

DETECTION OF PRUNUS NECROTIC RINGSPOT VIRUS (PNRSV) IN APRICOT AND CHERRY AND ITS POTENTIAL ELIMINATION AT NURSERY STAGE

Chahat Kaundal^{1*}, Aishwarya Nayar², Shalaka Ahale³, Akanksha⁴, Aman Sharma⁵, Rajesh Kumar⁶, Anil Handa⁷

¹Young Professional-II at ICAR Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, chahat.k4128@gmail.com

²Office of District Project Management Unit, HP-CDP JICA ODA, Chambaghat, Solan, Himachal Pradesh-173213, aishwaryatwinkz96@gmail.com

³Assistant Professor, Plant Pathology, Chhatrapati Shivaji Maharaj Krishi Vigyan Sankul Kashti, Malegaon, Nashik, Maharashtra, shalakaahale135@gmail.com

⁴Assistant Professor, Plant Pathology, School of Agriculture, Sanskaram University, Jhajjar, Haryana, akanksha.walia12345@gmail.com

⁵Research Scholar DR YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh-173230, as1942207@gmail.com

⁶Horticulture Development Officer, Head office, Navbahar, Shimla, Himachal Pradesh -171002, bhagtaray17@gmail.com

⁷Professor and Head, DR YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh-173230, anilhandamsn@gmail.com

¹Corresponding Authors: Chahat Kaundal, chahat.k4128@gmail.com

ABSTRACT

Aims: Detection of prunus necrotic ringspot virus (PNRSV) in apricot and cherry and its potential elimination at nursery stage

Study design: Laboratory work

Place and Duration of Study: Lab experiments were conducted at Plant Virology Lab, department of Plant Pathology, DR YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during apricots and cherries active growing season of 2020–2021.

Methodology: Leaf samples exhibiting typical symptoms were collected from the marked plants in the selected nurseries and carried to the laboratory in separate polythene bags in an ice box to keep the leaf samples fresh for serological detection of ilarviruses via alkaline phosphatase (ALP) based direct antigen coating (DAC) and double antibody sandwich-enzyme linked immune sorbent assay DAS-ELISA

Results: Apple mosaic virus (ApMV) and Prunus necrotic ringspot virus (PNRSV) were found to be associated with the majority of apricot and cherry cultivars' notably symptomatic plants, as confirmed by DAC-ELISA. Out of total 10 plants each of apricot and cherry, 6 plants of apricot and 8 plants of cherry were infected with ilarviruses thus confirming the association of ilarvirus. Additionally, DAS-ELISA provided conclusive evidence that among ilarvirus, PNRSV was more common than ApMV in the infected apricot and cherries. Out of total 10 plants each of apricot and cherry, 6 plants of apricot and 8 plants of cherry were infected with PNRSV. Further, in order to select virus-free clones for apricot and cherry nurseries, serological indexing of evidently healthy plants of significant cultivars of major apricot and cherry cultivars was also carried out using DAS-ELISA. The results indicated that Harkot cultivar of apricot at Experimental Farm, Department of Fruit Science at Dr. YS. Parmar University of Horticulture and Forestry, Nauni, Krishi Vigyan Kendra, Kandaghat, PCDO Gaura and Kashyap nursery were found to be completely free from infection of PNRSV.

Conclusion: Given that apricots and cherries are frequently used as seedling rootstock for commercial cultivation of apricots and cherries in India, serological indexing is extremely significant. Since PNRSV is a graft transmissible pathogen, it spreads from rootstock to scion. If either the scion or the stock has an infection with PNRSV, the virus will spread throughout the plant. Therefore, it is crucial to choose healthy seedling rootstock. The significance of serological methods in cultivating robust cherry and apricot cultivars in the nurseries has been emphasized by the DAS-ELISA tests carried out in these investigations.

