Serological Characterization of Major Viruses infecting 
Strawberry in India

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ABSTRACT

Strawberry (Fragaria x ananassa Duchesne) is one of the most important commercial 
plants representing Rosaceae family. Like other fruit crops, strawberry is also known to be 
susceptible to diseases caused by fungi, bacteria and viruses. Since strawberry is 
propagated through runners year after year, the mother stocks often get infected with 
viruses pass on these viruses to the next progenies. Reports indicate that more than 30 
viruses and phytoplasmas infect strawberry naturally. The most important viruses are 
strawberry latent ringspot virus (SLRSV), tobacco ringspot virus (TRSV), raspberry 
ringspot virus (RRSV), tobacco streak virus (TSV) and strawberry Mild yellow edge virus 
(SMYEV). A detailed investigation was conducted to characterize the major viruses 
infecting strawberry on serological basis using both Direct Antigen Coating (DAC) and 
Double Antibody Sandwich (DAS) forms of ELISA. The studies found DAS-ELISA to be 
more precise than DAC-ELISA for serological characterization of strawberry viruses.

Keywords
Serological Characterization, Strawberry, DAS-ELISA

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Introduction

Strawberry is an important fruit crop of India and its commercial production is possible in 
temperate and sub-tropical areas of the country. It is one of the most favourite fruits 
of the temperate world. Strawberry cultivation is gaining more popularity in the plains of 
Punjab and northern states due to higher yields resulting in lucrative returns. In India, total 
area and production under strawberry is 1000 ha and 5000MT, respectively and in Himachal 
Pradesh, strawberry is cultivated over an area of 55 ha with a production of 559MT (NHB, 
2017). There are reports indicating that about 30 viruses infect strawberry crop under natural 
condition. 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). A detailed and critical study of the symptoms observed on strawberry 
plants infected with viruses revealed a combination of wide range of symptoms 
extending from mild mottle to cupping of leaves with majority of the plants exhibiting 
puckering, necrotic rings, ringspots, marginal necrosis, reddening of leaves, oak leaf pattern, 
leaf deformation and small sized misshapen fruits (Fig. 1–9). Virus diseases are a major 
limiting factor in the production of certified virus- free planting material of strawberry.
Serological characterization of viruses infecting strawberry will help in the production of virus indexed planting material of strawberry which in turn will go a long way for developing a sound certification programme in this commercially important crop.

Materials and Methods

Planting material

Leaves from strawberry cv. Chandler with virus like symptoms were collected during the cropping season of 2017 from HRTS & KVK kandaghat, Solan and IARI Regional Station Dhanda Farm, Shimla.

ELISA detection

DAC (Direct Antigen Coating) and DAS (Double Antibody Sandwich) forms of ELISA were used for the detection of viruses in the test samples. The procedure for conducting DAC-ELISA and DAS-ELISA is presented in the following paragraphs.

DAC-ELISA

In case of DAC-ELISA, the modified procedure given by Handa and Bhardwaj (1994) was followed. Wells of the microtitre plate (NUNC maxisorp certified micro plates) except those of the top and bottom rows and rows on the extreme left and right, were filled with 100 µl aliquots of infected sap (each sample in duplicate) diluted in 1X extraction buffer (1: 10 ratio w/v) besides positive and negative control wells. The plate was incubated in humid box for 2 hours at 37°C. The contents of the plate were removed by shaking out the plate over the washbasin. The wells were filled with 1X PBS-Tween and kept for 2 minutes with gentle shaking. The plate was emptied and filled again with PBS-Tween. The washing was repeated three times. The coating antibodies were diluted in 1X coating buffer (1:500 ratio v/v). The wells were filled with 100 µl aliquots of antibodies. The plates were incubated for 2 hours at 37°C. The washing steps were repeated as mentioned above. The alkaline phosphatase (ALP) conjugated goat-antirabbit IgG were filled in each well with 100µl aliquots after diluting it in 1X ECI (enzyme conjugated immunoglobulin) buffer at a ratio of 1: 200 (v/v). The plates were incubated in humid box for 90 minutes at 37°C. Washing was done as mentioned above. The p-nitrophenyl phosphate (pNPP) substrate was dissolved in 1X substrate buffer by dissolving 5 mg pNPP tablet in 5ml of 1X substrate buffer. Each well was filled with 100 µl aliquots of substrate. The plates were kept in humid box in the dark condition at room temperature until a yellow colour was clearly visible in the positive control (usually between 30 minutes to 60 minutes). If desired, the reaction was stopped by adding 50 µl of 3M NaOH to each well. The results were assessed by measurement of the absorbance value of the hydrolysed substrate (p-nitrophenyl) at 405 nm wavelength in a microtitre plate reader (Micro Scan MS 5608A, Electronics Corporation of India Limited, Hyderabad). The results of ELISA for the detection were interpreted as per Dijkstra and Jager (1998) as samples were considered infected when their absorbance values (A405 nm) exceeded two times the mean value of respective healthy control samples.

