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Correlating Field Symptoms of PNRSV with DAC and DAS-ELISA based Serological Detection

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A B S T R A C T

Field surveys were conducted between 2015-2018 to determine the occurrence and distribution of PNRSV infecting peach in 40 orchards representing major peach producing districts of Himachal Pradesh Symptomatic trees typical of viral infection present in all the orchards surveyed exhibited chlorotic spots, necrotic rings, leaf deformation and shot hole symptoms with an incidence ranging from 1 to 47 percent. DAC-ELISA confirmed the association of an *Ilarvirus* with peach cv. July Elberta infected plants and DAS-ELISA test further conclusively proved that the *Ilarvirus* associated with infected plants was PNRSV.

Introduction

India produces considerable quantity of all stone fruits including peach, plum, apricot and cherry. These are mainly grown in the North-Western Indian states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand.

Besides, North-Eastern hilly region, comprising of the states of Arunachal Pradesh, Nagaland, Meghalaya, Manipur and Sikkim also grows stone fruits on a limited scale. Due to introduction and adaptation of low chilling cultivars, peach and plum are also now being grown commercially in certain areas of the north Indian plains. Among all stone fruits, peach is the most important in terms of production and area under cultivation.

Routine surveys conducted for recording the natural occurrence of virus diseases in different peach growing areas of Solan, Shimla, Sirmour and Kullu district of Himachal Pradesh revealed the presence of prunus necrotic ring spot disease with typical viral etiology. Prunus necrotic ring spot virus, is a member of the genus *Ilarvirus* belonging to the family *Bromoviridae* and thought to be transmitted by vegetative propagation. In India, natural occurrence of prunus necrotic ring spot disease was earlier confined to roses and cherries but it is now considered to be a serious emerging problem in the peaches and pose a threat to its cultivation in the state. Serological detection of a virus infecting peaches in Himachal Pradesh revealed the prevalence of PNRSV (Chandel *et al.,* 2013;

Kapoor and Handa 2017a). Serological and molecular methods of detection by employing enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) are widely used all over the world in different virus indexing and certification programmes of peach (Mink 1992). However, for virus indexing on mass scale, ELISA is widely employed because of its rapidity, accuracy and sensitivity.

Survey

Surveys were conducted during active growing seasons of 2015, 2016, 2017 and 2018 in different peach growing districts of Himachal Pradesh to record the occurrence, distribution and incidence of viral diseases in peach orchards situated at different locations. Percent disease incidence was calculated after recording observations on symptoms as per the formula given below:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Marking of symptomatic and disease free trees

Peach trees existing in the surveyed orchards were critically observed for recording the symptoms on each cultivar. Symptomatic trees of different cultivars were marked for recording the observations on the symptoms as well as serological detection of viruses by following enzyme-linked immunosorbent assay (ELISA) technique. Alkaline phosphatase (ALP) based direct form of double antibody sandwich (DAS) ELISA was used to detect the infection of PNRSV by following Clark and Adam (1977). The procedures of detection are described in this chapter. Additionally, true to type, good quality, apparently healthy trees of peach cv. July Elberta were marked in the selected orchard for serological indexing by using ELISA.

Serological detection of virus

Leaf samples exhibiting typical symptoms were drawn from the marked plants in the selected orchard and brought to the laboratory in separate polythene bags in an ice box so as to keep the leaf samples fresh for serological detection of prunus necrotic ring spot virus (PNRSV) through Alkaline phosphatase (ALP) based DAC (direct antigen coating) and DAS (double antibody sandwich) forms of ELISA as per the procedure given below:

Serological detection through DAC-ELISA

ALP based DAC form of enzyme-linked immunosorbent assay (ELISA) was used to detect the viruses in test samples as per the protocol proposed by Handa and Bhardwaj (1994). Young leaves exhibiting typical viral symptoms were harvested and brought to the laboratory in polythene bags. Leaf extract of each sample in extraction buffer (1:20 w/v) was prepared by crushing the leaves in a tissue homogenizer (SEDIAG, France) and used for coating the wells of microtitre ELISA plates. The wells of microtitre plate were filled with 200 µl aliquots of test sample. The coated plates were kept in a humid box and incubated overnight at 4±1 ^{0}C. The plates were washed by removing suspension of samples by vigorously shaking out the plate over the wash basin. The wells were filled with 1X PBS-Tween and kept for 2 minutes with gentle shaking and emptied the plate and in this way the washing was repeated 3 times or simply by washing in ELISA plate washer. Then the wells were filled with 200 µl aliquots of coating antibodies diluted in 1X coating buffer. The plate was incubated in humid box for 2 hours at 37^{0}C the washing of the plates was done as mentioned before. ALP labeled goat anti-rabbit IgG conjugate (GeNei,

