Diagnostics, Interactions And Impact On Host. *Journal Of Plant Diseases And Protection, 128*(2), 353-368.

Soler, S., Debreczeni, D. E., Vidal, E., Aramburu, J., López, C., Galipienso, L., & Rubio, L. (2015). A New Capsicum Baccatum Accession Shows Tolerance To Wild-Type And Resistance–Breaking Isolates Of Tomato Spotted Wilt Virus. *Annals Of Applied Biology, 167*(3), 343-353.

Syller, J. (2020). Interspecific And Intraspecific Interactions Among Plant Viruses In Mixed Infections. In *Applied Plant Virology* (Pp. 437-453). Academic Press.

Takahashi, H., Fukuhara, T., Kitazawa, H., & Kormelink, R. (2019). Virus Latency And The Impact On Plants. *Frontiers In Microbiology, 10*, 2764.

Tatineni, S., Riethoven, J. J. M., Graybosch, R. A., French, R., & Mitra, A. (2014). Dynamics Of Small RNA Profiles Of Virus And Host Origin In Wheat Cultivars Synergistically Infected By Wheat Streak Mosaic Virus And Triticum Mosaic Virus: Virus Infection Caused A Drastic Shift In The Endogenous Small RNA Profile. *Plos One, 9*(11), E111577.

Tomašechová, J., Hančinský, R., Predajňa, L., Kraic, J., Mihálik, D., Šoltys, K., & Glasa, M. (2020). High-Throughput Sequencing Reveals Bell Pepper Endornavirus Infection In Pepper (Capsicum Annum) In Slovakia And Enables Its Further Molecular Characterization. *Plants, 9*(1), 41.

Truniger, V., & Aranda, M. (2009). Advances In Virus Research. *Adv Virus Res, 75*, 119-159.

Untiveros, M., Fuentes, S., & Salazar, L. F. (2007). Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with carla-, cucumo-, ipomo-, and potyviruses infecting sweet potato. *Plant disease*, *91*(6), 669-676.

Vargas-Mejía, P., Vega-Arreguín, J., Chávez-Calvillo, G., Ibarra-Laclette, E., & Silva-Rosales, L. (2020). Differential Accumulation of Innate-and Adaptive-Immune-Response-Derived Transcripts during Antagonism between Papaya Ringspot Virus and Papaya Mosaic Virus. Viruses, 12(2), 230.

Vanitharani, R., Chellappan, P., Pita, J. S., & Fauquet, C. M. (2004). Differential Roles Of AC2 And AC4 Of Cassava Geminiviruses In Mediating Synergism And Suppression Of Posttranscriptional Gene Silencing. *Journal Of Virology*, *78*(17), 9487-9498.

Verma, R., Gaikwad, P., Mungekar, D., Tripathi, S., Datar, V., & Singh, J. (2014). First Report Of Mixed Infection Of Papaya Ringspot Virus And Phytoplasma In Papaya In India. *Journal Of Plant Pathology*, *96*(2).

Wang, Y., Lee, K. C., Gaba, V., Wong, S. M., Palukaitis, P., & Gal-On, A. (2004). Breakage of resistance to Cucumber mosaic virus by co-infection with Zucchini yellow mosaic virus: enhancement of CMV accumulation independent of symptom expression. *Archives of virology*, *149*(2), 379-396.

Wege, C., & Siegmund, D. (2007). Synergism Of A DNA And An RNA Virus: Enhanced Tissue Infiltration Of The Begomovirus Abutilon Mosaic Virus (Abmv) Mediated By Cucumber Mosaic Virus (CMV). *Virology*, *357*(1), 10-28.

Whitfield, A. E., Falk, B. W., & Rotenberg, D. (2015). Insect Vector-Mediated Transmission Of Plant Viruses. *Virology*, *479*, 278-289.

Wintermantel, W. M., Cortez, A. A., Anchieta, A. G., Gulati-Sakhuja, A., & Hladky, L. L. (2008). Co-Infection By Two Criniviruses Alters Accumulation Of Each

Virus In A Host-Specific Manner And Influences Efficiency Of Virus Transmission. *Phytopathology*, *98*(12), 1340-1345.

Xu, Y., Li, S., Na, C., Yang, L., & Lu, M. (2019). Analyses Of Virus/Viroid Communities In Nectarine Trees By Next-Generation Sequencing And Insight Into Viral Synergisms Implication In Host Disease Symptoms. *Scientific Reports*, *9*(1), 1-12.

Zeng, R., Liao, Q., Feng, J., Li, D., & Chen, J. (2007). Synergy Between Cucumber Mosaic Virus And Zucchini Yellow Mosaic Virus On Cucurbitaceae Hosts Tested By Real-Time Reverse Transcription-Polymerase Chain Reaction. *Acta Biochimica et Biophysica Sinica*, *39*(6), 431-437.

Zhang, X. S., Holt, J., & Colvin, J. (2001). Synergism Between Plant Viruses: A Mathematical Analysis Of The Epidemiological Implications. *Plant Pathology*, *50*(6), 732-746.

Ziebell, H., & Carr, J. P. (2010). Cross-Protection: A Century Of Mystery. *Advances In Virus Research*, *76*, 211-264.