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Physical Properties of Potyvirus on Chilli (*Capsicum annuum)* of Doon Valley in Uttarakhand

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ABSTRACT

A virus is transmitted mechanically in the plant host. Potyviridae is a family of Potyvirus, consisting of a positive-sense RNA genome and filamentous particles. It has a single type of protein coat. Mostly, this virus is identified in *Leguminosae* and *Solanaceae* family. The plant virus is transmitted by mechanical inoculation and aphids. Chilli, cucurbits, potatoes, peas, tomatoes, and other plants were infected with the bean yellow mosaic virus, pea mosaic virus, bean yellow mosaic virus, sugarcane mosaic virus, and peanut mosaic virus. Thermal inactivation point, dilution endpoint, longevity *in vitro*, and longevity in vivo were used to identify the physical properties of a potyvirus. Several isolates mild and severe were collected in the chilli plant. The infected plant developed necrotic leaf spots and chlorotic symptoms.

Keywords: Necrotic, Chlorotic, Mechanical inoculation.

INTRODUCTION

Chilli comes under the family of *Solanaceae*. It is also known as pepper, bell pepper, hot pepper, sweet pepper, etc. The two most common species cultivated in India like *Capsicum annuum* and *Capsicum frutesences.* It contains a high amount of vitamins C, A, E, P, and allicin, beta-carotenoids, isothiocyanate capsin and piperine which creates pungent nature of chilli. The loss of production of chilli is caused by potyvirus, begomovirus, tymovirus causes curling of leaves, puckering, vein clearing and stunting of plants due to the spread of infections of a potyvirus and so the low production of the crop. India has the high producer of chilli in the world. Potyvirus belongs to the potato Y virus group and can be differentiated from most other seed-transmitted legumes family viruses (Hansen and Lesemann 1978). The Potato Y virus is a microscopic infectious agent. It belongs to the family Potyviridae, which replicates mostly in the plant host cell and is transmitted through vector-like insects that feed plant sap such as aphids. Genetic analysis of the potyvirus group has shown that the prerequisites for moving viruses from the parental virus in protoplasts, leaf to leaf through the phloem, or long-distance transport, are different from those for moving viruses from cell to cell (Doija et al, 1994; Cronin et al, 1995). Aphids and whiteflies, respectively, transmitted leaf curl illnesses and mosaic in chilli cultivars (Figure 1) (Khan et al, 2006). In other circumstances, potyviruses spread more frequently through seed (Gibbs et al, 2008). Viral RNA in the infected cell has the ability to compete with endogenous mRNAs for the translation machinery and related translation factors in the plant host cell. Potyvirus infection includes complex molecular processes that take place in several cellular compartments and involvement of numerous proteins and host genes. Understanding the interactions between multiple virus-induced processes and host gene expression in the nucleus and cytoplasm is one of the problems in the future (Ivanov et al, 2014).

A B

Figure 1: (A). Normal chilli (*Capsicum frutesences)* plant (B) Infected chilli (*Capsicum frutesences)* plants show leaf curl and severe mosaic disease.

METHODS AND MATERIAL

The physical properties of the potyvirus on chilli were isolated given below:

Dilution endpoint

The stock culture infected chilli leaves were extracted and put together with distilled water, to make dilution viz.1:10, 1:100, 1:1000, etc. The potyvirus lost infectivity of the dilution end point were10^{-3} to 10^{-4}.

Thermal inactivation point

To identify the physical properties of potyvirus, the determination of the thermal inactivation point on chilli which extracts crude sap of chilli was taken in 6 test tubes add 2 ml of mild and severe infected sap were taken after that heat the test tube at 40°C to 70°C. The observation was taken at lost the mild and severe infection of a potyvirus.

Longevity *in vitro*

Infected leaves of chilli were crushed and kept under dark room temperature in a conical flask. Every day 10 chilli seedlings were inoculated for up to one week. The experiment was performed in insect-proof glass chamber.

