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In vitro evaluation of different plant stages of blackgram to assess the susceptibility by Tobacco streak virus

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Abstract

Blackgram being an important pulse crop which is a rich source of protein. Tobacco streak virus causing necrosis on blackgram showing typical symptoms were identified in blackgram (Co- 8) fields in Killikulam, Tamil Nadu which is leading to severe yield loss. 10-day old plants were found to be most susceptible in which 93.12 percent transmission was observed. Necrosis in blackgram is caused by Tobacco streak virus produced systemic infection on all age of the plants in which up to 30-day old plants were produced local lesions. The present study was focused on to unravel the effect of age on susceptibility of blackgram plants to necrosis inciting Tobacco streak virus.

Keywords: tobacco streak virus, blackgram, necrosis, age of susceptibility

Introduction

Black gram (*Vigna mungo* L) is a protein rich food, containing about 26 percent protein, which is almost three times that of cereals (Peeta Gobi *et al.*, 2016) [11]. It ranks fourth among the major pulses cultivated in India and it also supplies a major share of protein requirement of vegetarian population of the country. Most emerging infectious diseases of plants are caused by viruses. Blackgram cultivation severely affected by viral diseases such as Mungbean yellow mosaic virus (MYMV) and Urdbean leaf crinkle virus (ULCV) (Narayanasamy & Jaganathan, 1973; Srivastava, 2010) [9, 13]. Tobacco streak virus (TSV) is a type member of Ilarvirus and reported mainly on herbaceous plants and causes necrosis diseases on tobacco, cotton dahlia, tomato, sunflower, groundnut, asparagus and legumes such as soyabean (Bhat *et al.*, 2002a; Bhat *et al.*, 2002b; Lava Kumar *et al.*, 2008; Vemana and Jain 2010) [2, 3, 7, 14]. The symptoms of the disease appear as necrosis of leaf lamina resulting in twisting of the leaf. The disease was found to infect the crop at all stages starting from seedling to maturity (Kannan, 2012) [5]. TSV infection during mid-stage of the plant growth may result in necrosis of the leaves and severe reduction in yield. Infection at late stage of the plant growth results in mild chlorotic symptoms, with little effect on plant growth and yield (Lavakumar *et al.*, 2008) [7]. TSV also produce leaf deformation on lettuce, necrotic lesions on *Chenopodium quinoa* and *Phaseolus vulgaris*, necrotic and chlorotic local lesion on *Nicotiana tabacum* cv. *Samsun* was reported by Abtahi (2009) [1]. The infected black gram plant shows brown, necrotic lesions on the young leaves and brown necrotic discoloration on the petiole and top portion of the stem. When the disease progresses, the stem and branches necrotized which lead to death of the plant was first reported by Ladhakshmi (2002) [6]. The present study focusses on influence of plant age on susceptibility to blackgram inciting TSV.

Materials and Methods

Plant Material and Source of the virus

Blackgram (CO-8) plants showing typical symptom of necrosis on leaves were collected from Agriculture College and Research Institute, Killikulam, Tamil Nadu, India and were used as source of virus. The infected plants were identified by the presence of brown, necrotic spots in the young leaves, typical veinal necrosis followed by brown streaks on the petiole and stem. Black gram (variety CO-8) seeds were obtained from Department of Plant Breeding and Genetics, Agriculture College and Research Institute, Killikulam. The seeds were sown in pots and raised under glass house condition.

Multiplication and propagation of the virus

One gram of blackgram leaves showing typical symptoms were taken and sap was extracted by using 3 ml of 0.1M sodium phosphate buffer (pH 7.0) containing mercaptoethanol (0.1%) in the pre-cooled pestle and mortar kept in an ice tray. The sap was inoculated on primary leaves of cowpea (6 days old), previously dusted with 600-mesh carborundum powder to serve as abrasive described by Hull (2009) [4]. The local lesions produced 6 to 8 days after inoculation on the cotyledonary leaves of cowpea CV C-152 were the source of inoculum throughout the period of study.

Susceptibility and yield of blackgram plants due to blackgram necrosis virus infection

A pot culture experiment was conducted to study the age of the plant in relation to susceptibility to TSV infection. The plants were mechanically inoculated at 10 days interval starting from 10 days after sowing (DAS) and up to 70 DAS. Percentage of the infected plants and yield of pods were recorded in each treatment. Percentage of the death of plants was calculated based on the mortality of the plants and pod yield was recorded in each treatment. In each treatment three replications and in each replication six plants were maintained

Result

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 systemically that spreads to the petiole and stem which leads to death of whole plant (plate 1a & 1b). The infected plant parts such as leaf, petiole, and stem were used as a source of virus.

Multiplication and propagation of the virus

Blackgram plant parts *viz.*, leaf, petiole, and stem exhibiting the typical symptom of necrosis were collected and inoculated on 6-day old cowpea (CV C-152) plants for multiplication. Invariably on the eight day after inoculation the leaves produced distinct necrotic local lesions (Plate 2a, 2b). The lesions were noticed more on the primary leaves which are subsequently dropped and the trifoliolate leaves exhibit necrotic local lesions and in about 10 days, the whole plant died. 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.