KEYWORDS: Apricot, cherry, prunus necrotic ringspot virus, virus free plants, enzyme linked immunosorbent assay

1. INTRODUCTION

Apricot (*Prunus armeniaca*) some wild relatives like chuli (*Prunus armeniaca*), paja (*Prunus cerasoides*), sweet cherries (*Prunus avium*) and sour cherries (*Prunus cerasus*) are a significant group of fruit crops grown across the world's temperate and sub-temperate zones. Both of these stone fruits are members of the Rosaceae family, with a basic chromosomal number of $n=8$ and a somatic chromosome number of $2n=16$. Commercial cultivation of apricots and cherries in India is limited to the mid hills of Himachal Pradesh, Jammu & Kashmir, Uttarakhand and certain hilly parts of the North-Eastern states of Arunachal Pradesh, Meghalaya, Manipur and Nagaland (Gangwar et al, 2008). Apricots and cherries are known to be a victim of several plant infections including fungus, bacteria and viruses, much like other horticulture crops. Viral infections in these fruits have a major impact on fruit's productivity and frequently take the lead

in reducing yield in nurseries. Apricots and cherries are infested with many viruses namely prunus necrotic ring spot virus (PNRSV), plum pox virus (PPV), apple mosaic virus (ApMV), apple chlorotic leaf spot virus (ACLSV), peach mosaic virus (PcMV) and prune dwarf virus (PDV) worldwide.

The two viruses that infest apricot and cherry nurseries the most in Himachal Pradesh include prunus necrotic ring spot virus (PNRSV) and apple mosaic virus (ApMV). Prunus necrotic ring spot virus is the most common virus prevalent in stone fruit nurseries. The detection of viruses during the early phases of plant growth is critical for designing a "Clean Stock Programme" that results in the production of virus-indexed plants (Handa and Kapoor, 2018). A range of molecular and serological techniques are used in stone fruit virus indexing and certification procedures, such as the dot immune binding assay (DIBA) and the enzyme-linked immunosorbent assay (ELISA) (Mink, 1992). However, due to its speed, precision, and sensitivity, ELISA is frequently used for viral indexing on a large scale.

2. MATERIAL AND METHODS

2.1 Surveys

Surveys were conducted during active growing season of the year 2021 in the stone fruit nurseries in district Shimla and Solan to record the occurrence, distribution and incidence of viral diseases in stone fruit nurseries. The percent disease incidence was calculated as per the following formula:

$$\text{Percent disease incidence} = \frac{\text{number of plants infected}}{\text{Total number of plants}} \times 100$$

2.2 Serological detection of ilarviruses

Leaf samples exhibiting typical symptoms were collected from the marked plants in the selected nurseries and carried to the laboratory in separate polythene bags in an ice box to keep the leaf samples fresh for serological detection of ilarviruses via alkaline phosphatase ALP based direct DAC and DAS-ELISA as described below.

2.2.1 DAC-ELISA

ALP based indirect DAC form of enzyme-linked immunosorbent assay (ELISA) was used to detect the virus(es) in test samples as per the protocol proposed by (Handa and Bharadwaj, 1994). Leaf extract of each sample using extraction buffer (1:20 w/v) was prepared by crushing the leaves in pestle using mortar and coated 200 µl to the wells of microtitre ELISA plate. The coated plate was kept in a humid box and incubated overnight at 40°C. The plate was 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 emptying the plate and the process was repeated three times. The wells were then filled with 200 µl aliquots of coating antibodies diluted in 1X coating buffer and the plate was incubated in humid box for 4 hours at 300°C. The washing of plate was done as mentioned earlier. ALP labelled 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 then incubated in humid box for 5 hours at 300°C and washing process was repeated as mentioned above. The pNPP substrate was dissolved in 1X substrate buffer (5mg pNPP tablet in 5ml of substrate buffer) under dark conditions and each well was filled with 200 µl aliquots of substrate. The plate was incubated in a humid box in dark at room temperature until a yellow colour started appearing in wells 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 20 substrate p-nitrophenyl at 405 nm wavelength in a microtitre plate reader (iMark Microplate reader, BIORAD, USA).

Leaf samples of symptomatic leaves were collected from the selected nurseries for serological detection of genera Iarvirus (PNRSV and ApMV) through DAC-ELISA since this genera have been earlier reported to be associated with stone fruits in India. For all serological tests of ELISA, immune reagents, buffers, positive and negative control supplied by BIOREBA (AG Switzerland) and were used as per the instructions issued by the supplier. The results of ELISA for detection were interpreted by following (Dijkstra and Jager, 1998); (Lemmetty, 1988) as samples were considered infected when their O.D. values at 405 nm exceeded two times the mean values of respective healthy and negative control samples.

