

Review Article

**VIRUSES OF STRAWBERRY AND PRODUCTION OF VIRUS FREE
PLANTING MATERIAL- A CRITICAL REVIEW**

**Abhilasha Sharma^{1*}, Anil Handa¹, Shelly Kapoor², Santosh Watpade³, Bhupesh Gupta¹
and Praveen Verma²**

¹Department of Plant Pathology, ²Department of Biotechnology,
Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan- 173 230 (HP)

³ICAR- IARI Regional Station, Shimla- 171 004 (HP)

E-mail: abhilashasharma094@gmail.com (*Corresponding Author)

Abstract: Strawberry (*Fragaria x ananassa* Duchesne) is a member of the Rosaceae family. Among all soft berries like brambles, strawberry and currants, strawberries are the most favourite soft berry fruits of the temperate world. Many viruses are involved and they can act to produce symptoms which vary according to the virus species involved, the relative proportions of each, the environmental conditions and the response of the particular host variety. There are about seven aphid transmitted viruses infecting strawberry in nature. These include strawberry crinkle virus (SCV), strawberry mottle virus (SMoV), strawberry vein bending virus (SVBV), strawberry pseudo mild yellow edge virus (SPMYEV), strawberry chlorotic fleck virus (SCFV) and strawberry latent c virus (SLCV). The virus diseases are the most prominent limiting factor in the production of certified virus- free planting material of strawberry. Key to success for achieving this goal lies in the development of a sound certification scheme that has the aim of providing strawberry plants which are true-to-type, free from virus diseases and substantially free from other pests.

Keywords: Strawberry, viruses, serology, molecular detection, certification.

Introduction

Strawberry (*Fragaria x ananassa* Duchesne) is a member of the Rosaceae family. The fleshy fruit of strawberry is classified as an aggregate fruit (Green, 1971). The cultivated strawberry is a hybrid of two Native American species, North American *Fragaria X chiloensis* and South American *Fragaria x virginiana* (Hancock et al 1999). Among all soft berries like brambles, strawberry and currants, strawberries are the most favourite soft berry fruits of the temperate world with its origin in Europe around 1750. The *Fragaria x ananassa* is a perennial soft berry which arises from a crown of meristematic tissues. The most popular cultivated strawberry is the dessert strawberry, *Fragaria x ananassa*. Annual world production of this species has steadily grown through the ages with quantities doubling in the last 20 years to over 2.5 million tonnes (FAO, 2016). Many viruses are involved and they can act to produce symptoms which vary according to the virus species involved, the relative

proportions of each, the environmental conditions and the response of the particular host variety. However, in practical terms the most important effect is the loss of vigour and yield caused by some viruses and virus combinations, which can render the crop worthless. Reports of initial detection, symptoms, severities, and/or vectors for more than 30 viruses, virus like diseases and phytoplasmas affecting *Fragaria spp.* have been reviewed (Martin and Tzanetakis, 2006). It can lead up to 30 percent yield reduction and losses can be up to 80 percent in mixed infections with other viruses (Thompson and Jelkmann, 2003; Martin and Tzanetakis, 2006). Out of these, viruses are the major limiting factor in commercial strawberry production. There are about seven aphid transmitted viruses, five nematode transmitted and two whitefly transmitted strawberry viruses. Among these, the most important viruses include strawberry mild yellow edge virus (SMYEV), strawberry vein bending virus (SVBV), strawberry latent ring spot virus (SLRSV) and tobacco streak virus (TSV). Of late, a new virus named strawberry pallidosis virus (SPaV) has emerged as the one of most serious viruses infecting strawberry after nematode transmitted viruses, often referred to as nepoviruses. A combination of these viruses poses a serious threat to strawberry cultivation and in the production of certified planting material in strawberry all over the world. A critical study of these viruses will therefore go a long way in understanding their spread in the planting material and will certainly help in developing sound strategies for managing the diseases caused by them. This manuscript highlights major viruses infecting strawberry in relation to their natural distribution, symptomatology, particle morphology, transmission, host range, serology and molecular detection.

Viruses in strawberry

Many viruses affect strawberries either alone or in combination. These may lead to strange appearances such as green petals, crinkling, yellow spotting and vein banding of the leaves. In strawberries the symptoms are variable and complex usually many viruses are involved at the same time and they can act to produce symptoms which vary according to the virus species involved, the relative proportion of each, the environmental conditions and the response of the particular host variety. However, in practical terms, the most important effect is the loss of vigour and yield caused by some viruses and virus combinations which can render the crop worthless.

Strawberry (*Fragaria x ananassa* Duch.) has been reported to be infected by a large number of viruses under natural conditions. A critical screening of the available literature on the occurrence of various viruses infecting strawberry under natural conditions in different parts

of the world revealed that this crop is infected by a number of viruses and their strains. The economically important viruses known to infect strawberry are discussed here under.

