Virus-vector relationships of chickpea chlorotic dwarf virus (CpCDV) and sesame jassid (*Orosius orientalis*) in chickpea

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ABSTRACT

Serological testing using tissue blot immunoassay (TBIA) showed a close association between CpCDV and stunt disease in chickpea. Therefore, a glasshouse investigation was initiated at Hudeiba Research Station, the River Nile State, Sudan, in 2006 to verify the role of *O. orientalis* as an insect vector in disease transmission. The results showed that disease transmission efficiency was increased as the number of leafhopper (*O. orientalis*) increased from one to 15 insects/ plant. The minimum virus acquisition and inoculation access periods (AAP and IAP) were found to be about 5 min. The latent and retention periods were 180 min and 17 days, respectively.

INTRODUCTION

Stunt disease is among the most serious biotic stresses limiting chickpea production in Sudan and causing serious economic losses. Although it was first observed in the early 1990s (Abdalla and Van Rheenen, 1991), the disease etiology was not known until mid 1990s when Makkouk and his colleages reported CpCDV as a major causal agent of stunt disease (Makkouk *et al*., 1995). Disease symptomatic features included leaf yellowing, internode shortening, overall dwarfing and phloem browning in the collar region (Kaiser, 1972; Nene and Reddy, 1976; Reddy *et al*., 1979; Jiha *et al*., 1981; Nene and Reddy, 1987; Kaiser *et al*., 1988; 1990; Horn *et al*., 1996). Inspite of importance of CpCDV, its vector has not been known in Sudan and no information is available on the CpCDV-vector relationship. This study was aimed at availing knowledge on virus/vector relationship as a pre-requiste for understanding disease epidemiology and ultimately adopting successful disease management strategies.

MATERIALS AND METHODS

All experiments were carried out under glasshouse conditions in 2006 at Hudeiba Research Station, the River Nile State, Sudan.

Source and maintenance of virus isolate

Infected chickpea plants (cv.ICCV-2) showing typical symptoms of stunt disease were used as a source of CpCDV. The virus was maintained on renewable chickpea host plants kept in cylindrical iron framed screened cages, 30 cm in length and 12 cm in diameter and tightly covered with a cloth.

Source and maintenance of leafhopper

A random culture of the leafhopper, *Orosius orientalis* (Matsumura) previously named *O. albicinctus* (Distant) was collected from chickpea fields at Hudeiba Research Station Farm and maintained on renewable ICCV-2 plants in cylindrical iron framed screened cages in a glasshouse.

Efficiency of leafhopper in transmitting CpCDV

To determine the identity and efficiency of leafhoppers in CpCDV transmission, non-viruliferous young adults of the leafhoppers, *O. orientalis*, *Agallia* spp and *Neolimnus aegyptiacus*, predominantly intercepted leafhopper species on chickpea, were kept on infected chickpea plants in tightly closed cylindrical iron framed cages for 72 hours as acquisition feeding periods. Thereafter, different groups of 1, 3, 5, 10, or 15 leafhoppers were transferred to healthy chickpea (cv.ICCV-2) seedlings for 72 hours after which the insects were eliminated by spraying with Pprimor ® insecticide. The plants were tested after 20 days from inoculation for CpCDV by tissue blot immunoassay (TBIA) (Lin *et al.*, 1990; Hsu and Lawsan, 1991). The estimated percentage of infective individuals of leafhopper was determined by the maximum likelihood estimator, P*, which is defined by Gibbs and Gower (1960) as the estimated probability of virus transmitted by a single individual vector.

$P^* = 1 - (1 - R/N)^{1/i}$ where R is the number of infected plants, N is the total number of target plants and i is the number of leafhoppers per target plant.

$P^* \times 100$ = the estimated percentage of the infective individuals of young adult leafhoppers.