2.1.2 DAS-ELISA

Alkaline phosphatase based direct double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was used to detect PNRSV as per the protocol of (Clark and Adams, 1977) with slight modifications. 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 and the plate was incubated in humid box for 4 hours at 300°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 for emptying the plate and filled again with PBS-Tween.

The washing step was repeated three times. The leaf extracts were prepared in 1X extraction buffer and were filled with 200 µl aliquots of test samples besides positive control and negative control wells. The plates were then incubated in humid box overnight at 4±10°C and the washing step was repeated as mentioned earlier. Specific alkaline phosphatase (ALP) based conjugated antibodies were filled in each well with 200 µl aliquots and the plate was incubated in humid box for 5 hours at 300°C. 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 dark conditions.

Each well was filled with 200 µl aliquots of substrate and the plates were kept in humid box under dark condition at room temperature after giving a brief incubation of 15 minutes at 300C. The plates were incubated until a yellow colour starts appearing in wells (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 hydrolyzed substrate (p-nitrophenyl) at 405 nm wavelength in a microtitre/(ELISA) plate reader (iMark Microplate reader, BIORAD, USA) and through visual screening.

3. RESULTS AND DISCUSSION

3.1 Occurrence, Distribution and Incidence of Viral Diseases in Major Stone fruit Growing Nurseries of Himachal Pradesh

Surveys were done from the year 2020 to 2021 to record the incidence of viruses in the major apricot and cherry nurseries of district Solan and Shimla of Himachal Pradesh. Observations on the disease incidence noted in these nurseries are given in Table 1. Though symptoms of ilarviruses are usually masked due to their latent nature, some of the typical symptoms recorded on main cultivars of stone fruits nurseries were chlorotic spots, necrotic rings, mid vein distortion line, oak leaf patterns, faint yellow ringspots and shot holes on leaves as shown in Plate II and III. Several workers had previously reported similar symptoms in stone fruits (Almaraz et al. 2014; Brunt et al. 1996; Choueiri et al. 2001; Fulton 1970; Kapoor and Handa 2017; Paunovic et al. 2011; Petrzik 2005; Wells et al. 1986; Winkowska et al. 2016).

Table 1: Incidence of viruses (PNRSV and ApMV) in different nurseries

District	Locality	Fruit crop	Cultivars	Incidence (%)
Solan	Experimental Farm, Department of Fruit Science, Dr. YS. Parmar University of Horticulture and Forestry.	Apricot	Kaisha	24.0
			New Castle	17.0
			Harkot	20.0
	Model Farm, Dr. YS. Parmar University of Horticulture and Forestry	Apricot	Kaisha	20.0
			New Castle	15.0
	Krishi Vigyan Kendra Kandaghat	Apricot	Kaisha	18.0
			New Castle	15.5
	PCDO Gaura	Apricot	New Castle	17.0
			Kaisha	19.0
Shimla	RHR&TS, Mashobra	Cherry	Stella	35.0
			Merchant	33.0
			Bing	29.5

The data presented in Table 1 showed the incidence of viral diseases in apricot and cherry cultivars surveyed across different nurseries in Solan and Shimla districts. In Solan district, the incidence of viral diseases in apricot ranged from 15 to 24 per cent across the surveyed locations, whereas in Shimla district, the incidence in cherry ranged from 29.5 to 35 per cent. At the Experimental Farm, Department of Fruit Science, Dr. Y.S. Parmar University of Horticulture and Forestry, the maximum disease incidence of 24 per cent was recorded in apricot cv. Kaisha, followed by 20 per cent in cv. Harkot, while the minimum incidence of 17 per cent was observed in cv. New Castle. At the Model Farm of Dr. Y.S. Parmar University of Horticulture and Forestry, the highest disease incidence of 20 per cent was recorded in apricot cv. Kaisha, whereas the lowest incidence of 15 per cent was observed in cv. New Castle. Similarly, at Krishi Vigyan Kendra, Kandaghat, apricot cv. Kaisha recorded a higher disease incidence of 18 per cent compared to cv. New Castle (15.5%). At PCDO, Gaura, the maximum disease incidence of 19 per cent was observed in apricot cv. Kaisha, while the minimum incidence of 17 per cent was recorded in cv. New Castle.