Bangalore) were filled in each well with 200 µl aliquots after diluting in 1X conjugate buffer. The plate was incubated in a humid box for 2 hours at 37^0C. The washing of plate was done as mentioned previously. The pNPP substrate was dissolved in 1X substrate buffer (5 mg pNPP tablet in 5 ml of substrate buffer) under dark conditions. Each well was filled with 200 µl aliquots of substrate. The plate was kept in humid box in dark at room temperature. The plate was incubated until a yellow colour was visible in the positive controls (usually between 30 and 90 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 MS5605A, Electronic Corporation of India Limited).

Leaf samples from fifty symptomatic trees were collected from the selected orchard surveyed in the experimental farm of the Departments of Fruit Science of Dr YS Parmar University Nauni, Solan for serological detection of two ilarviruses namely apple mosaic virus (ApMV) and prunus necrotic ring spot virus (PNRSV) through DAC-ELISA since these two viruses have been reported to be associated with stone fruits in India. For all serological tests of ELISA, immunoreagents, buffers, positive and negative control supplied by BIOREBA AG (Switzerland) were used as per the instructions issued by the supplier.

Serological detection through DAS-ELISA

Alkaline phosphatase based direct double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect the virus as per the protocol of Clark and Adams (1977) with slight modifications. The detailed procedure used for DAS-ELISA based serological detection of PNRSV is described hereunder: In DAS-ELISA, wells of the microtitre plate except those of the top and bottom rows on the extreme left and right were first filled with 200 µl coating antibody. The plate was incubated in humid box for 4 hours at 30 ^{0}C. The coating antibody suspension was removed by vigorously shaking out the plate over the wash-basin. The wells were filled with 1X PBS-Tween and kept for 2 min with gentle shaking emptied the plate and filled again with PBS-Tween. The washing was repeated three times or by washing in ELISA plate washer. The leaf extract from the test samples were prepared in buffer. All coated wells were filled with 200 µl aliquots of test samples (each sample at least in duplicate) besides positive control and negative control wells. The plates were incubated in humid box overnight at 4 ± 1^0C. The washing step was repeated as mentioned above.

The specific alkaline phosphatase (ALP) based conjugated antibodies were filled in each well with 200 µl aliquots. The plate was incubated in humid box for 5 hours at 30^0C. The 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 5 ml of 1X substrate buffer under the dark conditions. Each well was filled with 200 µl aliquots of substrate. The plates were kept in humid box in the dark condition at room temperature after giving a brief incubation of 15 minutes at 30^0C. The plates were incubated until a yellow colour was clearly visible in the positive controls (usually between 30 and 90 minutes). If desired the reaction was stopped by adding 50 µl of 3M NaOH to each well. The results were assessed either by measurement of the absorbance value of the hydrolyzed substrate (p-nitrophenyl) at 405 nm wavelength in a microtitre/(ELISA) plate reader (Micro Scan MS5605A, Electronic Corporation of India Limited) or through visual screening.

Leaf samples from twenty symptomatic trees which were found positive in DAC-ELISA were collected for further serological detection through DAS-ELISA in selected orchard surveyed in the experimental farm of the Departments of Fruit Science, Dr YS Parmar University Nauni, Solan. For all serological tests of ELISA, immunoreagents, buffers, positive and negative control supplied by BIOREBA AG (Switzerland) were used as per the instructions issued by the supplier. The results in both DAC and DAS forms of ELISA for detection of PNRSV were interpreted by following Lemmetty (1988) and Dijkstra and Jager (1998) as samples were considered infected when their OD values at 405nm exceeded two times the mean values of respective healthy and negative control samples.

Occurrence, distribution and incidence of viral diseases in major peach growing districts of Himachal Pradesh

Field surveys were conducted between 2015-2018 to determine the occurrence and distribution of PNRSV infecting peach in 40 orchards representing major peach producing districts of Himachal Pradesh (Table 1). Observations on incidence recorded are presented in (Table 2a, b. c and d). Owing to its latent nature, symptoms of PNRSV are largely masked.