There are about seven aphid transmitted viruses infecting strawberry in nature (Tzanetakis and Martin, 2013). These include strawberry crinkle virus (SCV), strawberry mottle virus (SMoV), strawberry vein bending virus (SVBV), strawberry pseudo mild yellow edge virus (SPMYEV), strawberry chlorotic fleck virus (SCFV) and strawberry latent c virus (SLCV). Out of these, SCV, SMYEV, SMoV and SVBV have been considered to be the most economically important viruses of strawberry in majority of production areas (Tzanetakis and Martin, 2013). Besides, Whitefly transmitted viruses are fast emerging as a major problem in commercial strawberry production. There are about five nematode transmitted viruses known to infect strawberry. Out of these, four viruses belong to the genus *Nepovirus* representing family *Comoviridae*; whereas strawberry latent ringspot virus (SLRSV) and tobacco ringspot virus (TRSV) are members of the family *Secoviridae*.

Nepovirus

Nepovirus is one of the important genera of viruses transmitted by nematodes. There are currently 36 species in this genus including the type species strawberry latent ringspot virus. Nepoviruses usually have a wide host range and induce diseases of economic importance in a variety of cultivated plant species. Symptoms induced by nepoviruses include chlorotic and necrotic lesions, although many hosts remain symptomless. *Nicotiana species* infected by Nepoviruses typically recover from infection at late stages. The major viruses in these genera are arabis mosaic, raspberry ringspot, tomato black ring, tobacco ringspot, strawberry latent ringspot, strawberry mottle virus, blueberry ringspot, peach rosette mosaic, peach yellow bud mosaic, cherry rasp leaf and grapevine yellow vein *Nepovirus* (Halbrendt and Brown, 1993; Vrain, 1993).

Strawberry Latent Ringspot Virus

Taxonomically, the virus belongs to the family *Secoviridae* and genus *Nepovirus*. The virus is also referred to as aesculus line pattern and rhubarb virus 5.

Natural occurrence

Strawberry latent ringspot virus (SLRSV) was first reported in strawberry in North America though the virus was previously reported in a single cherry tree in Ontario, Canada. SLRSV has also been reported from Egypt (EL-Morsy et al 2017). It is a member of the subgroup IV of the genus *Nepovirus* in the family *Comoviridae* (EPPO/CABI, 1996). SLRSV is widespread in many countries, especially in Europe (Tang et al 2013). In India, SLRSV has

been reported in Rose (Kulshrestha et al 2004). It occurs naturally in many species of wild and cultivated plants and infects (often symptomlessly) a wide range of commonly used herbaceous test plants.

Symptomatology

The disease is usually latent in strawberries and other fruit crops. Some strawberry Cultivars show varying degrees of mottling and decline and leaves become patchy and reddish in colour leading to yield loss. In Italy, isolates of SLRSV were found associated with russetting disease in peach (Belli et al 1980).

Transmission

The virus is transmitted mostly by mechanical means, via seed and pollens in several hosts including strawberry, and under natural conditions SLRSV is locally dispersed by the nematode *Xiphenema diversicaudatum* (EPPO/CABI, 1996).

Host range

SLRSV has a wide host range. It infects strawberries and raspberries, mostly without symptoms but resulting in various degrees of mottle and decline in some cultivars. Other fruit crop hosts are blackberries, black currants, red currants, cherries, grapes, plums, peaches and elderberry. Furthermore, it has been reported from asparagus, celery, gladiolus, narcissus, rhubarb and roses (EPPO/CABI, 1996). Schmelzer (1969) reported a wide host range of SLRSV among dicotyledonous plants.

Particle morphology and serology

Virions are icosahedral, non- enveloped 25-30 nm in diameter, genomes are linear, segmented and bipartite measuring around 24-7kb in length. SLRSV isolated from black locust (*Robinia Pseudoacasia*) has been separated into three serotypes (Borodynko et al 2007). Analysis of SLRSV from *Robinia* indicated that one isolate was remarkably different from other two. A different isolate was thus proposed as a new strain of SLRSV (Borodynko et al 2007). Strawberry latent ringspot virus (SLRSV), a tentative member of the *Nepovirus* group (Regenmortel et al 2000) with a worldwide distribution has isometric particles measuring 30 nm in diameter (Faggioli et al 2002).

Faggioli et al (2002) used one-step RT-PCR to detect SLRSV infection in samples from 87 trees of olive grown in Italy by application of designed primer pair and also worked for the detection of SLRSV by using DAS-ELISA. Mazyad *et al.* (2014) studied the characterization of SLRSV on strawberry and isolated SLRSV from symptomless strawberry plants identified with specific antiserum by using Double Antibody Sandwich-Enzyme Linked Immune

Sorbent Assay (DAS-ELISA). EL-Morsey *et al.* (2017) while studying the molecular identification of strawberry latent ringspot virus SLRSV in Egypt identified a specific antiserum using Double Sandwich ELISA (DAS-ELISA).

Tang *et al.* (2013) also worked on the diversity of strawberry latent ringspot virus in New Zealand. Double-antibody sandwich (DAS) enzyme-linked immune-sorbent assay (ELISA) was performed on selected symptomatic herbaceous indicator plants using commercial polyclonal antiserum raised against SLRSV.

