

Original Research Article

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Physical Characteristics of Black Gram Necrosis Inciting Tobacco Streak Virus on the Cowpea

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ABSTRACT

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Tobacco streak virus (TSV) causes necrosis on leaf, veins, petiole and stem in Blackgram. The sap extracted from the infected blackgram plants remains infectious upto 24 hrs at room temperature. Infectivity of TSV on cowpea progressively reduced in response to increasing in the temperature and dilution. The present investigation was carried out to test the physical properties of TSV such as longevity storage period, thermal inactivation point and dilution end point on Cowpea (CV C- 125) isolated from naturally infected Blackgram plants (CO-8).

Introduction

Blackgram or Urdbean (*Vigna mungo* L. Hepper) is an excellent source of easily digestible good quality protein. The Successful cultivation of Blackgram was hindered by both biotic and abiotic factors. Plant pathogens including fungi, bacteria and virus are causing multifarious diseases on Blackgram plants. Among them, viral diseases are causing a drastic reduction on seed yield and quality which leads to serious economic losses (Kang *et al.*, 2005). Urdbean leaf crinkle virus and Mungbean yellow mosaic virus frequently causing diseases on Blackgram were already reported by several

researchers (Narayanasamy and Jaganathan, 1973; Srivastava, 2010; Shyam Singh and L.P. Awasthi, 2009). Ladhakshmi (2002) reported the Tobacco streak virus (TSV) inciting stem necrosis on Blackgram and yield loss of about 20 percent in Coimbatore during 2001. Study on infected plant parts of leaves, stems and seeds of Blackgram necrosis shown a positive reaction with TSV antiserum and purified samples showed isometric particles with a diameter of 27 nm under Transmission Electron Microscope (Ladhakshmi *et al.*, 2002). TSV was assigned by the ICTV to the Iarvirus group as the type member in the family Bromoviridae. TSV was described first by Johnson (1936) and its properties were

reviewed by Fulton (1970). The variability in the particle size is caused by the encapsulation of the different size of RNAs into separate virions. The size of the coat protein varies from 25 to 30 kDa (Guo *et al.*, 1999). TSV having worldwide distribution, was first discovered in tobacco (*Nicotiana tabacum*) in Brazil during 1940 (Costa, 1945). Over the 40 years, TSV was reported in crops like cotton, tomato, soybean, peanut, sunflower and some weeds in Brazil (Gracia and Feldman, 1974). In India TSV have been reported in various field crops, including sunflower, peanut, okra and cucurbits (Bhat *et al.*, 2002 a; Bhat *et al.*, 2002b; Prasada Rao *et al.*, 2000; Lava Kumar *et al.*, 2008; Vemana and Jain, 2010).

This study focusses on testing the sap of naturally infected Blackgram plants (CO-8) on Cowpea (CV C- 125) for the physical properties of TSV such as longevity storage period, thermal inactivation point and dilution end point.

Materials and Methods

Plant materials and source of the virus

TSV susceptible Cowpea variety CV C-125 seeds were obtained from Department of pulses, Tamil Nadu Agricultural University, Coimbatore, India. The seeds were sown in pots and raised under glass house condition. Blackgram plants showing typical symptom of necrosis on leaves were collected from Agricultural College and Research Institute, Killikulam, India and were used as source of virus.

The infected plants were identified by the presence of brown, necrotic spots on the young leaves, typical veinal necrosis followed by brown streaks on the petiole and stem. When the disease progresses, the tip of the stem start necrotized and leads to the death of plant.

Virus culture: isolation and extraction

One gram of infected blackgram leaves exhibiting typical symptoms were taken and sap was extracted by utilizing 3 ml of 0.1M sodium phosphate buffer (pH 7.0) containing mercaptoethanol (0.1%) in the precooled pestle and mortar kept in a frozen tray. The sap was mechanically transmitted to primary leaves of cowpea (6 days old), anteriorly dusted with 600-mesh carborundum powder to accommodate as abrasive, described by Hull (2009). The local lesions were produced 6 to 8 days after inoculation on the cotyledonary leaves of cowpea cv C-152 which was the source of inoculum throughout the period of study.