Some of the typical symptoms were however recorded during specific period of the year on major cultivars like July Elberta, Glo Haven and Sun Haven growing in several orchards in Solan, Sirmour, Shimla and Kullu districts. Predominant symptoms prevalent in most of the cultivars observed were leaf damage in the form of mosaic, chlorotic spots, necrotic rings, shot holes, mid vein distortion and oak leaf pattern on leaves (Plate I). Such type of symptoms have been reported to be associated with peach trees infected with PNRSV by a number of workers (Fulton 1970; Wells *et al.*, 1986; Brunt *et al.*, 1996; Hammond 2011; Almaraz *et al.*, 2014; Winkowska *et al.*, 2016; Kapoor and Handa 2017a).

Data presented in Table 2 (a, b, c and d) clearly indicate the prevalence of PNRSV infection in all the orchards surveyed with an incidence ranging from 2 to 17 percent in Shimla district, 5 to 18 percent in Kullu district, 4 to 28 percent in Solan district and 5 to 37 percent in Sirmour district of Himachal Pradesh. In Shimla district, maximum incidence of PNRSV was recorded at NBPGR Phagli (17 percent) followed by IARI regional station Dhanda with an incidence of 15 percent whereas minimum incidence of 2 percent was recorded at Dhami.

In Kullu district, maximum incidence of PNRSV was recorded at Manali (18 percent) followed by Seo Bagh (15 percent) and Sharabhai recorded the minimum disease incidence (5 percent). Maximum incidence was recorded at Patta Mehlog (28 percent) in Solan district which was followed by Deothi with an incidence of (15 percent) and Kuthar had minimum incidence of 4 percent. In Sirmour district, maximum incidence of 37 percent was recorded at Sanaura followed by Ratoli (23 percent) whereas Phagu recorded minimum incidence of 5 percent.

Recent studies conducted in different parts of the world on incidence levels of PNRSV revealed a huge variation. Incidence of PNRSV was reported to be 60 percent in Georgia (Wells *et al.*,1986); 25 percent in Turkey (Gumus *et al.*, 2007); 70 and 100 percent in South Carolina (Scott 2014); 30 percent in Canada (Pallas *et al.*, 2012); 25 percent in Mexico (Almaraz 2008); 32 percent in Saudi Arabia (Alhudaib and Rezk 2011); 15 percent in Central Bohemia, Czech Republic (Winkowska *et al.*, 2016) and 18 percent in India (Kapoor and Handa 2017a).

Plate.1 Symptoms of PNRSV on peach cv. July Elberta leaves

Diffused chlorotic spots

Deformed leaves

Necrotic rings

Oak leaf pattern

Mid vein distortion

Table.1 Different peach growing areas selected for survey

District	Area
Shimla	IARI regional station Dhanda, NBPGR Phagli, Suni, Basantpur, Dhami, Shoghi, Jathiya Devi, Pujarli, Fagu, Gumma
Kullu	Bajaura, Seo Bagh, Manali, Panarsa, Gadsa, Sainj Valley, Sharabhai, Jhiri, Shamshi, Raison
Solan	Dr YS Parmar University Nauni, Deothi, HRTS & KVK Kandaghat, Jatoli, Kuthar, Patta Mehlog, Sadhupul, Sabathu, Jaunaji, Chail
Sirmour	Pabyana, Habban, Kotli, Ratoli, PCDO Gauda, Sanaura, Bhanat, Phagu, Shannaghat, Kwagdhar

Table.2a Incidence of PNRSV on peach trees in different orchards of Shimla district

Orchard No.	Location	Incidence (%)
Orchard-1	IARI regional station Dhanda	15
Orchard-2	NBPGR Phagli	17
Orchard-3	Suni	09
Orchard-4	Basantpur	05
Orchard-5	Dhami	02
Orchard-6	Shoghi	11
Orchard-7	Jathiya Devi	04
Orchard-8	Pujarli	10
Orchard-9	Fagu	12
Orchard-10	Gumma	04

Table.2b Incidence of PNRSV on peach trees in different orchards of Kullu district