Molecular detection and characterization

Faggioli *et al.* (2002) used one-step RT-PCR to detect SLRSV infection in samples from 87 trees of olive grown in Italy by application of designed primer pair and also worked for the detection of SLRSV by using DAS-ELISA. It was concluded that direct detection of SLRSV can reliably be done by One-step RT-PCR and was capable of detecting SLRSV in some symptomless plants grown in the same orchard.

Martin and Tzanetakis (2004) first reported SLRSV in US and Canada and used RT-PCR by using primers SLRSV F and SLTSV R which amplify a 497-bp fragment of RNA 2 of SLRSV. No amplicon was obtained from the non-inoculated control plant. Kumari (2009) also used one-step reverse transcriptase chain reaction (One-step RT-PCR) for the detection of SLRSV from apricot and peach plants by the application of different pairs of primers. The studies concluded that one-time RT-PCR protocol is rapid and sensitive and has the potential to be used for the diagnosis of SLRSV in routine diagnostic laboratories.

Mazyad *et al.* (2014) studied the characterization of SLRSV on strawberry and isolated SLRSV from symptomless strawberry plants identified with specific antiserum by using DAS-ELISA. RNA from isolates of SLRSV was detected using RT-PCR by utilizing specific primers designed for coat protein genes.

Molecular identification of SLRSV by using DAS- ELISA isolates from symptomatic and symptomless strawberry plants. RT PCR was conducted by El-Morsy *et al.* (2016) used to amplify 497 bp fragment using specific primers for the viral coat protein gene. It was concluded that SLRSV should be considered as an important pathogen of strawberry and one-step RT-PCR allows the detection of SLRSV on the early stage of strawberry cultivation.

A simple and rapid diagnostic method for strawberry latent ringspot virus in plants using LAMP assay (loop mediated isothermal amplication assay) was developed by Kim *et al.* (2016). This method was found to be more effective than RT-PCR and nested-PCR which are

the standard methods of detecting SLRSV. LAMP assay showed sensitivity similar to that of currently used methods and was more rapid, simple and specific.

Tobacco ringspot virus

Tobacco ringspot virus is a plant pathogenic virus in the plant virus family *Secoviridae* under genus *Nepovirus*. The virus is also referred to as Anemone necrosis virus (Hollings, 1965), blueberry necrotic ringspot virus and tobacco ringspot virus 1.

Natural occurrence

TRSV was observed for the first time in tobacco fields of Virginia (Fromme et al 1927). This virus also spreads in the North American regions, China, Australia, U.K., Germany and New Zealand. TRSV occurred naturally on cowpea in India and was identified as the cause of ringspot disease of cowpea on the basis of transmission studies, host range, physical properties and serology (Patil and Mali, 1982). The occurrence of TRSV was also found in the North Carolina in tobacco crop (Rush and Gooding, 1970). Tobacco Ringspot Virus was first described in the United States in 1941 and has been reported in Canada, Cuba, Brazil, India, Australia, the former Soviet Union, and China (Hill and Witham, 2014)

Symptomatology

The characteristic symptoms evoked by TRSV are necrotic spots, mottling, chlorotic ringspots and vein banding. Symptoms vary depending on the plant affected. In general, symptoms are more severe on young plants at the beginning of the growing season and are less noticeable later in the season. In soyabean (soyabean bud blight, the most severe disease caused by TRSV), symptoms are severe stunting, curvature of the terminal bud and necrosis of the other buds. Reduction in pod development may occur (Frison et al 1990). (Similar symptoms will also develop upon infection with *Tobacco streak virus*.) In tobacco (widespread disease in North America), symptoms are stunting of the plant, ring and line patterns on the leaves, and reduction in yield and quality of leaves. In blueberry, the symptoms are general stunting of the plant, chlorotic and necrotic spots on the leaves, and stem dieback. In *Rubus* spp., symptoms can vary and can include chlorotic mottling, line-pattern, mosaic, vein yellowing and leaf curling. Necrotic symptoms are also observed on roots upon infection of seedlings by TRSV (Leggat and Teakle, 1976).

Transmission

The virus is transmitted non-specifically by insects and mites (*Aphis gossyphi*, *Myzus persici* and *Thrips tabaci*) and nematode vector (*Xiphinema americanum*). The virus is lost by the vector when it moults. It does not multiply in the vector and is not transmitted

congenitally to the progeny of the vector. It does not require any helper virus for vector transmission and is transmitted by mechanical inoculation. The virus is not transmitted by contact between plants, besides transmission by seeds and pollen. TRSV can reach the embryo where the virus multiplies in the developing embryo during germination leading to a rapid systemic infection of the plantlets (Lecoq and Katis, 2014). The virus has been reported to be readily transmissible by mechanical means but not through seeds or aphids (Patil and Mali, 1982).

Host range

Diagnostically susceptible host species of TRSV are *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativa*, *Phaseolus vulgaris*, *Nicotiana clevelandii*, *Vigna unguiculata*, *N. glutinosa*, *N. tabacum*, *Calendula officinalis*, *Datura metel*, *Glycine max*, *Medicago sativa*, *Pisum sativum*, *Solanum tuberosum*, *Vigna raiata*, *Zea mays* and *Zinnia elegans* (Schneider et al 1972).

