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Characterisation of Blackgram Leaf Crinkle Virus.

By

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ABSTRACT

The blackgram leaf crinkle virus (BLCV) lost its activity at dilutions above 1:5000, at temperatures above 60°C, and in storage after 48 hours at room temperature. The optimum pH was found to be 7.2. BLCV was antigenic, and capable of reacting specifically with its antiserum. Of the 24 plant species tested cowpea, cluster beans greengram, groundnut and redgram were infected by BLCV.

INTRODUCTION

The transmissibility of Blackgram Leaf Crinkle virus (BLCV), through sap inoculation and seeds was reported by Kolte and Nene (1972). The white flies *Bemisia tabaci* Genn. were found to be the vectors of BLCV(Narayanasamy and Jaganathan, 1973). With the aim of characterising BLCV, the physical and serological properties and host range of the virus were studied and the results are reported in this communication.

MATERIALS AND METHODS

Infected blackgram (*Phaseolus mungo* CO1 plants were used as virus sources. The standard infective sap was prepared by grinding BLCV- infected blackgram leaves with O.IM phosphate buffer added at the rate of I ml/g of leaf tissue and expressing the sap through cheese cloth. The dilution end point, longevity *in vitro*, thermal inactivation point and pH stability of BLCV were determined by following standard procedures.

The antiserum against BLCV was prepared by injecting purified BLCV into the rabbit. One intramuscular and four intravenous injections through marginal veins of the ear were given at weekly intervals. The animal was bled after giving a rest period of two weeks after the last injection. The blood was allowed to clot and after 4 hours, the supernatant serum was removed and cenrtifuged at 3000 rpm for 30 minutes to remove the blood cells and stored with sodium azide added as a preservative in small vials. The precipitation test was carried out as per the procedure described by Matthews (1957).

For host range studies, the different plant species were inoculated by rubbing the standard infective sap on carborundum-dusted leaves.

RESULTS AND DISCUSSION

Physical properties: The dilution end point of BLCV was detemined by prepa ring dilutions of 1: 10, 1: 100, 1: 1,000, 1: 5.000 and 1: 10.000 of infective sap and inoculating healthy test plants with each dilution of the inoculum.

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TABLE 1. Dilution end point of BLCV

Dilution	Number of plants infected/inoculated
1:1	8/10
1:10	5/10
1:100	3/10
1.1,000	2/10
1:5,000	1/10
1:10.00	0/10

The results presented in Table I show that BLCV lost its activity at dilutions above 1: 5,000. The BLCV of Pantnagar had a dilution end point between 1: 10,000 and 1: 100,000 (Nene and Kolte, 1972).

To find out the longevity *in vitro* of BLCV, the sap expressed from infected leaves was stored at room temperature (28-30°C). The infected leaves were also stored at room temperature and the inoculum was prepared when required. The test plants were inoculated after different periods of storage.

TABLE 2. Longevity *in vitro* of BLCV.

Period of storage	Type of inoculum	
	Expressed sap	Leaf tissue
0 hours	8/10*	7/10
24 ,,	2/10	4/10
48 ,,	1/10	2/10
72 ,,	0/10	0/10
96 ,,	0/10	0/10

* Number of plants infected/number of plants inoculated.

It was observed that BLCV was not active after 48 hours of storage at room temperature (Table 2). Nene and Kolte (1972) reported that BLCV of Pantnagar could retain infectivity for 3 days at room temperature (30-33°C).

The thermal inactivation point of BLCV was determined by subjecting the standard sap kept in thin-walled test tube to different temperatures for 10 minutes and immediately immersing the tubes in ice water. The virus was inactivated at temperatures above 60°C (Table 3).

TABLE 3. Thermal inactivation point of BLCV.

Temperature (°C)	Number of plants infected/ inoculated
40	3/10
50	2/10
60	1/10
70	0/10
80	0/10
Contro	8/10

Nene and Kolte (1972) also observed that BLCV was inactivated at temperatures between 60° and 70°C.

To find out the effect of different pH levels on the infectivity of BLCV, inocula of the virus were prepared with buffers adjusted to pH 6.0, 6.5, 7.0, 7.2, 7.5 and 8.0 and sets of 10 healthy plants were inoculated with the diffe-

TABLE 4. pH stability of BLCV.

pH	Number of plants infected/inoculated
6.0	4/10
6.5	4/10
7.0	5/10
7.2	8/10
7.5	5/10
8.0	2/10

rent inocula. The results presented in Table 4 indicate that the maximum infectivity of BLCV was retained when the buffer with pH 7.2 was used to prepare the inoculum.

Serological property: The precipitin test indicated that BLCV was antigenic, capable of stimulating the production of specific antibodies with which it united forming a precipitate. The titre of the antiserum was found to be 1/8 beyond which dilution no visible precipitate was formed (Table 5).

TABLE 5. BLCV antiserum titre

Dilutions of antiserum	Relative amount of precipitate
1:1	+++
1:2	++
1:4	+
1:8	+
1:16	no precipitate
1:32	"

Different leguminous plants, vegetable crop plants and weeds present in blackgram fields were inoculated to study the host range of BLCV. Of the 24 plant species inoculated, clusterbeans, cowpea greengram, groundnut and redgram developed symptoms of virus infection (Table 6). The BLCV induced mild mosaic patterns on cowpea leaves. The margins of leaves of infected redgram exhibited crinkling, while narrowing of leaves and crinkling were observed in clusterbeans. Infected groundnut plants showed cupping of leaves which turned chlorotic later.

Table 6. Host range of BLCV.

Name of plant	Number of plants infected/total	Incubation period (days)
Clusterbeans *(Cyamopsis tetragonoloba L.)*	3/18	28-30
Cowpea (*Vigna sinensis* (L) Savi ex Hassk.)	3/17	20-23
Greengram *(Phaseolus aureus* (L) Roxb.	3/45	25
Groundnut *(Arachis hypogaea* (L.)	4/20	26-28
Redgram (*Cajanus cajan* Millsp.)	4/20	25-26

The following plant species did not develop any symptom of infection: *Abelmoschus esculentus* Moend., *Acanthospermum hispidum* DC, *Capsicum annuum* L., *Chenopodium amaranticolor* Coste & Reyn., *Cicer arietinum* L., *Ciitoria ternatea* L., *Crotalaria juncea* L., *Cucurbita papo* L., *Dolichos lablab* L., *Glycine max* (L) Merr *Lycopersicon esculentum* Mill., *Malvastrum coromandalianum* Garcke., *Medicago sativa* L., *Momordica charantia* L., *Nicotiana glutinosa* L., *Nicotiana tabacum* L., *Sesbania grandiflora* Pers., *Solanum melongena* L., and *Trianthema portulacastrum* L.

The present BLCV as well as that of Pantnagar had similar thermal inactivation point. But the present BLCV differed from Pantnagar BLCV, in having lower dilution end point and shorter longevity *in vitro*. The host range studies revealed that the present virus could infect clusterbeans, cowpea, greengram, groundnut and redgram, while Nene and Kolte (1972) observed that BLCV of Pantnagar infected cowpea, greengram and *Phaseolas aconitifolius*. The differnces in some of physical properties and host range indicate that the present virus may be different strain of blackgram leaf crinkle virus.

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