In Shimla district, cherry cultivars surveyed at RHR&TS, Mashobra showed comparatively higher disease incidence, ranging from 29.5 to 35 per cent. Among the cherry cultivars, the highest disease incidence of 35 per cent was recorded in cv. Stella, followed by cv. Merchant (33%), whereas the lowest incidence of 29.5 per cent was observed in cv. Bing. Overall, the observations indicated that viral disease incidence was higher in cherry compared to apricot under the surveyed conditions. Among the apricot cultivars, cv. Kaisha consistently recorded higher disease incidence across different locations in Solan district.

Research carried by several workers across worldwide reported a large variation in the level of incidence of ilarviruses such as 60 percent in Georgia (Wells et al. 1986); 25 percent in Turkey (Gumus et al. 2007); 30 percent in Canada (Pallas et al. 2012); 25 percent in Mexico (Almaraz 2008); 32 percent Saudi Arabia (Alhudaib and Rezk 2011); 15 percent Central Bohemia Czech Republic (Winkowska et al. 2016) and 18 percent in India (Kapoor and Handa 2017).

3.2 Serological detection of ilarviruses (ApMV and PNRSV) in apricot and plum through DAC-ELISA

Leaf samples with recognizable symptoms such as diffused chlorotic spots, leaf tattering, necrotic rings and shot holes were collected and carried to the laboratory in an icebox inside different polythene bags. DAC-ELISA was first used to describe and broadly categorize virus groups. Through DAS-ELISA, the precise identification of the virus was further confirmed. Pictures of symptomatic plants are shown in figure 1. Several workers had previously reported similar symptoms in stone fruits (Almaraz et al, 2014; Brunt et al, 1996; Choueiri et al, 2001; Fulton, 1970; Kapoor and Handa, 2017; Kaundal et al, 2026; Paunovic et al, 2011; Petrzik, 2005; Wells et al, 1986; Winkowska et al, 2016).

Fig. 1. Shot holes and chlorotic spots in apricot leaves with leaf tattering and chlorotic spots in cherry leaves



Tables 2 and 3 report the findings from the serological detection of viruses using DAC and DAS-ELISA. Alkaline phosphatase based DAC-ELISA was performed to discover if any ilarviruses that may be present in the marked symptomatic leaf samples which were taken from 10 plants each of apricot and cherry from their nurseries at model farm and experimental farm of the Department of Fruit Science at Dr. YS Parmar University of Horticulture and

Forestry Nauni, PCDO, Gaura, Krishi Vigyan Kendra, Kandaghat, Kashyap nursery, Kanda. It was evident from the data displaying O.D. readings and serological reactions that both ilarviruses were positively detected in the test sample. Positive samples had O.D. values that were at least double the O.D. value of negative control. The DAC-ELISA results checked the presence of Ilarvirus in apricot and cherry nurseries. In comparison to ApMV, the samples' O.D. values for PNRSV were greater. In order to determine if PNRSV was present alone or in combination with ApMV as a mixed infection, additional testing was conducted utilizing DAS-ELISA.

Data shown in Table 2 and Figure 2 revealed that out of total 10 plants each of apricot and cherry, 6 plants of apricot and 8 plants of cherry were infected with ilarviruses as indicated by their O.D. values. Maximum O.D. values of 0.412 was recorded in plant No. 4 of apricot followed by plant No. 1 of apricot with O.D. value of 0.397 for PNRSV however for ApMV, maximum O.D. value of 0.389 was recorded in apricot plant No. 1 followed by apricot plant No. 4 with O.D. values of 0.382. For cherry maximum OD value (0.434) for PNRSV was recorded in plant No.2 followed by plant No. 5 and plant No. 10 with O.D. value of 0.414. Whereas for ApMV, maximum O.D. value of 0.392 was recorded in plant No.10 followed by cherry plant No. 5 with O.D value of 0.391. Current research supports the conclusions of several workers (Bashir et al, 2017; Digiario et al, 1991; Kaundal et al, 2026; Roussel et al, 2004; Salem et al, 2004) have effectively detected ilarviruses in stone fruits using DAC-ELISA.