Particle morphology and serology

Virions are isometric, non-enveloped and about 25-29 nm in diameter, angular in profile and without a conspicuous capsomere arrangement. Genome consists of RNA which is single stranded and a total genome size measuring about 11.2kb and the genome is of two parts: largest genome part of 6.8 kb and the other one is of 4.3 kb. Genomic nucleic acid was isolated by Rezaian and Fracki (1974). 5' terminus of RNA has a VPg, Poly A region present on it and genome has no rRNA like activity.

Rush and Goodling (1970) studied the occurrence of tobacco ringspot virus strains and tomato ringspot virus in hosts indigenous to North Carolina. Six isolates were identified serologically distinct from the common strain from Tobacco in North Carolina, which is identical serologically to the American Type Culture Collection isolate Number 98.

Molecular detection and characterization

A PCR based diagnostic system for the detection of the seed-transmitted tobacco ringspot virus in quarantines was developed by Lee et al (2015). In this study, RT-PCR and nested PCR systems for TRSV detection in quarantine sites and modified –positive control for proving laboratory contamination and false positive reactions were developed.

Raspberry ringspot virus (RRSV)

As per the preferred classification this virus belongs to the family *Secoviridae* and genus *Nepovirus*. This virus is also known as raspberry Scottish leaf curl virus and red currant ringspot virus.

Natural occurrence

Bargen et al (2015) observes RRSV for the first time in mosaic diseased hybrid roses in Germany. This virus is prevalent in Iran, Turkey, Finland, France, Germany, Greece and Ireland.

Symptomatology

The characteristic symptoms evoked by RRSV are abnormal shape of fruits, leaves with abnormal colour pattern and the dwarfed plants showing dieback symptoms. On raspberries young expanding leaves develop a conspicuous, yellowish-green, ringspot or oak-leaf pattern, chlorotic blotches and a net-like chlorosis along the smaller veins may also be present (Murant, 1970). On strawberries Symptoms vary according to season and strain. In general, progressive dwarfing and ultimate death may be expected (Lister, 1970).

Transmission

The virus can be transmitted by nematode vectors. The Scottish strain is mostly spread by *Longidorus elongates* and the English strain by *Longidorus macrosoma* (Dhingra and Niazi, 1972). RRSV is transmitted in the soil via nematode vectors, different serotypes of RRSV were found to be transmitted by different species (Wellink et al 2000).

Host range

Digonostically susceptible host plants of RpRSV are *Narcissus*, *Phlox*, *Prunus avium*, *Rosa hybrida*, *Rubus idaeus*, *Sambucus nigra*, *Vitis vinifera*, *Weigela* etc. RRSV also occurs naturally in many species of wild and cultivated dicotyledonous and monocotyledonous plants. A number of other hosts infected experimentally includes *Chenopodium amaranticolor*, *Cucurbits spp.*, *Iberis amara*, *Nicotiana spp.* etc. (Harrison, 1957).

Particle morphology and serology

The virus particles are isometric and about 28nm in diameter with an angular outline. Particle sediment as two nucleoprotein components M and B with sedimentation coefficient of 92 and 130 S, respectively. The complete nucleotide sequence of the 3928 nt of RNA-2 of a Scottish isolate of virus has been determined (Blok et al 1992).

Bargen et al (2015) detected Raspberry ringspot virus in hybrid roses exhibiting mosaic symptoms in Germany. This virus was detected by DAS-ELISA in bud and leaf sample of eight diseased roses.

Molecular detection and characterization

Complete nucleotide sequence of an isolate of the nepovirus raspberry ringspot virus was identified by Ebel et al (2003) from grapevine. A maximum level of 34 percent identity was found between the RNA-1 encoded polypeptides of RpRSV-ch and other nepoviruses. For RNA-2 encoded polypeptides, about 88n percent identity was found between RpRSV-ch and RpRSV-S (a Scottish isolate). RT-PCR in RpRSV infected roses was carried out successfully by Wei and Clover (2008) by applying two different primer pairs specific for the amplification.

Potexvirus

Potexvirus is a genus of pathogenic viruses in order *Tymovirales* in the family *Alphaflexiviridae*. It include viruses like *Allium virus x*, *Alstroemeria virus x*, *Asparagus virus 3*, *Clover yellow mosaic virus*, *Daphne virus x*, *Lettuce virus x*, *Potato virus x*, *Tulip virus x* and *Strawberry mild yellow edge virus* (Lesemann & Koenig, 1977).

Strawberry mild yellow edge virus

According to the taxonomical classification, the virus belongs to the family *Alphaflexiviridae* and genus *Potexvirus*. This virus is also known by name strawberry virus 2.

Natural occurrence

Strawberry mild yellow edge is one of the widespread and common virus diseases in cultivated strawberries. There are records of SMYEV in china and in several other countries like Belgium, Bulgaria, Czech Republic, France, Germany and in some parts of America (EPPO, 1994). The presence and identification of the main strawberry aphid-borne viruses in five European countries Italy, Poland, Czech Republic, Germany and Lithuania has been reported by Babini et al (2004). The virus has also been reported from Japan (Martin and Tzanetakis, 2006).