DAS-ELISA

The protocol given by Clark and Adams (1977) was followed for conducting DAS-ELISA tests. Wells of the microtitre plate (NUNC maxisorp certified microplates) except those of the top and bottom rows and rows on the extreme left and right, were filled with 100 µl aliquots of coating antibodies diluted in 1X coating buffer (1:500 ratio v/v). The plate was incubated in humid box for 4 hours at 37°C. The coating antibody suspension was removed by shaking out the
plate over the wash basin. The wells were filled with 1X PBS-Tween and kept for 2 minutes with gentle shaking. The plate was emptied and filled again with PBS-Tween. The washing was repeated three times. The test samples were ground in 1X extraction buffer (1:10 ratio w/v). All coated wells were filled with 100 µl aliquots of test sample (each sample in duplicate) besides positive and negative control wells. The plates were incubated in humid box overnight at 4±1˚C. The washing steps were repeated as mentioned above. The alkaline phosphatase (ALP) conjugate antibodies were filled in each well with 100 µl aliquots after diluting it in 1X conjugate buffer at a ratio of 1:500 (v/v). The plate was incubated in humid box for 2 hours at 37˚C. The washing was done as mentioned above. The p-nitrophenyl phosphate (pNPP) substrate was dissolved in 1X substrate buffer. Each well was filled with 100 µl aliquots of substrate. The plates were kept in humid box in dark condition at room temperature until a yellow colour was clearly visible in the positive control (usually between 30 to 60 minutes). If desired, the reaction was stopped by adding 50 µl of 3M NaOH to each well. The results were assessed in the same manner as for DAC-ELISA.

Results and Discussion

In order to characterize the viruses infecting strawberry on serological basis, DAC-ELISA was performed initially to broadly identify the virus groups to which the causal viruses belong.

For this purpose, antibodies against viruses representing each of the three genera namely Nepovirus, Ilarvirus and Potexvirus were used. It is evident from the data in Table 1 that TRSV (a member of the genus Nepovirus), TSV (a member of Ilarvirus) and SMYEV (a member of Potexvirus) were found to be prevalent in all the isolates collected from the two locations, Kandaghat and Dhanda.

These results were further confirmed in DAS-ELISA using antibodies against these three viruses in addition to SLRSV and RRSV both members of the genus Nepovirus. Strawberry virus isolates collected from Kandaghat had the highest OD value for all the virus genera for nepoviruses 1.182, Ilarviruses 1.736 and for potexviruses 1.386 as compared to the virus isolates collected from Dhanda.