Table 2. Serological detection of prunus necrotic ring spot virus (PNRSV) and apple mosaic virus (ApMV) through DAC-ELISA in apricot and cherry

Apricot				Cherry			
Plant	Symptoms	O.D. Value		Plant	Symptoms	O.D. Value	
		PNRSV	ApMV			PNRSV	ApMV
Plant 1	Mosaic, chlorotic spots	0.397(+)	0.389(+)	Plant 1	Necrotic rings, shot holes	0.412(+)	0.303(+)
Plant 2	Necrotic rings, oak leaf pattern	0.224(+)	0.219(+)	Plant 2	Shot holes, oak leaf pattern, mid vein distortion	0.434(+)	0.385(+)
Plant 3	Shot holes	0.170(-)	0.072(-)	Plant 3	Mosaic, mid vein distortion, necrotic rings	0.389(+)	0.281(+)
Plant 4	Mosaic, necrotic rings,	0.412(+)	0.382(+)	Plant 4	Mosaic, chlorotic spots	0.395(+)	0.390(+)
Plant 5	Chlorotic spots, necrotic rings, mid vein distortion	0.309(+)	0.306(+)	Plant 5	Necrotic rings, mid vein distortion	0.414(+)	0.391(+)
Plant 6	Mosaic, chlorotic spots, necrotic rings	0.173(-)	0.067 (-)	Plant 6	Mosaic, mid vein distortion,	0.172(-)	0.136(-)
Plant 7	Chlorotic spots, shot holes,	0.162(-)	0.053(-)	Plant 7	Shot holes, chlorotic spots	0.327(+)	0.208(+)
Plant 8	Shot holes, necrotic rings, oak leaf pattern	0.154(-)	0.046(-)	Plant 8	Chlorotic spots, necrotic rings, shot holes	0.385(+)	0.274(+)
Plant 9	Mosaic, necrotic rings mid vein distortion	0.329(+)	0.293(+)	Plant 9	Shot holes	0.168(-)	0.151(-)
Plant 10	Chlorotic spots, mid vein distortion	0.385(+)	0.287(+)	Plant 10	Chlorotic spots, shot holes, mid vein distortion	0.414(+)	0.392(+)
						Positive Control	Negative Control
						0.272	0.087

* O.D. Value (A405nm)/ (Serological Reaction)

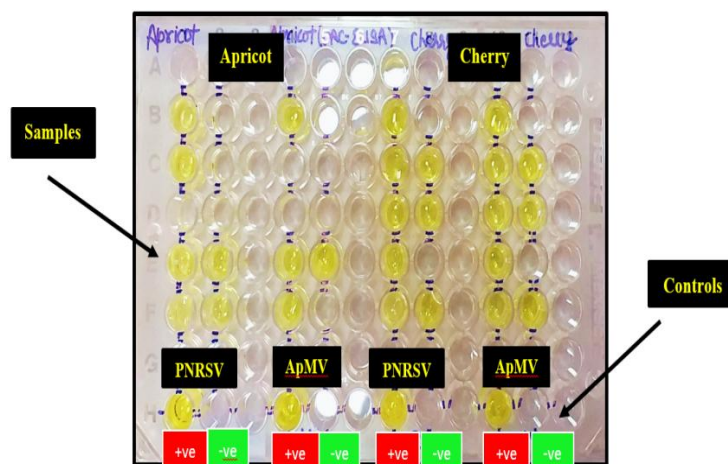


Fig. 2. Serological indexing of PNRSV and ApMV through DAC-ELISA for apricot and cherry cultivars

3.3 DAC-ELISA Serological Detection at Model Farm through DAS-ELISA

Leaf samples were tested for PNRSV and ApMV using an alkaline phosphatase-based direct DAS-ELISA from 10 symptomatic plants each of peach, plum apricot and cherry that were marked and Tables 3 provides their marked and recorded O.D. values.