Symptomatology

The characteristic symptoms of SMYEV include cupped leaflets, chlorotic mottling, interveinal necrosis of older leaves and stunting (Jelkmann, 1991). This virus do not induce distinct symptoms in commercial cultivars but often cause a loss of vigour, stunting, and decreased yield in infected plants (Babini et al 2004).

Transmission

SMYEV is transmitted in a semi-persistent manner by an aphid spp. in the *Chaetosiphon* genus mainly *C. fragaefolii*, *C. thomasi*, *C. thomasi Jacobi*. (Martin and Tzanetakis, 2006). This virus possibly requires vector transmission and a helper virus i.e.

luteovirus (Jelkmann, 1991). The virus cannot be transmitted through seeds, contact, grafting or pollen.

Host range

Jelkmann (1991) observed that susceptible host spp. of this virus mainly belong to Rutaceae and Rosaceae families. The virus was first reported in *Fragaria vesca* from California, U.S.A, and England and diagnostically susceptible host species of the virus were *Fragaria vesca*, *F. virginiana* and *F. ovalis* (Martin and Converse, 1982).

Particle morphology and serology

Virions are isometric non enveloped and about 23-28 nm in diameter. It is angular in profile without a conspicuous capsomere arrangement (Jelkmann, 1991). RNAs are encapsidated in filamentous particle usually flexuous particle of 13nm in diameter (Jelkman et al 1990).

Monoclonal and polyclonal antibodies were produced against the Potexvirus associated with SMYEV. For Polyclonal antibody, SMYEV was purified from *Chenopodium quinoa* co-infected with *Japonicus* ilarvirus and for monoclonal antibody SMYEV was purified from *Fragaria vesca* UC-5 plants. Results indicated that SMYEV was detected in the samples of *F.vesca* infected with the homologues virus isolates and results for polyclonal viruses were similar and indicate a broad reactivity. In another report, the virus has been purified from strawberry and antiserum was developed against the virus (Yoshikawa and Inouye, 1986).

Molecular detection and characterization

Thompson and Jelkmann (2003) reported that there are several distinct isolates of SMYEV around the world that can make molecular detection of this virus rather challenging. Jelkmann et al (1990) studied the mapping of some selected clones prepared from *dsDNA* associated with SMYEV by restriction fragment analysis. The larger open reading frame product showed considerable amino acids homology to coat protein cistrons of six potexviruses and two carlaviruses. Another product encoded completely within the coat protein cistron, but in a different frame, had similarities to two potexviruses polypeptides. The resulting polyclonal antiserum reacts strongly and resembled with those of potexviruses. It was concluded that the potexvirus is hitherto undescribed and name SMYE virus associated was proposed. Conci et al (2009) used ELISA positive plants which were analyzed by immunocapture reverse transcription semi nested polymerase chain reaction (IC-RT-semi nested PCR) with specific primers i.e. A, B and C for SMYEV. The results showed a

fragment of predicted size (406bp) in 5 of 6 symptomatic plants tested thereby confirming the SMYEV infection in strawberry plants.

Crinivirus

Crinivirus, formerly the lettuce infectious yellows virus group, is a genus of viruses, in the family closteroviridae. Martelli et al (2002) reported major 7 definitive and 3 tentative species were reported in this genera. Different species like abutilon yellows, beet yellows disorder, beet pseudo yellows, cucurbit yellow stunting disorder, diodia vein chlorosis, lettuce chlorosis, lettuce infectious yellows, potato yellow vein, strawberry pallidosis-associated, sweet potato chlorotic stunt, tomato chlorosis, tomato infectious chlorosis *Crinivirus*.

Strawberry pallidosis Virus (SPaV)

This virus is the type member of the family *Clostoviridae* and represents the genus *Crinivirus*.

Natural occurrence

Frazier and Stubbs (1969) observed the presence of strawberry pallidosis virus (SPaV) in the 1950s in both Australia and the United States, though the disease was largely confined to the Western US.

Symptomatology

Since the symptoms of SPaV disease resemble with those induced by nutritional deficiencies, it becomes difficult to diagnose the presence of SPaV. Leaves of strawberry plants with virus decline turn purple to red in color. Diseased plants have greatly reduced fruit production and roots become brittle with reduced number of absorptive rootlets. Symptoms like marginal leaf chlorosis and stunting were of common occurrence in *F. virginiana* clones ('UC-10' and 'UC-11') and the virus may cause runner reduction (Converse, 1992).

Transmission

In commercial strawberry cultivation, the virus is transmitted mostly by mechanical and graft-transmittable agents. However, the main modes of transmission of this virus include pollen, seed and whitefly transmission (Tzanetakis et al 2006). Anecdotal reports suggested that the pallidosis agent may be pollen-borne (Converse, 1992).

Host range

Strawberry pallidosis virus (SPaV) has a narrow host range, primarily limited to strawberry and related species but most of the common weeds growing in and around

strawberry fields often remain free from the virus (Tzanetakis, 2004). In addition to strawberry, the virus infects cucurbits.

Particle morphology and serology

Virion is non-enveloped with bipartite filamentous geometry, 10-13nm in diameter and 700-900nm in length. Genomes are linear and bipartite (Tzanetakis and Martin, 2003).

