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First Report of Tobacco Streak Virus on Strawberry (*Fragaria* x *ananassa* Duchesne) in India

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Abstract
Strawberry (*Fragaria* x *ananassa* Duchesne), a member of the Rosaceae family, is infected by wide range fungal, bacterial and viral pathogens. Unlike fungal and bacterial pathogens that express typical symptoms on strawberry leaves and fruits, the presence of viruses in strawberry is quite distinct as they remain largely symptomless or the symptoms produced are often similar to those resulting from nutritional deficiencies. Such plants are symptomless carriers of viruses that spread with vegetatively propagated plant material or could be vectored by insects, mites, nematodes, fungi and seeds. Multiple virus infections are frequently encountered in strawberry and may cause a drastic reduction in yield and vigour. Thorough regular screening of strawberry germplasm revealed the presence of a new *Ilarvirus*, Tobacco streak virus (TSV) which was not reported earlier in strawberry from India. Keeping in view the importance of this new *Ilarvirus* in India, the present studies were conducted to establish the exact identity of the virus on the basis of DAS-ELISA tests conducted for the detection of the causal virus. On the basis of results obtained in DAS-ELISA, it can be concluded that majority of the virus isolates collected and subjected to ELISA confirmed the presence of TSV in strawberry. This is the first report of TSV infecting strawberry in India.

Keywords: Strawberry, tobacco streak virus, DAS-ELISA

1. Introduction
Strawberry production is increasing logarithmically with a total world production of strawberry fruits exceeding 4 million tonnes. Many viruses and phytoplasmas affect strawberries, either singly or in combination. These may lead to the appearance of striking symptoms such as green petals, crinkling, yellow spotting, vein banding, stunting, poor growth and loss of yield. With a shift from conventional fruit crops to strawberry in new regions and the advent in detection techniques has led to the emergence of new viruses. Merely a decade ago, there were not more than a dozen viruses known to infect strawberry but of late the number of viruses found associated with strawberry has now gone very high. Visual symptoms have indicated that the new *Ilarvirus* infecting strawberry in India could be tobacco streak virus (TSV) which has not been reported earlier**.** The characteristic symptoms observed under present investigations comprised of necrotic spots, mottling, chlorotic ringspots and vein banding which are similar to those evoked by TSV reported from other parts of the world.

2. Materials and Methods
2.1 Planting Material
Leaves from strawberry cv. Chandler with virus like symptoms were collected during the cropping seasons of 2016 and 2017 from HRTS & KVK kandaghat, Solan and IARI Regional Station Dhanda Farm, Shimla.

2.2 ELISA Detection
DAS (Double Antibody Sandwich) form of ELISA was used for the detection of viruses in the test samples.

DAS-ELISA
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) [1]. 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 30° 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±1° 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 30° 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) [2] as samples were considered infected when their absorbance values (A_{405nm}) exceeded two times the mean values of respective healthy control samples.

3. Results and Discussion

Data based on O.D. values presented in Table 1 indicate the presence of tobacco streak virus in the leaves of all the strawberry virus isolates except for the two virus isolates K_2 and D_3 from the two locations surveyed for the collection of isolates. A critical analysis of the OD values recorded in DAS-ELISA revealed that Kandaghat virus isolate K_3 recorded the highest OD value of 1.078 closely followed by K_5. It is also evident from the data that the concentration of TSV was relative higher in Kandaghat isolates in comparison to Dhanda isolates. Viruses infecting strawberry have been critically reviewed by Sharma *et al.* (2018) [3] and Tzanetakis and Martin, (2012) [4] with regard to symptomatology, virion properties, serology and molecular characterization.Though there are reports of TSV in legumes from India (Vermana and Jain, (2011) [5], Patil and Mali, (1982) [6]) there is no report of TSV infecting strawberry in India. This is the first report of TSV 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.

Table 1: Detection of Tobacco streak virus with DAS- ELISA

Antibody	Locality	Isolate	Mean OD at 405nm		
			Test Sample	Positive control	Negative control
TSV	Kandaghat	K_1	0.977(+)	1.109(+)	0.332(-)
		K_2	0.450(-)		
		K_3	1.078(+)		
		K_4	0.959(+)		
		K_5	1.035(+)		
	Dhanda	D_1	0.560(+)	0.576(+)	0.223(-)
		D_2	0.477(+)		
		D_3	0.396(-)		
		D_4	0.690(+)		
		D_5	0.320(+)		

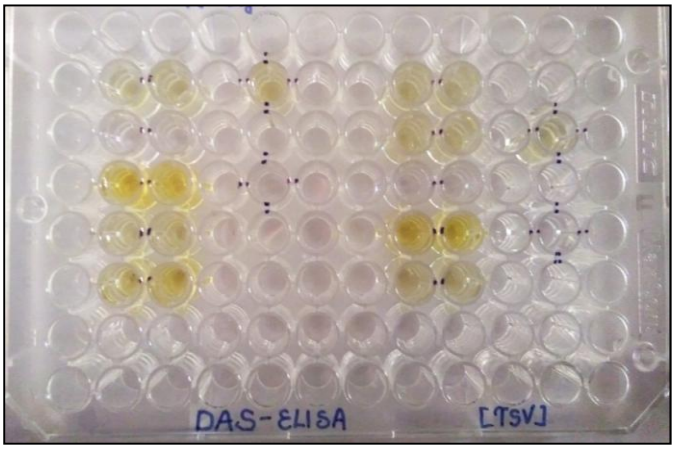

Fig 1: ELISA plate showing reaction of virus isolates to TSV antibodies in DAS-ELISA

Fig 2: Fruits from serologically indexed healthy plants of strawberry

Fig 3: Fruits from serologically indexed TSV infected plants

Fig 4: Virus indexed healthy plants of strawberry

Fig 5: Leaf crinkling accompanied by chlorosis in TSV infected strawberry leaves

Fig 6: Leaf crinkling and marginal necrosis of strawberry leaves

Fig 7: Severe leaf deformities in leaves of TSV infected strawberry plant

4. Conclusion

On the basis of the visual symptoms appearing in the form of green petals, crinkling, yellow spotting, vein banding, stunting, poor growth and positive reactions recorded in DAS-ELISA tests it can be concluded that a number of virus isolates collected during the present investigations were infected with tobacco streak virus, a new *Ilarvirus* hitherto not reported from strawberry in India.

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