Since the leaf samples from the majority of the tested plants produced a positive reaction with antibodies against PNRSV but not with those against ApMV, it is evident from the data indicating O.D. values in Table 2 and Figures 3 that the Ilarvirus associated with test samples is PNRSV and not ApMV. Table 3 indicates that Plant number 4 of apricot recorded highest O.D. value of 0.430 followed by plant No. 2 of apricot with O.D. value of 0.341 for PNRSV, whereas in cherry plant No. 10 recorded highest O.D. value of 0.452 followed by plant No. 2 with O.D. value of 0.442 for PNRSV. The results of this study are consistent with those of several researchers who have found that DAS-ELISA is a useful method for identifying PNRSV in stone fruits. (Almaraz et al, 2008; Chandel et al, 2013; Ghanem, 2000; Kapoor and Handa, Kaundal et al, 2026; 2018; Myrta et al, 2001; Salam et al, 2007; Salem et al, 2003; Scott et al, 2001; Szyndel et al, 2006; Vemulapati et al, 2014; Virscek and Mavric 2005).

Table 3. Serological detection of prunus necrotic ring spot virus (PNRSV) and apple mosaic virus (ApMV) through DAS-ELISA for apricot and cherry

Apricot				Cherry			
Plant	Symptoms	O.D. Value		Plant	Symptoms	O.D. Value	
		PNRSV	ApMV			PNRSV	ApMV
Plant 1	Mosaic, chlorotic spots	0.309(+)	0.102(-)	Plant 1	Necrotic rings, shot holes	0.429(+)	0.106(-)
Plant 2	Necrotic rings, oak leaf pattern	0.341(+)	0.130(-)	Plant 2	Shot holes, oak leaf pattern, Mid vein distortion	0.442(+)	0.125(-)
Plant 3	Shot holes	0.143(+)	0.077(-)	Plant 3	Mosaic, mid vein distortion, necrotic rings	0.348(+)	0.085(-)
Plant 4	Mosaic, necrotic rings,	0.430(+)	0.112(-)	Plant 4	Chlorotic spots, shot holes	0.337(+)	0.093(-)
Plant 5	Chlorotic spots, necrotic rings Mid vein distortion	0.329(-)	0.101(-)	Plant 5	Mid vein distortion, necrotic rings	0.425(+)	0.116(-)
Plant 6	Mosaic, chlorotic spots, necrotic rings	0.157(-)	0.063(-)	Plant 6	Shot holes, chlorotic spots	0.161(-)	0.069(-)
Plant 7	Shot holes, necrotic rings, oak leaf pattern	0.151(-)	0.058(-)	Plant 7	Mosaic, mid vein distortion	0.334(+)	0.110(-)

Plant 8	Mosaic, necrotic rings, mid vein distortion	0.160(-)	0.049(-)	Plant 8	Chlorotic spots, necrotic rings, shot holes	0.320(+)	0.071(-)
Plant 9	Mosaic, chlorotic spots, mid vein distortion	0.316(+)	0.099(-)	Plant 9	Shot holes	0.159(-)	0.058(-)
Plant 10	Chlorotic spots, mid vein distortion	0.321(+)	0.085(-)	Plant 10	Chlorotic spots, shot holes, mid vein distortion	0.452(+)	0.128(-)
		Positive Control PNRSV	Negative Control PNRSV			Positive Control ApMV	Negative Control ApMV
		0.285	0.186			0.082	0.079

* O.D. Value (A405nm)/ (Serological Reaction)