Molecular detection and characterization

Tzanetakis et al (2003) studied the association of *Crinivirus* (strawberry pallidosis virus) with strawberry plants by detecting it on molecular level using reverse transcription-polymerase (RT-PCR). The studies concluded the geographical distribution of SPaV in major strawberry producing regions of the United States.

Tzanetakis et al (2004) studied the genome of two criniviruses i.e. SPaV and BPYV and also conducted studies on the molecular detection of both viruses by using RT-PCR. The presence of the virus in the test plants was confirmed by either RT-PCR or dot blot (Sambrook et al 2001). The results indicated that both these viruses are closely related.

Ilarvirus

The term Ilar is derived from the description "isometric labile ringspot" first used to identify a few viruses of stone fruits that were unstable in sap and thought to be spherical. The genus *Ilarvirus* has a number of viruses associated with temperate fruits and ornamental crops. The major viruses in this genera are apple mosaic, asparagus 2, blueberry necrotic shock, citrus leaf rugose, citrus variegation, elm mottle, hydrangea mosaic, lilac ring mottle, pareitaria mottle, plum American line pattern, prune dwarf, prunus necrotic ringspot, spinach latent and tobacco streak (Mink, 1992).

Tobacco streak virus

As per taxonomy, the virus belongs to the family *Bromoviridae* and genus *Ilarvirus*. The other referred names for this virus are European plum line pattern virus, hop B virus, strawberry necrotic shock virus, and red currant necrotic ringspot virus, rose vein bending virus, rose yellow vein mosaic virus and cherry necrotic ringspot virus (Fulton, 1985).

Natural occurrence

The virus is probably distributed worldwide and is prevalent in the North American region and the pacific regions like Australia, Canada, Peru and the USA (Converse and Bartlett, 1979). In India, the first report of strawberry latent ringspot virus was from Karnataka in Niger (*Guizotia absynnia*) leaves (Kumar et al 2007)). It has spread extensively through the Indian subcontinent (Ahmed et al 2003; Kumar et al 2008; Prasada Rao et al

2000). In Africa, it has been reported from Sudan (Ali et al 2009) and South Africa (Cook et al 1999). It has been reported in several States within the United States of America, Brazil (Costa and Carvalho, 1961) and Peru (Salazar et al 1982) . In the Australasian region, the virus occurs in Australia (Greber, 1971) and New Zealand (Pappu et al 2008).

In Australia, Tobacco streak virus (TSV) was first described from tobacco in the early 1970s (Greber, 1971). An ilarvirus isolated from the M9 clone of strawberry (*Fragaria vesca*) cv. Redlands Crimson, designated TSV-S, was first described by Greber in 1979. Klose et al (1996) suggested that the strain may have originated from imported North American strawberry clones. Sdoodee (1989) demonstrated serological and host range differences between three strains of TSV found in Australia. One notable difference being that while strains TSV-Ag and TSV-A both produced dentate leaves in systemic infections of *Nicotiana tabacum* cv. Turkish (Greber, 1971; Sdoodee, 1989), TSV-S failed to do so (Greber, 1979). First report of SNSV from Australia by Klose et al (1996) also demonstrated different rates of transmission efficiency between the three strains using different thrips species as vectors. The close nucleotide identity of the Australian SNSV isolates with isolates from Mississippi, USA is in agreement with the suggestion by Klose et al (1996) that strain TSV-S may have originated from North American strawberry plants introduced into Australia.

Symptomatology

The characteristic symptoms evoked by the strawberry necrotic shock virus include systemic necrosis, leaf mottling, bud blight, leaf vein yellowing, stunting and reddening of nodes. Stem become woody and develop canker, discoloration of bark, flecking, streak and witches broom are other prominent symptoms evoked by the virus (Kumar et al 2008; Jagtap and Utpal, 2013).

Transmission

This virus is transmitted by insect vectors (*Frankliniella occidentalis* and *Thrips tabaci*) possibly by allowing virus from the surface of infected pollen to enter through feeding wounds (Sdoodee and Teakle, 1987). This virus is transmitted by mechanical inoculation, grafting, seeds and pollens to the pollinated plants and not by contact between plants. While members from at least 15 virus genera are reported to be transmitted via pollen (Card et al 2007), only a limited number, including TSV, appear to be able to infect the leaves of healthy plants via virus-infected pollen with the aid of an insect vector (Mink, 1993; Hull, 2014). Vertical transmission of viruses via pollen where fertilization results in infection of the embryo and subsequent seed transmission is more common than horizontal

transmission where virus in pollen infects non gametophytic tissues (Card et al 2007; Hull 2014). A review by Mink indicated little evidence that fertilization by virus-infected pollen can result in infection of the mother plant and generally only results in embryo infection and potential seed transmission.