Acquisition access period (AAP)

This experiment was conducted in the glasshouse at 25-30^{0}C and relative humidity of 40-50%, at Hudeiba Research Station, the River Nile State, Sudan, to determine the minimum time needed by *O. orientalis* to acquire CpCDV from diseased plants. Groups of non- viruliferous leafhoppers were allowed to feed on infected chickpea plants at different acquisition access periods of 5, 15, 30, 60 or 120 min on infected chickpea plants. Fifteen to twenty insects/ plant from each group were then removed and kept on healthy chickpea plants for 72 hours to inoculate the virus. The plants were tested for the virus 20 days later. The experiment was repeated three times.

Inoculation access period (IAP)

This expeiment was conducted in the glasshouse at the temperature and relative humidity mentioned above, to determine the minimum time required by the vector to inoculate the virus. Large numbers of *O. orientalis* were given an acquisition feeding period of 72 hours on infected chickpea plants. Then groups of viruliferous leafhoppers were allowed to feed on healthy plants (at the seedling stage) at different inoculation access periods of 5, 15, 30, 60, or 120 min. At the end of each inoculation period, the plants were sprayed with an insecticide. The experiment was replicated three times.

Retention period

The aim of this experiment was to determine the period during which the virus could persist within the body of its vector and transmit the virus after feeding only once on the virus source. Groups of the last instar nymphs of non- viruliferus *O. orientalis* were given a 2-day acquisition period on infected chickpea plants. Then groups of 5 insects/plant were transferred serially at one- day interval to healthy chickpea plants at seedling stages. The experiment was replicated five times.

Latent period

In this experiment groups of young adult leafhoppers were given acquisition feeding periods for one hour on infected plants. Ten individuals/plant were then transferred serially to healthy plants at one hour intervals.

RESULTS

Efficiency of CpCDV transmission

When three species of leafhoppers; Agallia spp, Neolimnus aegyptiacus and Orosius orientalis were used to test their ability to transmit CpCDV, only *O. orientalis* was found to transmit the virus. Thus *O. orientalis* was used to study the relationship between the virus and the vector in all other experiments. Successful transmissions by a single insect of *O. orientalis* reached 30%. The transmission rate increased with increasing the number of leafhoppers. The use of 3 and 5 leafhoppers per plant gave rates of transmission of 70% and 88%, respectively. CpCDV transmission efficiency increased to 100% when a group of 10 and 15 insects were used. The estimated percentages of infective individuals ranged from 18.1% to 45.1% (Table 1). All plants expressing characteristic symptoms gave positive reactions when tested by TBIA.

Table 1. Transmission efficiency of CpCDV using different numbers of *O. orientalis*

Expt	No. of leafhoppers per plant	No.of infected plants/no. of inoculated	Successful transmission (%)	Infective individuals (%)
	1	3/10	30	30.0
	3	6/10	60	26.3
	5	8/10	80	27.5
1	10	10/10	100	25.9
	15	10/10	100	18.1
	1	4/10	40	40.0
	3	8/10	80	41.5
2	5	8/10	80	27.5
	10	10/10	100	25.9
	15	10/10	100	18.1
	1	2/10	20	20.0
	3	6/10	60	26.3
3	5	10/10	100	45.1
	10	10/10	100	25.9
	15	10/10	100	18.1
	1	3/10	30	30.0
	3	8/10	80	41.5
4	5	9/10	90	36.9
	10	10/10	100	25.9
	15	10/10	100	18.1
	1	12/40	30	30.0
	3	28/40	70	33.1
Total	5	35/40	88	34.0
	10	40/40	100	25.9
	15	40/40	100	18.1

Acquisition access period

Results of this experiment indicated that the minimum period required by *O. orientalis* to acquire CpCDV from infected chickpea plants was about 5 minutes with 13% successful transmission (Table 2). An acquisition period of 2 hours led to 86% transmission. The transmission rate increased with increasing AAP. Only 3% of leafhoppers individuals used for 5 mintues could transmit the virus, while 33% of the individuals transmitted the virus after an acquisition access period of 2 hours.

