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First report of strawberry mild yellow edge virus in India

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Abstract
Garden strawberry (*Fragaria ananassa* Duch.) plants with symptoms typical of viral infection in the form of mild mottling, cupping of leaflets and leaf deformation were observedatone of the two locations surveyedin Solan and Shimla districts of Himachal Pradesh, India. The main objective of this study was to characterize the causal agent of this diseaseserologically using DAS-ELISA. Infected plants exhibited symptoms that have not been reported so far in strawberry plants from India. The virus was detected using DAS-ELISA in both symptomatic and symptomless plants. Keeping in view the importance of thisrelativelyunknown virus from India, the present studies were conducted to characterize the causal virus serologicallyon the basis of direct DAS-ELISA tests conducted for the purpose. On the basis of the results obtained in direct DAS-ELISA, it was concluded that only three isolates collected from the location in Solan district yielded positive results for SMYEV whereas none of the isolates from Shimla district tested positive for the virus. This is the first report of SMYEV infecting strawberry in India. This valuable information can be put to use in the development of certification programmes aimed at producing healthy planting material in the larger interest of commercial strawberry units functional in India

Keywords: First report, strawberry, mild, yellow edge virus, India

Introduction
Strawberry production is increasing logarithmically with a total world production of strawberry fruits now exceeding 4 million tonnes (Sharma *et al*., 2018) [1]. Many viruses and phytoplasma affect strawberries, either singly or in combination. The characteristic symptoms of SMYEV include cupped leaflets, chlorotic mottling, interveinal necrosis of older leaves and stunting (Jelkmann, 1991) [2]. This virus does 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)[3].The virus can cause yield losses up to 30% in the field (Spiegel and Martin, 1998)[4] while the synergistic effect when found in complexes with other viruses increases impact. SMYEV belongs to the family *Alphaflexiviridae* and genus *Potexvirus*. This virus is also known by name strawberry virus 2 (Sharma *et al*., 2018) [5].Virions are isometric non-enveloped and about 23-28 nm in diameter. It is angular in profile without a conspicuous capsomere arrangement (Jelkmann, 1991) [2]. Like fungal and bacterial pathogens, viral diseases cannot be managed by chemical treatment and moreover their infection passes to successive generations through vegetatively propagating planting material (Cieszlinska and Malinowski, 2002 [6]; Pupola *et al.*, 2011 [7] resulting in decline of plant health and poor productivity over a number of years. Only virus, phytoplasma and viriod free plants can produce high yields of quality fruits. Therefore, selection and propogation of disease free planting material is the only way to tackle such problems and this is dependent on the technology for quick and reliable detection of viruses and related pathogens.

Materials and Methods
Planting Material
Leaves from strawberry cv. Chandler with virus like symptoms such as mottling, cupping of leaflets and leaf deformation were collected during the cropping season of 2017 from HRTS & KVK kandaghat, Solan and IARI Regional Station Dhanda Farm, Shimla.

ELISA Detection
DAS (Double Antibody Sandwich) form of ELISA was used for the detection of

viruses in the test samples. The procedure followed for conducting DAS-ELISA is presented in the following paragraph.

Infected leaves showing symptoms of necrotic spots and vein bending were collected and brought to the laboratory in ice bucket for conducting DAS-ELISA tests as per the protocol given by Clark and Adams (1977) [8]. Wells of the microtitre plate (BIOREBA, Switzerland certified microplates) except those of the top and bottom rows and rows on the extreme left and right, were filled with 200µl aliquots of coating antibodies diluted in 1x coating buffer (1:1000 ratio v/v). The plate was incubated in humid box for 4 hours at 30̊ 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 grounded in 1x extraction buffer (1:10 ratio v/v). All coated wells were filled with 200µl aliquots of test samples (each sample in duplicate) besides positive and negative control wells. The plate was incubated in humid box overnight at 4±1̊ C. The washing steps were repeated as mentioned above. Alkaline phosphate (ALP) conjugated antibodies were filled in each well with 200µl aliquots after diluting it in 1x ECI (enzyme conjugated immunoglobin) buffer at a (ratio of 1:1000 v/v). The plate was incubated in humid box for 5 hours at 30̊ C. The washing was done as mentioned above. p-Nitrophenyl phosphate (pNPP) substrate was dissolved in 1x substrate buffer by dissolving 5mg pNPP tablet in 5ml of 1x substrate buffer. Each well was filled with 200µl aliquots of the substrate. The plate was kept in humid box in the dark condition at room temperature until a yellow colour was clearly visible in the positive control (usually between 30-60 minutes). The results were assessed either by visual observations or by measurement of the absorbance value of the hydrolysed substrate (p-nitrophenyl) at 405 nm wavelength in a microtitre plate reader (Micro Scan MS 5605A, Electronics Corporation of India Limited, Hyderabad). The results of ELISA for the detection were interpreted as per Dijkstra and Jager (1998)[9] as samples were considered infected when their absorbance values (A_{405nm}) exceeded two times the mean values of respective healthy control samples.

Results

Table 1: Detection of Strawberry Mild Yellow Edge Virus (SMYEV) in virus isolate with DAS-ELISA

Antibody	Locality	Isolates	Mean OD at 405nm		
			Test Sample	Positive control	Negative control
SMYEV	Kandaghat	K_1	0.042(-)	0.375(+)	0.035(-)
		K_2	0.378(+)		
		K_3	0.174(+)		
		K_4	0.063(-)		
		K_5	0.218(+)		
	Dhanda	D_1	0.170(-)	0.445(+)	0.117(-)
		D_2	0.103(-)		
		D_3	0.217(-)		
		D_4	0.189(-)		
		D_5	0.041(-)		

Data based on O.D. values presented in Table 1 indicate the presence of strawberry mild yellow edge virus in the leaves of all the strawberry virus isolates except for the two virus isolates K_1 andK_4 from Kandaghat locations surveyed for the collection of isolates. A critical analysis of the OD values recorded in DAS-ELISA revealed that Kandaghat virus isolate K_2 recorded the highest OD value of 0.378 closely followed by K_5. It is also evident from the data that SMYEV tested negative for all the isolates collected from

Fig 1: Puckering and leaf deformation in strawberry cv. Chandler

Fig 2: Chlorosis and cupping of leaves in a severely infected plant.

Discussion

Strawberry is a member of the Rosaceae family and is an important commercial crop. Strawberry is a soft-berry fruit and is prone to many viruses in addition to a number of fungal and bacterial pathogens. Viruses infecting strawberry have been critically reviewed by Sharma *et al.,* (2018) [5] with regard to Symptomatology, Virion properties, serology and molecular characterization. Though there are reports of

SMYEV in strawberry from Argentina Conci *et al*., (2009) [10], South America (Hepp and Martin, 1991) [11] EPPO region, China, Japan, Canada, Australia and New Zealand (EPPO\CABI, 1996) [12] there is no report of SMYEV infecting strawberry in India. This is the first report of SMYEV infecting strawberry in India and the valuable information emerging out of the present studies can be put to use in the development of certification programmes aimed at producing healthy planting material in the larger interest of commercial strawberry units functional in India.

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