A number of workers have found DAC-ELISA to be very efficient in the detection of nepoviruses, ilarviruses and potexviruses in different crops (Fromme et al., 1927; Abtahi and Habibi, 2008; Vemana and Jain, 2011; Sharma et al., 2018; Hepp and Martin, 1991; Conci et al., 2009; EPPO\CABI, 1996). DAC-ELISA results in the present study are in line with these findings and have also proved to be very efficient as it detected all three viruses successfully.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Antibody</th>
<th>Locality</th>
<th>Mean OD at 405nm</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Test sample</td>
</tr>
<tr>
<td>Nepovirus</td>
<td>TRSV</td>
<td>Kandaghat</td>
<td>1.182(+)</td>
</tr>
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<td></td>
<td></td>
<td>Dhanda</td>
<td>0.602(+)</td>
</tr>
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<td>Ilarvirus</td>
<td>TSV</td>
<td>Kandaghat</td>
<td>1.736(+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dhanda</td>
<td>1.227(+)</td>
</tr>
<tr>
<td>Potexvirus</td>
<td>SMYEV</td>
<td>Kandaghat</td>
<td>1.386(+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dhanda</td>
<td>0.168(+)</td>
</tr>
</tbody>
</table>

Table 1: DAC-ELISA detection of strawberry viruses
### Table 2 DAS-ELISA detection of strawberry viruses

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Locality</th>
<th>Mean OD at 405nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test sample</td>
</tr>
<tr>
<td>SLRSV</td>
<td>Kandaghat</td>
<td>0.405 (+)</td>
</tr>
<tr>
<td></td>
<td>Dhanda</td>
<td>0.449 (+)</td>
</tr>
<tr>
<td></td>
<td>Nauni</td>
<td>0.206 (+)</td>
</tr>
<tr>
<td>TRSV</td>
<td>Kandaghat</td>
<td>0.403 (+)</td>
</tr>
<tr>
<td></td>
<td>Dhanda</td>
<td>0.449 (+)</td>
</tr>
<tr>
<td></td>
<td>Nauni</td>
<td>0.244 (+)</td>
</tr>
<tr>
<td>RRSV</td>
<td>Kandaghat</td>
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</tr>
<tr>
<td></td>
<td>Dhanda</td>
<td>0.575 (+)</td>
</tr>
<tr>
<td></td>
<td>Nauni</td>
<td>0.398 (-)</td>
</tr>
<tr>
<td>TSV</td>
<td>Kandaghat</td>
<td>1.078 (+)</td>
</tr>
<tr>
<td></td>
<td>Dhanda</td>
<td>0.690 (+)</td>
</tr>
<tr>
<td></td>
<td>Nauni</td>
<td>0.206 (+)</td>
</tr>
<tr>
<td>SMYEV</td>
<td>Kandaghat</td>
<td>0.378 (+)</td>
</tr>
<tr>
<td></td>
<td>Dhanda</td>
<td>0.217 (-)</td>
</tr>
<tr>
<td></td>
<td>Nauni</td>
<td>0.147 (+)</td>
</tr>
</tbody>
</table>

**FIG.1 Deformed fruits from infected plants**
FIG.2 & FIG.3 Healthy leaves and fruits of strawberry

FIG.4 Severe leaf deformity

FIG.5 Leaf crinkling and marginal necrosis
FIG. 6 Necrotic ringspots

FIG. 7 Savoying

FIG. 8 Cupping and leaf deformation

FIG. 9 Typical ringspots
For more accurate and specific detection of strawberry viruses, three viruses namely SLRSV, TRSV and RRSV representing the genus nepovirus were used for DAS-ELISA based serological characterization of the causal viruses. Additionally, antibodies against TSV (*Ilarvirus*) and SMYEV (*Potexvirus*) were also used for exact identification of the virus. Data set out in Table 2 indicate the presence of all the viruses for which antibodies were used for DAS-ELISA based characterization in the virus isolates collected from the all localities except for the SMYEV (*Potexvirus*) at Dhanda where concentration of the virus was found to be below detectable limits. DAS-ELISA was thus considered to be an effective and efficient method for the detection of strawberry viruses.

A number of workers have found DAS-ELISA to be very efficient in the detection of nepoviruses, ilarviruses and potexviruses in different crops (Sharma et al., 2018; Bargen et al., 2015; Tang et al., 2013). DAS-ELISA results in the present study are in line with these findings and have also proved to be very efficient as it detected all three viruses successfully.

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**References**


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