Physical properties of virus

Dilution end point

Crude sap was prepared using 0.1 M phosphate buffer at the ratio of 1:2 (w/v) in a precooled pestle and mortar from artificially inoculated cowpea leaves. By serial dilution method, 10^{-1} to 10^{-6} dilutions were prepared. The plants were inoculated with the sap from each dilution. Each dilution was inoculated on a set of four replication of test plants (cowpea cv C152). Undiluted sap was inoculated on another set of test plants to serve as control. Number of local lesions produced on the plants are observed and compared with the control to determine the dilution end point (Lavanya, 2001).

Thermal inactivation point

Thermal inactivation point of the virus in a crude juice was found out by taking 20 ml of extract. Two ml sap was pipetted out from the 20 ml extract into a thin-walled test tube, while pipetting out care should be taken to evade contamination in the walls of the test tube with the sap. Then the tubes were immersed in the water bath at temperature

ranges from 40°C, 45°C, 50°C, 55°C, 60°C and 65°C for 10 min. After 10 min., tubes were removed and cooled immediately and inoculated on cowpea plants.

Freshly extracted sap that is not subjected to any temperature treatment was inoculated on a set of test plants to serve as control. Number of local lesions produced on the plants are observed and compared with the control to determine the thermal inactivation point (Ladhalakshmi, 2002).

Longevity of the tobacco streak virus under *in vitro* condition

The infective sap was extracted and divided into several aliquots of 2 ml in each test tube and stored at room temperature and at 4°C. At different time intervals, starting from 0 to 32 h, the sap was inoculated on test plants of cowpea cv C-152 at room temperature. Observation was recorded on the third day after inoculation.

Freshly extracted sap inoculated to a set of test plants served as control. Number of local lesions produced on the test plants were observed and compared with the control to determine the longevity of the virus under *in vitro* condition (Lavanya, 2001).

Results and Discussion

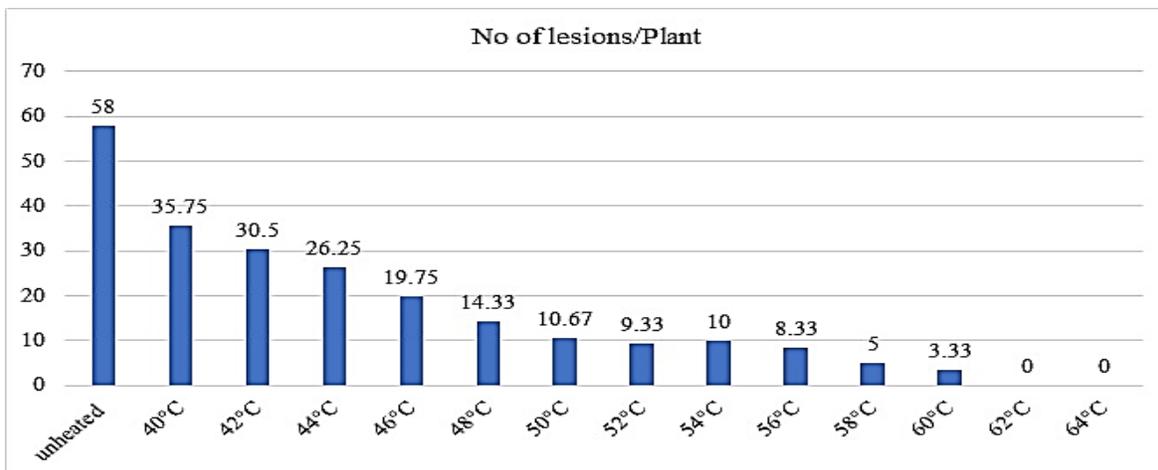
Identification and source of the virus

The Blackgram necrosis infected plants were identified by the presence of brown and necrotic spots on the young leaves, brown streaks on the petiole and stem. This virus causes typical veinal necrosis that systemically spreads to the petiole and stem which leads to death of whole plant. The leaves, petiole and stem of infected plant parts were used as a source of virus (Figure 1 and 2).