Table 2. Effect of different acquisition access periods (AAP) on transmission of CpCDV by *O. orientalis*.

Expt	AAP (min)	No. of infected plants/no.of inoculated	Succeful transmission (%)	Infective Individual (%)
	5	0/10	0	0.0
	15	2/10	20	4.4
1	30	4/10	40	9.7
	60	7/10	70	21.4
	120	8/10	80	27.5
	5	2/10	20	4.4
	15	3/10	30	6.9
2	30	4/10	40	9.7
	60	8/10	80	27.5
	120	8/10	80	27.5
	5	2/10	20	4.4
	15	3/10	30	6.9
3	30	4/10	40	9.7
	60	10/10	100	45.1
	120	10/10	100	45.1
	5	4/30	13.3	2.8
	15	8/30	26.7	6.1
Total	30	12/30	40.0	9.7
	60	25/30	83.3	30.1
	120	26/30	86.7	33.2

Inoculation access period

The results of this experiment showed that the shortest period needed by the viruliferous *O. orientalis* to reach the tissues of healthy chickpea plants and inoculate CpCDV was about 5 minutes (Table 3).

An inoculation access period of 5 minutes led to 16% transmission, whereas IAP of 2 hours gave about 100% transmission. As in acquisition period trials, only 3% of the leafhopper individuals transmitted the virus when given an inoculation period of 5 minutes, whereas about 40% of the leafhoppers transmitted the virus when they had an inoculation period of 2 hours.

Table 3. Effect of different inoculation access periods (IAP) on transmission of CpCDV by *O. orientalis*

Expt	IAP (min)	No.of infected plants/no. of inoculated	Successful transmission (%)	Infective individuals (%)
	5	1/10	10	2.1
	15	3/10	30	6.9
1	30	6/10	60	16.7
	60	9/10	90	36.9
	120	9/10	90	36.9
	5	2/10	20	4.4
	15	4/10	40	9.7
2	30	6/10	60	16.7
	60	10/10	100	45.1
	120	10/10	100	45.1
	5	2/10	20	4.4
	15	5/10	50	12.9
3	30	6/10	60	16.7
	60	9/10	90	36.9
	120	10/10	100	45.1
	5	5/30	16.7	3.6
	15	12/30	40.0	9.7
Total	30	18/30	60.0	16.7
	60	28/30	93.3	41.8
	120	29/30	96.7	42.4

Latent period

The results of this trial showed that *O. orientalis* can transmit CpCDV only three hours after the starting of an acquisition feeding (Table 4). This period of time may be needed by the virus to circulate from the stylet to the salivary glands.

Retention period

In this trial, last instar nymphs and young adults of *O. orientalis* were used to secure a higher number of transfers of leafhoppers to the tested plants. Thus, the maximum period for leafhoppers to retain and transmit the virus could be determined. The results showed that CpCDV could be transmitted successfully during 17 days and before the death of most of the insects initially used.

The last transfer was done by using the only 3 remaining leafhoppers before they died and it led to a 30% successful transmission.

Table 4. Serial transmission of CpCDV by *O. orientalis* following one hour acquisition access period.

Serial transfer (hr)	No. of infected plants/ no. of inoculated plants	Successful transmission (%)
1	0/5	0
2	2/5	40
3	3/5	60
4	3/5	60
5	5/5	100
6	5/5	100

DISCUSSION

The presence of cultivated and wild hosts throughout the year, plus favourable weather conditions for vector populations, make virus diseases one of the most important problems for growers. Therefore, a better understanding of virus/vector relationships and vector transmission efficiency is necessary for the development of effective control measures.