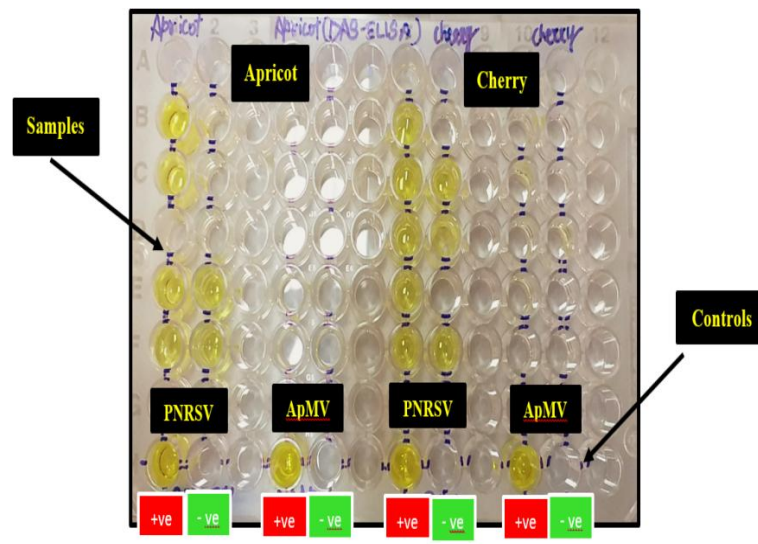


Fig. 3. Serological indexing of PNRSV and ApMV through DAS-ELISA for apricot and cherry cultivars

3.4 Screening of germplasm

After visual screening of the available germplasm of apricot and cherry in different nurseries, serological indexing of available cultivars was done through DAS-ELISA in order to ascertain the source(s) possessing resistance against the prevalent viruses, if any. The results of serological indexing are presented in Table 4.

The highest O.D. value of 0.414 for PNRSV was recorded in plant No. 3 of apricot (Kaisha) followed by plant No. 3 of apricot (Harkot) with an O.D. value of 0.367 at experimental farm Department of Fruit Science. Minimum O.D. value of 0.133 was detected in plant No. 3 of apricot cultivar Kaisha and in plant No. 3 of apricot cultivar New Castle with an O.D. value of 0.135 at PCDO, Gaura. At RHR&TS, Mashobra (Shimla), maximum O.D. value of 0.430 was recorded in plant No. 1 of cherry (Stella) followed by O.D. value of 0.422 in plant No. 2 of cherry (Merchant) and minimum O.D. value of 0.132 was recorded in Bing cultivar of cherry (No.3).

Table 4. Serological screening of germplasm of apricot and cherry through DAS-ELISA

Sr. No.	Location (District)	Fruit Crop & Variety	Plant 1 (Value)	Plant 2 (Value)	Plant 3 (Value)
1.	Experimental Farm, Department of Fruit Science, Dr. YS Parmar University of Horticulture and Forestry, Nauni (Solan)	Apricot Kaisha	0.275 (+)	0.321 (+)	0.414 (+)
		New Castle	0.137 (-)	0.307 (+)	0.143 (-)
		Harkot	0.330 (+)	0.150 (-)	0.367 (+)
2.	Model Farm, Dr. YS Parmar University of Horticulture and	Apricot	0.329 (+)	0.314 (+)	0.295 (+)

	Forestry, Nauni (Solan)	Kaisha			
		New Castle	0.143 (-)	0.294 (+)	0.149 (-)
3.	Krishi Vigyan Kendra, Kandaghat (Solan)	Apricot Kaisha	0.290 (+)	0.151 (-)	0.267 (+)
4.	PCDO, Gaura	Apricot New Castle	0.308 (+)	0.147 (-)	0.135 (-)
		Kaisha	0.142(-)	0.297 (+)	0.133 (-)
6.	RHR&TS, Mashobra (Shimla)	Cherry Stella	0.430 (+)	0.348 (+)	0.390 (+)
		Merchant	0.339 (+)	0.422 (+)	0.150 (-)
		Bing	0.356 (+)	0.143 (-)	0.132 (-)
				Positive Control	0.265
				Negative Control	0.076

* O.D. Value (A405nm)/ (Serological Reaction)