Orchard No.		Location	Incidence (%)
Orchard-1		Bajaura	12
Orchard-2		Seo Bagh	15
Orchard-3		Manali	18
Orchard-4		Panarsa	10
Orchard-5		Gadsa	07
Orchard-6		Sainj Valley	11
Orchard-7		Sharabhai	05
Orchard-8		Jhiri	10
Orchard-9		Shamshi	07
Orchard-10		Raison	11

Table.2c Incidence of PNRSV on peach trees in different orchards of Solan district

Orchard No.	Location	Incidence (%)
Orchard-1	Dr YSP University Nauni	13
Orchard-2	Deothi	15
Orchard-3	HRTS & KVK Kandaghat	08
Orchard-4	Jatoli	05
Orchard-5	Jaunaji	10
Orchard-6	Kuthar	04
Orchard-7	Patta Mehlog	28
Orchard-8	Sadhupul	10
Orchard-9	Sabathu	06
Orchard-10	Chail	11

Table.2d Incidence of PNRSV on peach trees in different orchards of Sirmour district

Orchard No.	Location	Incidence (%)
Orchard-1	Pabyana	11
Orchard-2	Habban	10
Orchard-3	Kotli	07
Orchard-4	Ratoli	23
Orchard-5	PCDO Gauda	17
Orchard-6	Sanaura	37
Orchard-7	Bhanat	14
Orchard-8	Phagu	05
Orchard-9	Shanaghat	11
Orchard-10	Kwagdhar	13

Table.3 Serological detection of Ilarviruses (PNRSV or ApMV) in the selected orchard through DAC-ELISA

Tree No.	Symptoms	OD Value (A_{405}nm)/ (Serological Reaction)	
		PNRSV	ApMV
1	Mosaic, chlorotic spots	0.102 (-)	0.009 (-)
2	Mosaic, necrotic rings, shot hole	0.333 (+)	0.267 (+)
3	Mosaic, mid vein distortion	0.008 (-)	0.123 (-)
4	Chlorotic spots, mid vein distortion	0.144 (-)	0.156 (-)
5	Mosaic, necrotic rings, shot hole, oak leaf pattern	0.356 (+)	0.265 (+)
6	Mid vein distortion	0.036 (-)	0.020 (-)
7	Chlorotic spots, mid vein distortion	0.045 (-)	0.097 (-)
8	Mosaic, necrotic rings, shot hole	0.445 (+)	0.319 (+)
9	Chlorotic spots, mid vein distortion, necrotic rings	0.367 (+)	0.266 (+)
10	Mosaic, chlorotic spots	0.044 (-)	0.038 (-)
11	Mosaic, necrotic rings, shot hole	0.377 (+)	0.236 (+)
12	Chlorotic spots, mosaic, mid vein distortion	0.099 (-)	0.077 (-)
13	Mosaic, chlorotic spots, oak leaf pattern	0.353 (+)	0.256 (+)
14	Mid vein distortion, mosaic	0.056 (-)	0.087 (-)
15	Mosaic, chlorotic spots, necrotic rings	0.279 (+)	0.234 (+)
16	Chlorotic spots	0.061 (-)	0.054 (-)
17	Mosaic, mid vein distortion	0.178 (-)	0.163 (-)
18	Chlorotic spots, necrotic rings	0.301 (+)	0.259 (+)
19	Chlorotic spots, mid vein distortion	0.126 (-)	0.132 (-)
20	Mosaic, mid vein distortion	0.093 (-)	0.075 (-)
21	Mosaic, chlorotic spots, necrotic rings, mid vein	0.323 (+)	0.267 (+)
	Distortion		
22	Chlorotic spots, mid vein distortion	0.045 (-)	0.064 (-)
23	Mosaic, necrotic rings	0.146 (-)	0.137 (-)
24	Mosaic, necrotic rings, oak leaf pattern	0.280 (+)	0.258 (+)
25	Mosaic, chlorotic	0.068 (-)	0.074 (-)
26	Mosaic, necrotic rings, mid vein distortion	0.308 (+)	0.251 (+)
27	Chlorotic spots, necrotic rings	0.280 (+)	0.209 (+)
28	Mosaic, mid vein distortion	0.124 (-)	0.098 (-)
29	Mid vein distortion, chlorotic spots	0.142 (-)	0.128 (-)
30	Chlorotic spots, mid vein distortion	0.106 (-)	0.115 (-)
31	Mosaic, mid vein distortion, necrotic rings, oak leaf	0.278 (+)	0.234 (+)
	Pattern		
32	Mid vein distortion	0.053 (-)	0.065 (-)
33	Mosaic, chlorotic spots, mid vein distortion	0.098 (-)	0.049 (-)
34	Chlorotic spots, necrotic rings, oak leaf pattern, mid	0.297 (+)	0.269 (+)
	vein distortion		
35	Mosaic, chlorotic spots, necrotic rings	0.105 (-)	0.089 (-)
36	Mosaic, shot hole, necrotic rings, oak leaf pattern, mid	0.333 (+)	0.287 (+)