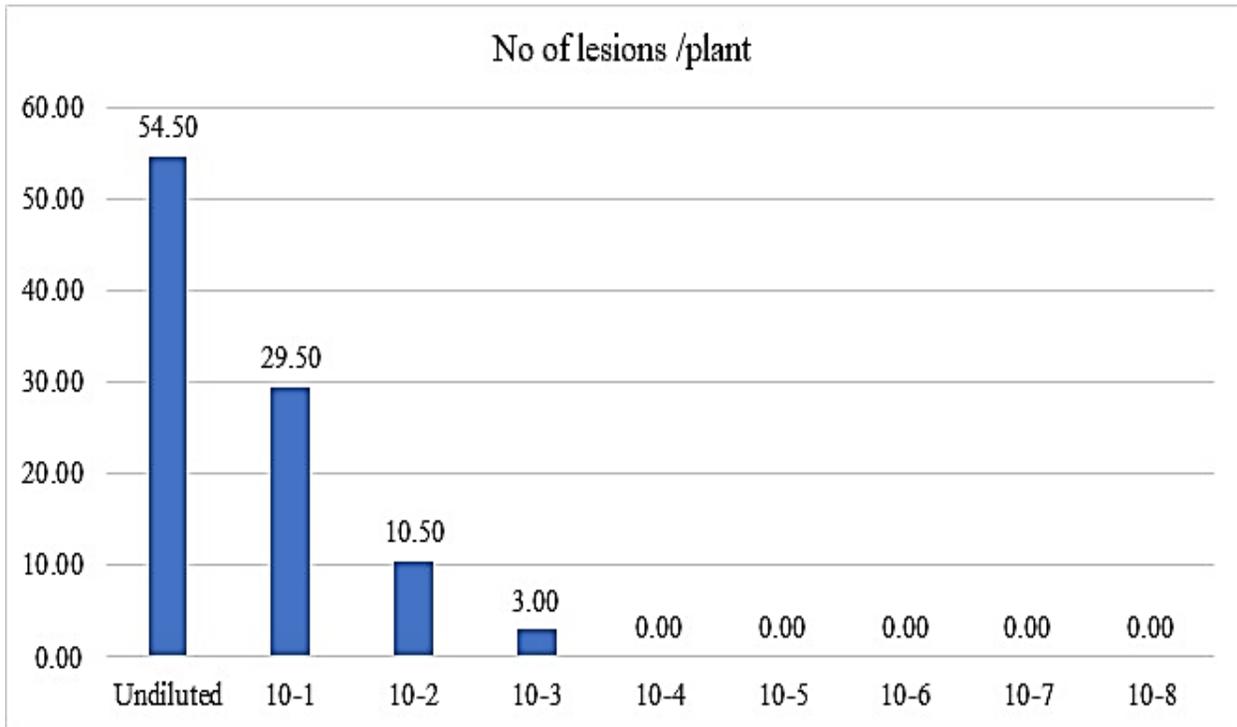
Isolation and maintenance of virus culture

Parts of Blackgram exhibiting the typical symptom of necrosis were collected and inoculated on 6-day old cowpea (C152) plants for multiplication. Invariably on the 8th day after inoculation the leaves produced distinct necrotic local lesions (Figure 3 and 4). The lesions were noticed more on the primary leaves which are subsequently dropped and the trifoliate leaves exhibit necrotic local lesions and the whole plant was died after 10 days.