Longevity *in vivo*

To determine the longevity duration of the time of infection virus on chilli plant under the in-vivo condition at 20°C to 25°C at room temperature. The inoculated sap was kept in a dark place at room temperature. Each ten chilli plant seedling was inoculated with each isolate inocula stored. Crude sap was used within 15 days. The Observation was recorded for up to 15-20 days after inoculation in each lot of plant seedlings.

RESULT

In two trials, the dilution endpoint observed that the physiological properties of the mild symptoms lost their infectivity at the dilution end point was10^{-3} whereas severe isolates lost their infectivity at 10^{-3} (Table 1).

Table 1: Number of plants infected out of 10 and 20 inoculated plants on chilli. Dilution end point of potyvirus on chilli mild and severe isolates storage

Dilutions	No. of plants infected out of 10 inoculated plants				Total No. of Plants infected out of 20 inoculated plants	
	Trial 1		Trial 2			
	Mild	Severe	Mild	Severe	Severe	Mild
1:10	7	8	7	8	14	16
1:100	4	5	3	4	7	9
1:1000	-	1	_	_	-	1
1:10000	-	-	-	-	-	-
Control	10	10	10	10	20	20

Thermal inactivation point

The potyvirus isolates were treated at different temperatures and the virus became inactive. The infectivity of mild isolates was entirely lost at 53°C to 55°C and that of severe isolates at 55°C to 60°C (Table 2).

Table 2: Number of plants infected out of 10 and 20 inoculated plants on chilli. Thermal inactivation point of potyvirus on chilli mild and severe isolates storage

Temperature	Number of plants out of 10 inoculated plants				Total number of plants infected out of 20 inoculated plants	
	Ist Trial		IInd Trial			
	Mild	Severe	Mild	Severe	Mild	Severe
40°C	10	6	7	7	17	13
50°C	5	3	5	3	10	6
55°C	-	2	-	1	-	3
60°C	-	-	-	-	-	-
Control	10	10	10	10	20	20

Longevity *in vitro*

In the case of mild isolates of potyvirus, infection was achieved 2 days whereas severe isolates infection showed within 3 days Results are given in following Table 3.

Table 3: Number of plants infected out of 10 and 20 inoculated plants on chilli. Longevity *in vitro* of potyvirus on chilli mild and severe isolates storage

Storage days	No. of plants infected out of 10 inoculated plants				Total No. of Plants infected out of 20 inoculated plants	
	Trial 1		Trial 2		Mild	Severe
0	Mild	Severe	Mild	Severe		
1	5	6	5	5	10	11
2	1	3	-	2	1	5
3	-	-	-	-	-	-
Control	10	10	10	10	20	20

Longevity *in vivo*

Longevity in vivo results obtained from two trials for conformation showed that mild isolates were 3 days while severe isolates periods of time were 4 days (Table 4).

Table 4: Number of plants infected out of 10 and 20 inoculated plants on chilli. Longevity *in vivo* of potyvirus on chilli mild and severe isolates storage.

Storage days	No. of plants infected out of 10 inoculated plants				Total No. of Plants infected out of 20 inoculated plants	
	Trial 1		Trial 2		Mild	Severe
	Mild	Severe	Mild	Severe	-	-
1	8	9	7	8	15	17
2	4	6	4	5	8	11
3	-	2	-	1	-	3
Control	10	10	10	10	20	20