	vein distortion		
37	Chlorotic spots, mosaic, mid vein distortion	0.109 (-)	0.112 (-)
38	Mosaic, mid vein distortion	0.067 (-)	0.100 (-)
39	Chlorotic spots, shot hole, oak leaf pattern, mid vein	0.290 (+)	0.258 (+)
	Distortion		
40	Necrotic rings, chlorotic spots	0.103 (-)	0.068 (-)
41	Mosaic, necrotic rings, chlorotic spots	0.114 (-)	0.126 (-)
42	Mosaic, chlorotic spots, shot hole, mid vein distortion	0.312 (+)	0.276 (+)
43	Necrotic rings, mid vein distortion	0.095 (-)	0.076 (-)
44	Mid vein distortion, chlorotic spots	0.116 (-)	0.104 (-)
45	Chlorotic spots, necrotic rings, mid vein distortion	0.290 (+)	0.257 (-)
46	Chlorotic spots, necrotic rings, mid vein distortion	0.278 (+)	0.289 (+)
47	Chlorotic spots, necrotic rings	0.126 (-)	0.134 (-)
48	Mid vein distortion	0.067 (-)	0.059 (-)
49	Necrotic rings, oak leaf pattern, mid vein distortion	0.299 (+)	0.301 (+)
50	Mosaic, mid vein distortion	0.097 (-)	0.065 (-)

Table.4 Serological detection of PNRSV and ApMV in the selected orchard through DAS-ELISA

Tree No.	**Symptoms**	**O.D. Value A_{405}nm**	
		PNRSV	**ApMV**
1	Necrotic rings, oak leaf pattern	0.345 (+)	0.102 (-)
2	Chlorotic spots, necrotic rings	0.321 (+)	0.068 (-)
3	Shot hole	0.467 (+)	0.112 (-)
4	Necrotic rings, shot hole	0.328 (+)	0.079 (-)
5	Shot hole, necrotic rings, chlorotic spots	0.279 (+)	0.057 (-)
6	Shot holes, oak leaf pattern, mid vein distortion	0.378 (+)	0.146 (-)
7	Necrotic rings, shot hole, mid vein distortion	0.296 (+)	0.131 (-)
8	Mosaic, mid vein distortion	0.304 (+)	0.115 (-)
9	Chlorotic spots, necrotic rings, shot hole	0.386 (+)	0.102 (-)
10	Chlorotic spots, shot hole	0.265 (+)	0.098 (-)
11	Mosaic, necrotic rings, mid vein distortion	0.411 (+)	0.056 (-)
12	Chlorotic spots, mid vein distortion	0.260 (+)	0.109 (-)
13	Chlorotic spots, necrotic rings, mid vein distortion	0.312 (+)	0.089 (-)
14	Chlorotic spots, necrotic rings	0.294 (+)	0.111 (-)
15	Chlorotic spots, shot hole	0.285 (+)	0.102 (-)
16	Shot hole, oak leaf pattern, mid vein distortion	0.342 (+)	0.076 (-)
17	Necrotic rings, shot hole	0.279 (+)	0.132 (-)
18	Chlorotic spots, shot hole, mid vein distortion	0.342 (+)	0.126 (-)
19	Necrotic rings, mid vein distortion	0.299 (+)	0.094 (-)
20	Chlorotic spots, shot hole, mid vein distortion	0.345 (+)	0.106 (-)

Selection of orchard

Peach orchard located in the Experimental Farm of the department of Fruit Science, Dr YS Parmar University Nauni was selected for conducting the present studies. The orchard had young plants in the age group of 5-8 years and had only one variety of peach (July Elberta). The orchard was easily accessible from the laboratory which was favourable from the point of collecting samples and bringing them to the laboratory for further studies without causing any degradation of the samples, a major concern for PNRSV as the virus is a thermos-labile latent virus.