Susceptibility of blackgram plants to blackgram necrosis virus infection with respect to age and yield loss

Ten-day old plants were found to be most susceptible in which shows symptoms on 7.67 days after the inoculation followed by 20-day old plants (8.33 DAI) whereas 70-day old matured plants take 11.67 days for the expression of symptoms (Figure 1). Higher virus transmission percentage was observed on 10-day old plants (95.08) followed by 20-day old plants (86.62%). The lower transmission percentage was noticed on 70 days old plants (74.42) followed by 60-day old plants (76.17). Percentage of death plants due to this virus was higher while inoculated on 10-day old plants (95.08%) followed by 20-day old plants (92.83%) whereas 14.4 % plants only lead to death while inoculating on the 70-day old plants (Figure 2). If the virus infects the plants within 30 day old, yield would be completely affected. Blackgram inciting TSV produced systemic infection on all age of the Blackgram plants. Moreover within 6 to 12 days after the expression of multifarious necrosis symptoms on leaf, petiole, Stem incited by TSV lead to complete death of all age plants (table 1).



Plate 1: Typical veinal necrosis on Blackgram (CO-8)



Plate 2: Necrotic lesions on mechanically inoculated Cowpea (CV C-125)

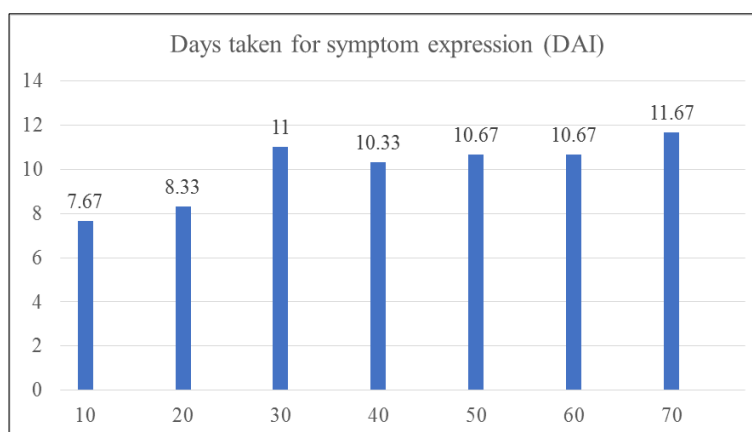


Fig 1: TSV inciting symptom expression on different age of plants

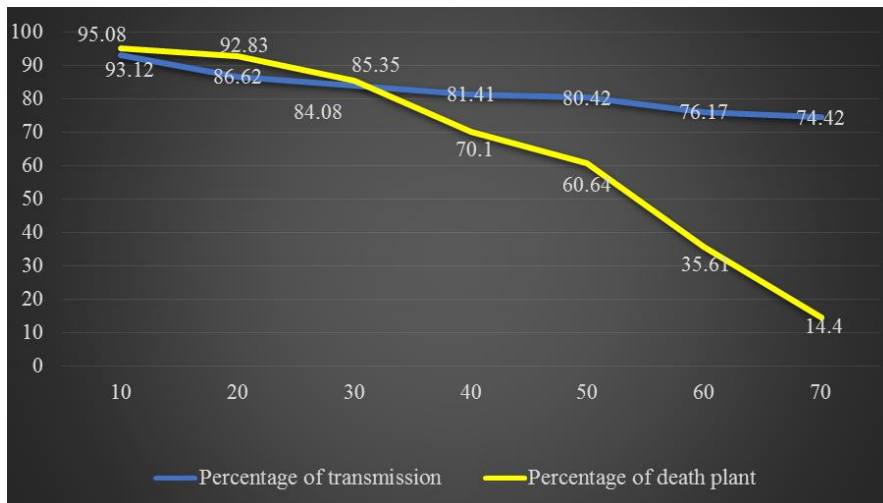


Fig 2: Transmission and death percentage of blackgram plants in response to TSV

Table 1: *In vitro* evaluation of yield at different age of the Blackgram plants in response to TSV

S. No	Plant age	Days taken for complete death of the plant (days after symptom expression)	Average yield/plant (g)	Symptoms on observed
1	10	6.33	0.013	systemic
2	20	6.67	0.02	systemic
3	30	7.33	0.04	systemic
4	40	8.67	1.45	systemic
5	50	9.00	1.96	systemic
6	60	11.00	2.54	systemic
7	70	11.67	2.96	systemic

Discussion

Infected blackgram plants were identified by presence of typical veinal necrosis and necrotic streaks on young leaves leads to drying of plants from top. The stem and petiole exhibited brown discoloration. Plants infected at early leads to premature death of the plants and cause severe yield loss. The infected plants also produced shrivelled grains which correlates the symptoms described by Ladhakshmi (2002) [6]. The virus sap was inoculated on the cotyledonary leaves of six days old cowpea (cv-152) plants. Eight days after inoculation, inoculated plants exhibited showed distinct necrotic symptoms including typical necrotic lesions. Similar characteristic symptoms of tobacco streak virus (TSV) on cowpea were described on sunflower (Papiah *et al.*, 2012) [10], cotton (Waqar Ahmed *et al.*, 2003) [15] and soybean (Rajamanickam *et al.*, 2016) [12]. 10-day old plants were more susceptible as compared with other age plants. When the Blackgram necrosis virus was inoculated on 10-day old plants the death of the plants will be higher (95.08 %). Whereas it was only 14.42 percent in 70-day old plants. Blackgram plants infected earlier (up to 30 days after sowing) with TSV leads to complete reduction in yield which agrees with the blackgram plants infected earlier were found to be highly susceptible to TSV whereas susceptibility was found decreasing with increase in age reported by Ladhakshmi (2002) [6].

Conclusion

In this present study, TSV could systemically infected all age of the plants. Since all the ages of the plants are vulnerable to infection, the peak infection period falls up to 30 days after sowing and it leads to drastic changes in plant performance in terms of growth and development. Hence, it has essential to incorporate management strategies at earlier stages of the crop growth to avoid the yield loss.

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