DISCUSSION

Potyvirus plant host-specific virus RNA virus. Several isolates were collected in mild and severe groups. The mild isolates showed characteristics of chlorosis, curling of leaves, spotting in leaves, and pale color of leaves. The flowering and seed formation was delayed and reduced. The severe isolates showed dark green color patches on chilli leaves and reduced size of leaves or internodes. Physically, potyvirus lost its ability to infect at concentrations of 10^{-3} to 10^{-4}, and at temperatures of 53°C to 55°C for mild isolates and 55°C to 60°C for severe isolates, respectively. In vitro potyvirus infection of mild isolates lasted for two days while infection of severe isolates lasted for three days. Longevity in vivo the mild isolates were observed in 3 days while in severe isolates periods of time were 4 days. The protein component of Bromegrass mosaic virus has a determined molecular weight of 3.6x106. Preliminary research indicates that Bromegrass mosaic virus has subunits with a molecular weight of around 20,000 (Bockstahler et al, 1962). The cucumber mosaic virus protein was produced by digesting the virus with calcium chloride and was determined to have a molecular weight of 32,200 by equilibrium sedimentation (Van et al, 1967). The potyvirus could survive in expressed sap for up to three days and had a thermal inactivation threshold of 62°C, a dilution endpoint of 10^{-3}, and a dilution range of 10^{-4} (Lana et al, 1975). 12 potyvirus isolates were generated with monoclonal antibodies (MAbs) against to differentiate diverse potyviruses (Jordan and Hammond, 1991). The biological and physicochemical features of viruses spread by mites, aphids, whiteflies, and fungi (Brunt, 1992). The results showed that the majority of potyvirus proteins are multifunctional and that both coding and noncoding portions of the potyvirus genome play roles in diverse biological processes. An in-depth understanding of the functions of these viral proteins will be possible with knowledge of the interacting host elements involved in susceptibility and defense (Revers et al, 1999).

Phylogenetic investigation of the Potyvirus papaya ringspot virus reveals mutation, long and local distance movement, and contribution to population variation (Bateson et al, 2002).The virus's physical characteristics included a thermal inactivation point between 60 and 65 degrees Celsius, a dilution endpoint between 1:5000 and 1:10000, and in vitro longevity of 2-4 days at room temperature (Prakash et al, 2002). Scrolls, pinwheels, laminated aggregates, and amorphous inclusions caused by the potyvirus were observed (Kim et al, 2005). Utilized for the management of viral diseases in chilli plants are several insecticide sprayings that increased biomass and red fruit production. With a thermal inactivation threshold of 65°C, a dilution endpoint of 10-3, and an in vitro lifetime of 24 hours at room temperature, ZYMV's physical characteristics showed that the virus is highly transmissible (Khan et al, 2006; EL-Shafi et al, 2006). The potyvirus isolates and phylogeographic analyses provide some evidence of Bean common mosaic virus lineage (Gibbs et al, 2008). Some isolates of these viruses can evade the RTM mechanism, even if RTM-mediated resistance is effective against other potyviruses including the Plum pox virus (PPV) and Lettuce mosaic virus (LMV). The N-terminal portion of the potyvirus CP gene controls how the virus interacts with RTM-mediated resistance. Lysine mutations resulted in lower positive surface charge, decreased in vitro NTP binding, uridylylation process, and unspecific RNA binding efficiency, and decreased *in vivo* infectiousness (Decroocq et al, 2009; Rantalainen et al, 2011). The dilution endpoint, heat inactivation point, and in vitro longevity of a potyvirus strain were all discovered to be 4 days at 65^{o}C, respectively (Reham et al, 2016). The research aims to determine the impact of silicon treatment on plant development and disease resistance in treated chilli plants (Lob et al, 2017). Thermal inactivation point of pepper mottle virus isolate is 60-65o C, DEP is 10-2 - 10-3, and LIV is 7 days at room temperature and 8 days under refrigeration (Sharma et al, 2018). To investigate neurodegenerative diseases and the possible use of natural plant products or plant extracts in the prevention or treatment of different diseases (Pohl et al, 2018).Molecular insights into the begomovirus-caused Euphorbia yellow mosaic virus that infects chilli peppers (Catarino et al, 2020).Various stain analyses have been conducted on potyviruses, including the Johnsongrass mosaic virus (JGMV), Sorghum mosaic virus (SrMV), and Sugarcane dwarf mosaic virus (MDMV-MDB) (Seifers et al, 2000). Cucumber mosaic virus host range studies or genome characterization and recombination study of CMV-Ko genome revealed that RNA2 and RNA1 and not RNA3 was the virus's genetic carriers (Ashwathappa et al, 2021).

Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

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