In the present study, CpCDV could be transmitted using a single leafhopper and the successful transmission increased with increasing numbers of leafhoppers. Seventy percent, 88% and 100% CpCDV infection was obtained when, respectively, 3, 5 and 10 viruliferous *O. orientalis* were used per plant. Symptoms appearance and severity seems to be correlated with the number of insects used. Ten days were enough for symptoms appearance when groups of 10 or 15 leafhoppers were used, while they needed a longer time, about 20 days with the use of 1 or 3 leafhoppers. Consequently, for screening chickpea genotypes for resistance to CpCDV in Sudan, it would be adequate to use 10 insects/plant. It is only *O. orientalis* that is, up to now, confirmed to be the vector of CpCDV, but the invlovement of other leafhopper species in CpCDV transmission is needed to be investigated.

O. orientalis as a pest seems to have importance in sesame in some countries like India, but it has no direct damage on winter legume crops or on sesame in Sudan. The importance of *O. orientalis* as a vector of phytoplasma, particularly those causing phyllody of sesame and faba bean is still needed to be studied.

Table 4. Serial transmission of CpCDV by *O. orientalis* following one hour acquisition access period.

Serial transfer (hr)	No. of infected plants/ no. of inoculated plants	Successful transmission (%)
1	0/5	0
2	2/5	40
3	3/5	60
4	3/5	60
5	5/5	100
6	5/5	100

Chickpea is seldom colonized by *O. orientalis* in the field probably due to the fact that chickpea exudes a fluid containing a high concentration of malic acid which is known to deter insect attack, however, the crop is still liable to high incidence of CpCDV infection, particularly the early sown crop.

Although *O. orientalis* can acquire and inoculate CpCDV into chickpea plants in less than five minutes, longer periods of time are required for efficient transmission. The vector also has the capability to retain the virus for more than two weeks within its body. So, the ability of the leafhopper to remain viruliferous for a long time without feeding on infected plants suggested possible involvement in long distance spread of CpCDV. Horn (1994) and Horn *et al*. (1993) showed that CpCDV was acquired and inoculated by *O. orientalis* in less than two minutes and was retained within the vector for up to three weeks. Studies on transmission of other geminiviruses transmitted by leafhoppers as beet curly top virus (BCTV) was found to be acquired and inoculated by *Circulifer tenellus* in about one minute with latent period of five hours (Bennet and Wallacae, 1938). The retention of CpCDV within the body of the vector for more than 20 days, the transmission of the virus after ecdysis and the latent period of three hours indicated that the virus was transmitted in a circulative persistent rather than a nonpersistent manner and this has important implications in terms of virus spread and control. The quick acquisition and inoculation of the virus may indicate that either *O. orientalis* can reach the phloem very rapidly or that CpCDV is not a phloem limited virus. However, two facts suggested that CpCDV is concentrated in the phloem tissues of diseased plants and these facts are; (1) leafhoppers are known to be phloem- feeders (Day *et al*., 1952) and (2) the extensive phloem browning of diseased chickpea plants.

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علاقة تقزم واصفرار الحمص مع حشرة نطاط الاوراق *Orius orientalis* فى محصول الحمص

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الخلاصة

أوضح استعمال اختبار الوصمة النسيجية السيرولوجى العلاقة الوطيدة بين فيروس تقزم واصفرار الحمص و مرض التقزم. تم بمحطة بحوث الحديبة بولاية نهر النيل في عام 2006 وذلك لمعرفة دور حشرة نطاط اجراء عدد من التجارب بالبيت الزجاجى أوضحت النتائج ان فاعلية الحشرة فى نقل المرض ازدادت مع زيادة عدد فى نقل المرض.*Orius orientalis* الاوراق الحشرات المستعملة من واحد الى 15 حشرة، و كذلك أوضحت النتائج أن أقل فترة لاكتساب ونقل الفيروس بواسطة الحشرة هى حوالى 5 دقائق و فترة حضانة الفيروس حوالى 3 ساعات. وجد كذلك ان للحشرة القدرة للاحتفاظ بالفيروس لمدة 17 يوماً وحتى موتها.