Graph.1 Thermal inactivation point of the blackgram inciting TSV



Graph.2 Dilution end point of the blackgram inciting TSV



Graph.3 Longevity *in vitro* of the blackgram inciting TSV

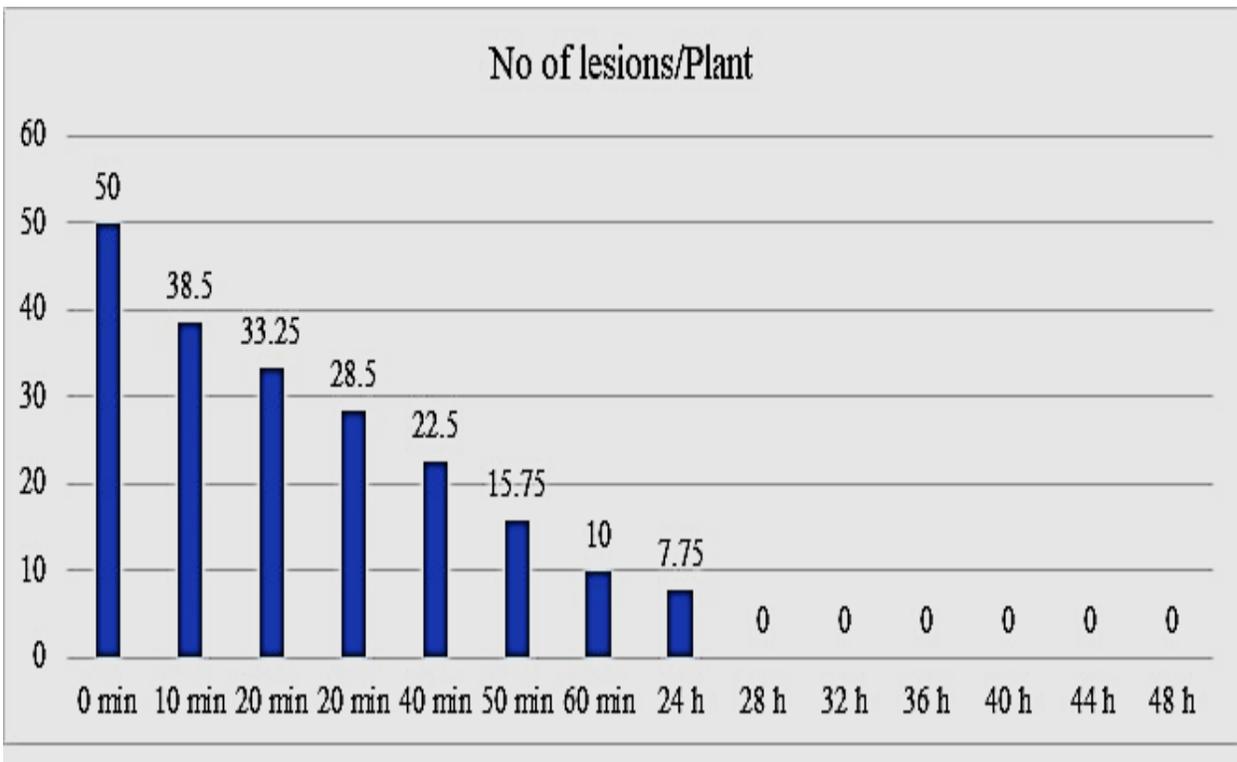


Fig.1 Veinal necrosis along with chlorosis on blackgram



Fig.2 Veinal necrosis along with necrotic streaks on blackgram



Fig.3 Chlorotic spots on cowpea



Fig.4 Veinal necrosis along with chlorosis on cowpea



There was also brown necrotic discoloration starting from top of the plant towards the stem where it caused stem necrosis. The virus culture was maintained regularly on cowpea plants and used for further studies.

Physical properties

The maximum lesions were observed on cowpea plants inoculated with unheated sap extracted from blackgram (58 lesions)

followed by 40°C (35.75 lesions). There was a progressive reduction in lesion development in response to increasing temperature. At 62°C, sap containing TSV lost its infectivity and shows no lesions (Graph 1). The undiluted infective sap shows maximum number of lesions (54.5) followed by 10⁻¹ dilution (29.50). Minimum number of lesions was observed at 10⁻³, above which there is no lesions were found to be produced. Infectivity were found to be decreased with the increase in dilution (Graph 2). Longevity test revealed that the sap immediately extracted from the Blackgram leaves were capable of producing maximum number of lesions (50) followed by 10 min storage (38.5). The infective sap remains viruliferous upto 24 h from the extraction after the infectivity were found lost (Graph 3).

The study of physical properties of blackgram inciting tobacco streak virus revealed that the virus infectivity was completely lost at a dilution of 10⁻⁵ and infectivity were lost in response with the increased dilution. Similar reports were given by Lavanya (2001) where sunflower necrosis virus lost its infectivity at 10⁻⁴ and 10⁻⁵.

Thermal inactivation point of TSV was found at 62°C. This agrees with Ramaiah *et al.*, (2001) in case of sunflower necrosis virus in which the thermal inactivation point was at the range of 50°C to 60°C. After the extraction TSV remains infective upto 24 h but, the maximum number of lesions were observed on the sap that used immediately after the extraction which was correlated with the findings of Ladhakshmi (2002). From the present study it is concluded that TSV causing necrosis diseases in blackgram, remains active and produces a greater number of lesions only at the temperature below 62°C and in addition the increase in dilution progressively reduces the symptom expression while the sap which were

inoculated immediately after extraction was found to cause numerous lesions with high infectivity.

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