Host range

This virus includes hosts from more than 9 families which are susceptible to this virus (Berkeley and Phillips, 1943). Diagnostically susceptible hosts are *Beta patellaris*, *Cyamopsis tetragonoloba*, *Phaseolus vulgaris*, *Vigna unguiculata*, *Nicotiana tabacum*. Maintenance and propagation hosts include *Catharanthus roseus*, *Cucumis sativus* and *Datura starmonium*. Among dicotyledonous hosts, *Amaranthes caudatus*, *Beta patellaris*, *Chenopodium quinoa*, *Fragaria vesca*, *Gomphrena globosa*, *Petunia hybrid*, *Nicotiana* and *Vicia faba* (Kaiser et al 1982). Diseases caused by TSV in India have been reported in at least 18 different field crops with some of the most severely affected including sunflower, peanut, okra and cucurbits (Bhat et al 2002; Prasada Rao et al 2000). Atleast 16 naturally infected alternative weed hosts have also been identified as TSV hosts in India (Prasada Rao et al 2003). Weeds found near crops with the highest incidence of TSV were *Ageratum conyzoides*, *Corchorus trilocularis* and *Parthenium hysterophorus*. Parthenium was a non-symptomatic host and was considered the principal source of TSV infected pollen leading to disease epidemics in nearby crops (Prasada Rao et al 2003).

Particle morphology and serology

Virions are isometric and non-enveloped about 27-35nm in diameter, round in profile and without a conspicuous capsomere arrangement (Brunt et al 1996). Tobacco streak virus (TSV) is the type member of the genus Ilarvirus (family: Bromoviridae) which have a positive sense single stranded RNA genome with a total length of approximately 8,600 nucleotides (nt), divided into 3 linear segments designated as RNA-1, -2 and -3. The 1a (viral replicase) protein is encoded by RNA-1, the 2a (RNA-dependent RNA polymerase) protein and the 2b protein are encoded by RNA-2. Two proteins are encoded by RNA-3, the 3a cell-to-cell movement protein and the 3b virus coat protein. The coat protein is translated from a sub-genomic RNA-4 which is derived from RNA-3. The first complete nucleotide sequence of RNA 3 for a strain of TSV was reported by Cornelissen et al (1984) with a total length of 2,205 nt. The complete RNA 1 and RNA 2 sequences were later reported for the same strain with total lengths of 3,491 nt and 2,926 nt, respectively (Scott et al 1998). All RNA segments

are encapsidated separately in quasi-isometric to bacilliform virions about 30 nm in diameter and 20 to 55 nm in length (Fauquet et al 2005).

Abtahi and Habibi (2008) studied the host range and characterization of Tobacco streak virus isolated from lettuce in Iran. TSV was tested in these plants by DAS-ELISA. Vemana et al (2014) first reported Tobacco streak virus infecting pigeon pea (*Cajanus cajan*) in India. DAC-ELISA was done and In direct antigen coating (DAC)-ELISA, all the infected pigeon pea and cowpea leaf samples were positive to a polyclonal antiserum specific to *Tobacco streak virus* (TSV).

Vemana and Jain (2011) established new experimental hosts of Tobacco streak virus and absence of true seed transmission in leguminous hosts. They detected groundnut seed infection with TSV for the first time by DAC-ELISA using whole seed. The seed infection ranged from 18.9 to 28.9 percent among the seeds collected from naturally infected and sap inoculated groundnut varieties.

Molecular detection and characterization

Tzanetakis et al (2004) studied a new virus previously thought to be tobacco streak virus isolates from *Fragaria* and *Rubus* by using RT-PCR. The studies indicated that strawberry and *Rubus* may not be the hosts of TSV. Furthermore, RT-PCR was used for detection of TSV in small fruit crops but was unsuccessful by using primers developed against the type isolate of TSV. An isolate from strawberry was however cloned and sequenced. Analysis of RNA 3 and part of RNA 2 revealed about 70 percent nucleotide sequence identity of this isolate with TSV.

Ho and Tzanetakis (2012) detected blackberry chlorotic ringspot virus, strawberry necrotic shock virus and blackberry yellow vein-associated virus from strawberry. TaqMan quantitative real time PCR assays were used with absolute quantification for the detection of all these viruses. The results concluded that the assays are attractive tools in blackberry virus diagnosis.

Li and Yang (2011) reported Strawberry necrotic shock virus in China for the first time. Reverse transcription (RT)-PCR was performed with the primer pair CPbeg F/CPend R (2). Amplified DNA fragments with the predicted size were obtained only in one strawberry sample which was further cloned and sequenced. The sequence (GenBank Accession No. HQ830017) was closely related and highly homologous (89.7 to 98.5 percent identity) to that of viral isolates (GenBank Accession Nos. AY363228-AY363242) from *Fragaria* and *Rubus* spp.

Serological and molecular identification of tobacco streak ilarvirus infecting onion (*Allium cepa* L.) on commercial fields in southern India was conducted by Asadhi et al (2016). The TSV infection was confirmed by DAC-ELISA using TSV antiserum. The CP gene was further amplified by using TSV coat protein primers. Total RNA from Elisa positive samples used for cDNA synthesis by using RT-PCR resulted in a single band of expected size (~717 bp) corresponding to the CP gene.

Sarovar et al (2010) detected tobacco streak virus by immunocapture-reverse transcriptase-polymerase chain reaction and conducted molecular variability analysis of a part of RNA3 of sunflower, gherkin, and pumpkin from Andhra Pradesh, India. The coat protein-coding and 3'UTR regions of the RNA3 of Tobacco streak virus infecting sunflower, gherkin, and pumpkin with the characteristic symptoms of necrosis were amplified by IC-RT-PCR. IC-RT-PCR was found to be more sensitive than RT-PCR, and hence could be used in quarantine programmes.

