Int.J.Curr.Microbiol.App.Sci (2018) *7*(5): 3674-3681

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 05 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.705.424

Serological Characterization of Major Viruses infecting Strawberry in India

Abhilasha Sharma* and Anil Handa

Plant Virology Laboratory, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, (HP), India

**Corresponding author*

Keywords

Serological Characterization, Strawberry, DAS-ELISA

Article Info

Accepted:
26 April 2018
Available Online:
10 May 2018

A B S T R A C T

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.

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 80percent 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 immunoglobin) 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 ($A_{405\ nm}$) 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.1 DAC-ELISA detection of strawberry viruses

Genera	Antibody	Locality	Mean OD at 405nm		
			Test sample	Positive control	Negative control
Nepovirus	TRSV	Kandaghat	1.182(+)	0.797(+)	0.070(-)
		Dhanda	0.602(+)	0.574(+)	0.058(-)
Ilarvirus	TSV	Kandaghat	1.736(+)	1.236(+)	0.123(-)
		Dhanda	1.227(+)	0.755(+)	0.131(-)
Potexvirus	SMYEV	Kandaghat	1.386(+)	0.912(+)	0.095(-)
		Dhanda	0.168(+)	0.513(+)	0.066(-)

Table.2 DAS-ELISA detection of strawberry viruses

Antibody	Locality	Mean OD at 405nm		
		Test sample	Positive control	Negative control
SLRSV	Kandaghat	0.405(+)	0.344(+)	0.048(-)
	Dhanda	0.449(+)	0.275(+)	0.112(-)
	Nauni	0.206(+)	0.236(+)	0.056(-)
TRSV	Kandaghat	0.403(+)	0.341(+)	0.061(-)
	Dhanda	0.449(+)	0.459(+)	0.262(-)
	Nauni	0.244(+)	0.170(+)	0.047(-)
RRSV	Kandaghat	0.340(+)	0.328(+)	0.069(-)
	Dhanda	0.575(+)	0.675(+)	0.046(-)
	Nauni	0.398(-)	0.417(+)	0.047(-)
TSV	Kandaghat	1.078(+)	1.109(+)	0.332(-)
	Dhanda	0.690(+)	0.576(+)	0.223(-)
	Nauni	0.206(+)	0.262(+)	0.056(-)
SMYEV	Kandaghat	0.378(+)	0.375(+)	0.035(-)
	Dhanda	0.217(-)	0.445(+)	0.117(-)
	Nauni	0.147(+)	0.153(+)	0.034(-)

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.

Acknowledgments

The authors sincerely acknowledge the Ministry of Agriculture, Government of India for funding the RKVY research project under which this research work was carried out. The authors also acknowledge the help received from Principal Scientist and Head, KVK Kandaghat and IARI Regional Station, Shimla for providing the planting material and research facilities.

References

Abtahi, F.S. and Habibi M.K. 2008. Host range and some characterization of Tobacco Streak Virus isolated from lettuce in Iran. *African Journal of Biotechnology.,* 7:4260-4264

Anonymous, 2017. 2nd www.hpagrisnet. gov.in/horticulture.

Bargen, S.V., Demiral, R. and Buttner C. 2015. First Detection of Raspberry Ringspot Virus in Mosaic Diseased Hybrid Roses in Germany. *New Disease Reports*., 32:18.

Clark, M.F. and Adams A.N. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology.,* 34:475-483.

Conci, V.C., Torrico, A.K., Cafrune, E. and Kirschbaum, B.S. 2009. First Report of Strawberry Mild Yellow Edge Virus in Argentina. *Acta Horticulturae*., 842(842): 303-306.

Dijksha, J. and Jager C.D. 1998: Practical Plant Virology. *Springer*., Lab Manuals. 145p.

EPPO/CABI, 1996. Strawberry veinbanding caulimovirus. In: Quarantine pests for Europe. 2nd edition (Ed. by Smith, I.M.; McNamara, D.G.; Scott, P.R.; Holderness, M.). CAB International, Wallingford, UK.

Handa, A. and Bhardwaj S.V.1994. Comparative study of the use of alkaline phosphatase and penicillinase based direct antigen coating ELISA for the detection of a potyvirus from faba bean. *FABIS.,* 34: 36-38.

Hepp, R.F. and Martin R.R., 1991. Occurrence of strawberry mild yellow-edge associated virus in wild *Fragaria chiloensis* in South America. *Acta Horticulturae*., No. 308, 57-60.

Martin, R.R. and Tzanetakis L.E. 2006. Chracterization and Recent Advances in Detection of Strawberry Viruses. *Plant Diseases.,* 90:384-396.

Sharma, A., Handa, A., Kapoor, S., Watpade, S., Gupta, B and Verma P., 2018 Viruses of strawberry and production of virus free planting material- A critical review. International Journal of Science, Environment and Technology., 7: 521-545.

Tang, J., Ward, L.I. and Clover G.R.G. 2013. The diversity of strawberry latent ringspot virus in New Zealand. *Plant Disease*., 97:662-667.

Thompson, J.R. and Jelkmann W. 2003. The detection and variation of *Strawberry mottle virus*. *Plant Disease* 87: 385-390.

Vemana, K. and Jain R.K.2011. New experimental hosts of Tobacco streak virus and absence of true seed transmission in leguminous hosts. Indian Journal of Virology 21: 117-127.

How to cite this article:

Abhilasha Sharma and Anil Handa. 2018. Serological Characterization of Major Viruses infecting Strawberry in India. *Int.J.Curr.Microbiol.App.Sci.* 7(05): 3674-3681.
doi: https://doi.org/10.20546/ijcmas.2018.705.424