Serological detection of PNRSV

Leaf samples from the marked trees exhibiting typical viral symptoms in the selected orchard were collected and brought to the laboratory in separate polythene bags in an ice box. These samples were further serologically detected for the presence or absence of ilarviruses (PNRSV or ApMV) through the use of DAC-ELISA. Further confirmation of the exact identity of the virus was established through DAS-ELISA. The results pertaining to serological detection are presented in Tables 3 and 4.

Serological detection of ilarviruses in the selected orchard through DAC-ELISA

Alkaline phosphatase based DAC-ELISA was performed to detect the presence of ilarviruses (PNRSV or ApMV) in the leaf samples drawn from 50 symptomatic trees marked in the selected orchard. The data on OD values and serological reactions set out in Table 3 clearly indicate the positive detection of both ilarviruses in test samples. Positive samples had OD values that were at least double the OD value of negative control. ELISA plate depicting serological reaction of the virus isolates against ilarviruses in DAC-ELISA is presented in Plate II. DAC-ELISA results confirmed the presence of *Ilarvirus* in the orchard surveyed. However, OD values of the samples in respect of PNRSV was higher than that of ApMV. Therefore, further investigations were carried out using DAS-ELISA to confirm whether PNRSV was present alone or in combination with ApMV as mixed infection.

Data presented in Table 3 on the basis of OD values clearly indicate the presence of ilarviruses (PNRSV and ApMV) as some of the samples reacted positively for both PNRSV and ApMV in DAC-ELISA. Out of 50 symptomatic trees, only 20 were found to be infected with ilarviruses as indicated by the OD values. Tree No. 8 recorded the maximum OD values of 0.445 followed by Tree No. 11 and 9 with OD values of 0.377 and 0.367, respectively for PNRSV whereas in case of ApMV, Tree No. 8 recorded maximum OD value of 0.319 followed by Tree No. 49 and 46 with OD values of 0.301 and 0.289, respectively. DAC-ELISA has been used widely for the detection of plant viruses (Ramiah *et al.,* 2001; Sujitha *et al.,* 2015; Rageshwari *et al.,* 2017). Successful detection of ilarviruses under present studies is in conformity with the findings of Roussel *et al.,* (2004) and Salem *et al.,* (2004) who have also used DAC-ELISA for the detection of ilarviruses in temperate fruits. Besides, a number of other workers have also reported the association of ilarviruses with stone fruits on the basis of DAC-ELISA tests (Digiaro *et al.,* 1991; Mink 1992; Hammond 2003; Bashir *et al.,* 2017).

Serological detection of PNRSV and ApMV in the selected orchard through DAS-ELISA

Alkaline phosphatase based DAS-ELISA was performed for the detection of PNRSV and ApMV in the leaf samples drawn from 20

symptomatic trees marked in the selected orchard and confirmed positive in DAC-ELISA test. Observations recorded on OD values are presented in the Table 4.

It is evident from the data in Table 4 and Plate III that the *Ilarvirus* associated with test samples is PNRSV and not ApMV as leaf samples from all 20 symptomatic marked trees reacted positively with antibodies against PNRSV and failed to record positive reaction with antibodies against ApMV. Tree number 3 recorded the maximum OD value of 0.467 followed by tree number 11 and tree number 9 with OD values of 0.411 and 0.386, respectively.

Use of DAS-ELISA for detecting viruses in peach is widely practiced and findings of the present studies are in line with those of a number of workers who have observed DAS-ELISA to be an effective technique for detecting PNRSV in peach (Ghanem 2000; Myrtra *et al.*, 2001; Salem *et al.*, 2003; Virscerk and Mavric 2005; Syzndel *et al.*, 2006; Salam *et al.*, 2007; Almaraz *et al.*, 2008; Chandel *et al.*, 2013; Vemulapati *et al.*, 2014; Kapoor and Handa 2017 a and b). The present studies have helped in correlating field symptoms of PNRSV with spectrophotometry based results obtained in DAC and DAS forms of ELISA and the finding can be very useful in linking field studies with ELISA tests.

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