Table 5 presents the DAS-ELISA based screening of apricot and cherry germplasm collected from different surveyed locations for the detection of PNRSV infection and identification of potential healthy planting material. Considerable variation in infection status was observed among the tested cultivars and locations. Among apricot cultivars, Kaisha exhibited a predominantly positive reaction across most surveyed sites, indicating a higher incidence of PNRSV infection. In contrast, New Castle displayed a mixed response, with several plants testing negative alongside occasional positive reactions, suggesting comparatively lower infection prevalence and the possible availability of healthier planting material within this cultivar. The apricot cultivar Harkot, evaluated at the Experimental Farm, Nauni, also showed a largely positive reaction, reflecting susceptibility to PNRSV infection. In cherry, the cultivar Stella recorded consistent positive reactions in all tested plants, clearly indicating widespread infection. Merchant and Bing, however, exhibited variable responses, with both infected and non-infected plants identified, pointing towards the presence of individual healthy plants that may be considered for further indexing and propagation. Overall, the study demonstrated the widespread presence of PNRSV in the surveyed stone fruit germplasm, while a few ELISA-negative plants were identified as potential candidates for the development of virus-free mother stock and the propagation of healthy planting material. The above findings are in concurrence with reports on stone fruits infected by PNRSV from many countries (Aouane, 2003; Chandel et al, 2007; Fajardo et al, 2015; Sucha and Savobodova, 2010) and these studies show that PNRSV has a broad host range in temperate fruits, as previously reported by (Kapoor et al, 2018).

Table 5. Reaction of PNRSV on stone fruits at surveyed nurseries for the selection of nuclear healthy planting material

Fruit Crops	Cultivars	Experiment alFarm, Nauni	Model Farm, Nauni	Krishi Vigyan Kendra	PCDO, Gaura	Kashyap Nursery	RHR&TS , Mashobra
Apricot	Kaisha	+++ (3/3)	+++ (3/3)	++ (2/3)	+ (1/3)	-	-
	New Castle	+ (1/3)	+ (1/3)	+ (1/3)	+ (1/3)	-	-
	Harkot	++ (2/3)	-	-	-	-	-
Cherry	Stella	-	-	-	-	-	+++ (3/3)
	Merchant	-	-	-	-	-	++ (2/3)
	Bing	-	-	-	-	-	+ (1/3)

4. CONCLUSION

There are several diseases of distinctive nature and significance including viral infections that are known to affect apricots and cherries. Serological indexing of major viruses linked to apricot and cherry nurseries in Solan district of Himachal Pradesh through ALP based DAC-ELISA confirmed the association of Ilarvirus viz., Apple mosaic virus (ApMV) and Prunus necrotic ring spot virus (PNRSV) among the marked symptomatic plants of most of the cultivars of apricot and cherries and DAS-ELISA further conclusively proved that PNRSV was more prevalent in the infected apricot and cherry plants as compared to ApMV. The research findings indicated that among both apricot and cherry plants, cherry proved out to be the most susceptible host for PNRSV. Survey findings showed higher disease incidence

in cherry cultivars ranging from 29.5 to 35 percent, compared to 15 to 24 percent in apricot across the surveyed locations. Among apricot Kaisha recorded higher disease incidence across different nurseries, whereas in cherry cv. Stella recorded highest incidence followed by cv. Merchant, while Bing showed the lowest incidence. Further germplasm screening through DAS-ELISA revealed cv. New Castle, Harkot and Kaisha of apricot at selected nurseries in Nauni, Kandaghat and Gaura and cv. Merchant and Bing of cherry at nursery in RHR&TS, Mashobra showed negative reaction for PNRSV. These virus free fruit cultivars were identified and maintained for the purpose of developing mother plants to provide virus-free healthy budwood that would prevent the spread of viral infections in stone fruits in the near future. Furthermore, future objectives should concentrate on developing a reliable and efficient certification process for the creation of virus-free and healthy planting material for apricot and cherry.

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Competing interests

“Authors have declared that no competing interests exist.”

Authors' Contributions

“ ‘Author 1 performed the experiments, calculations, wrote the manuscript’ wrote the protocol and wrote the first draft of the manuscript, Author 2 and 3 helped during conduction of research, author 4 and 5 contributed in manuscript writing and ‘Author 7’ designed the study and supervised the project. All the authors read and approved the final manuscript.”

Abbreviations

PNRSV- prunus necrotic ringspot virus, ApMV- apple mosaic virus, PPV- plum pox virus, ACLSV- apple chlorotic leaf spot virus, PcMV- peach mosaic virus, PDV- prune dwarf virus, ALP- alkaline phosphatase, DAC- direct antigen coating, DAS-ELISA- double antibody sandwich-enzyme linked immune sorbent aasay, PCDO- progeny cum demonstration orchard and DIBA- dot immune binding assay

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