Cloning of Tobacco streak virus

Cornelissen et al (1984) sequenced the complete nucleotide of tobacco streak virus 3. Double-stranded cDNA of *in vitro* polyadenylated tobacco streak virus (TSV) RNA was cloned and sequenced. The complete primary structure of 2,205 nucleotides revealed two open reading frames flanked by a leader sequence of 210 bases, an inter-cistronic region of 123 nucleotides and a 3'-extracistronic sequence of 288 nucleotides. Although the coat proteins of alfalfa mosaic virus (AIMV) and TSV were found to be functionally equivalent in activating their genomes, no homology between the primary structures of those two proteins was detectable.

Kumar et al (2007) reported the natural occurrence of Tobacco streak virus in niger (*Guizotia abyssinica*) from India. The resulting ~720-bp amplicon corresponding to the CP gene of TSV was cloned into pGem-T vector (Promega, Madison, WI) and sequenced. The resulting sequence of the TSV-niger isolate (TSV-NG) comprised of 717 nucleotides encoding 238 amino acid residues of the viral coat protein (Gene Bank Accession No. DQ864458). Comparison of the sequence with those of other TSV CP gene indicated 98.5 to 99.3 percent nucleotide and 97.9 to 99.6 percent amino acid sequence identity with TSV isolates from India.

Production of virus free planting material

With an increase in demand for healthy planting material of strawberry, there has been a sharp increase in the incidence of virus diseases affecting this crop. The last decade has

witnessed a significant work towards the detection and characterization of viruses infecting strawberry. As of now, the number of strawberry viruses has more than doubled compared to the number known at the turn of the century (Martin and Tzanetakis, 2006). Another significant change witnessed in the last few years is the presence of multiple virus infections that synergistically cause severe reduction in the fruit yield and deterioration of quality in strawberry planting material. It is an important commercial fruit grown in different parts of the world with a great potential for export when raised from virus indexed mother plants, strawberry plants can lead to higher yield and production of elite planting material. Virus indexed mother plants of strawberry thus raised will lead to the development of a sound certification programme in this commercially important crop.

Biotechnological approaches, primarily meristem tip culture holds the future for producing virus free strawberry plants with desirable horticultural traits. A number of workers have successfully demonstrated the protocol for meristem tip culture in strawberry (EPPO\CABI, 1991).

Mahmoud et al (2017) worked on the tissue culture techniques for clonal propagation, viral sanitation and germplasm improvement in strawberry (*Fragaria x ananassa* Duch.). It was concluded that in-vitro propagation of strawberry finds its main practical application in mass production of virus-free plants and meristem tip culture alone or combined with thermotherapy has been utilized to eliminate viruses from infected strawberries.

Nishi and Ohsawa (1973) demonstrated the commercial application of meristem tip culture in strawberry and concluded that conventionally only one plantlet can be obtained from one meristem. On the contrary, more than 50 plantlets can be secured in succession from one meristem by callus culture and the virus disease can be entirely eliminated from the plantlets raised through this system. Meristem tip culture can be expected to serve as a useful technique for the establishment of a virus-free plant production system. The major disadvantage with meristem tip culture is, however, the lack of trained scientific manpower especially in the third world countries (Nichodemus 2017) where the resources are limited.

Soil borne diseases, particularly nematode transmitted nepoviruses cause severe production losses in strawberry culture. Generally, an ideal rooting media combination can provide sufficient porosity, aeration and water holding capacity which can enhance crop growth and productivity (Ghazvani et al 2007; Jafarnia et al 2010; Hassan et al 2011). Soilless media combinations in particular, can additionally reduce soil borne diseases and prevent the spread of nematode transmitted viruses. Use of soilless substrates such as perlite, vermiculite and

cocopeat is fast emerging as an alternative strategy for the production of virus free planting material of strawberry particularly, those transmitted by nematode vectors namely SLRSV, TRSV and RRSV as soilless substrates discourage the transmission of nematode transmitted viruses besides improving the fruits quality parameters such as berry weight, size and total soluble solids (Shylla et al 2017). In this study, different substrates such as soil, FYM, perlite and cocopeat were compared in various combinations to study their effect on nematode transmitted viruses. Based on O.D. values obtained in DAS-ELISA, plants of strawberry cv. Chandler were found to be free from infection of nematode transmitted viruses namely SLRSV, TRSV and RRSV when produced on perlite, a soilless substrate. The studies also revealed that the plants raised on substrates such as soil, FYM and Cocopeat tested positive for all the viruses, thereby indicating that all these substrates did not help in checking the spread of nematode transmitted viruses in strawberry plants.

Conclusion

It is evident from the critical review of the major viruses infecting strawberry that the virus diseases are the most prominent limiting factor in the production of certified virus- free planting material of strawberry. Key to success for achieving this goal lies in the development of a sound certification scheme that has the aim of providing strawberry plants which are true-to-type, free from virus diseases and substantially free from other pests.

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