STUDIES ON LEAF CRINKLE DISEASE OF URD BEAN (VIGNA MUNGO (L.) Hepper)/MUNG BEAN (VIGNA RADIATA (L.)Wilczek) AND ITS CONTROL

By

OM PARKASH KADIAN

Dissertation submitted to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of :

DOCTOR OF PHILOSOPHY

in

PLANT PATHOLOGY

College of Agriculture

H i s s a r

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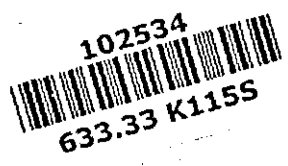
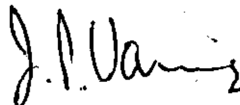


CERTIFICATE I

This is to certify that this dissertation entitled, "Studies on Leaf Crinkle Disease of Urd Bean (*Vigna mungo* (L.) Hepper)/Mung Bean (*Vigna radiata* (L.) Wilczek) and its Control" submitted for the degree of Doctor of Philosophy in the subject of Plant Pathology to the Haryana Agricultural University, is a bonafide research work carried out by Mr. Om Parkash Kadian under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been duly acknowledged.



3/XI/80

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CERTIFICATE II

This is to certify that the dissertation entitled, "Studies on Leaf Crinkle Disease of Urd Bean (Vigna mungo (L.) Hepper)/Mung Bean (Vigna radiata (L.) Wilczek) and its Control" submitted by Mr. Om Parkash Kadian to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the subject of Plant Pathology has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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OP Kadian 30/10/80

HISSAR
October 30,1980.

(OM PARKASH KADIAN)

DEDICATED TO MY MOTHER

EXPIRED DURING THIS STUDY

C O N T E N T S

Sr.No.	Description	Page No.
I	INTRODUCTION	1 - 3
II	REVIEW OF LITERATURE	4 - 12
III	MATERIAL AND METHODS	13 - 36
IV	RESULTS	37 - 95
V	DISCUSSION	96 - 111
VI	SUMMARY	112 - 117
	LITERATURE CITED	i - xiv

INTRODUCTION

Among pulses, mung bean (Vigna radiata (L.)Wilczek) and urd bean (Vigna mungo (L.) Hepper) occupy important position in Indian agriculture. Uttar Pradesh, Madhya Pradesh, Rajasthan, Punjab and Haryana are among the major mung bean and urd bean growing states in India.

In Haryana during the years 1974-75 and 1975-76, out of the total cropped area of 4842 and 5451 thousand hectares, mung bean and urd bean crops were grown in 23.1 and 25.8 thousand hectares and total production was 9.8 and 17.2 thousand tonnes,respectively (Anonymous, 1976, 1977 a).

These crops suffer due to several diseases of fungal, bacterial and viral origin. Of these, virus diseases are the most important limiting factors in their successful and profitable cultivation. Among viral diseases, yellow mosaic, leaf crinkle and mosaic (mottle) are important but yellow mosaic and leaf crinkle are the most destructive (Nene, 1972; Raychaudhuri et al., 1977). Leaf crinkle disease is reported to be wide spread in India (Williams et al., 1968; Nene, 1968; 1972; Khatri et al., 1971; Narayanasamy and Jaganathan, 1973; Gupta, 1974; Raychaudhuri et al., 1977; Singh et al., 1979). Information on yellow mosaic disease is available upto some extent but the information on leaf crinkle disease is scanty. Due to leaf

crinkle, there is either no pod formation or occasionally a few pods are formed on plants. The extent of losses due to this disease was estimated from 62 to 100 per cent at Pantnagar in Uttar Pradesh (Nene, 1972). This disease was also observed in International Mung bean Nurseries grown from the seed samples collected from India. It was considered to be caused by an unidentified virus causing the symptoms of leaf crinkling, dwarfing or flower abortion (Poehlman, 1977).

In Haryana, it has become serious on urd bean and mung bean during the recent years (Singh, 1974; Singh et al., 1979). However, this disease has been studied to a limited extent in India (Nene, 1972) but no studies seems to have been done abroad. Information regarding natural mode of transmission, host range, factors affecting disease development, control measures is scanty. Physical and chemical characteristics of the virus causing the disease are unknown since the virus was not purified.

Considering the growing importance of this disease and lacunae in knowledge about the virus and the disease, it was felt desirable to investigate leaf crinkle disease in detail. The studies as presented in this dissertation enabled us to understand the virus and various other aspects of the disease in order to devise the practical

methods of control. The present dissertation contains the results of investigations on leaf crinkle disease of urd bean and mung bean mainly on the following aspects:

1- Survey and epidemiology

2- Purification and identification of the virus

3- Changes in protein constitution of resistant and susceptible varieties of mung bean and urd bean

4- Control measures.

REVIEW OF LITERATURE

A number of elongated, isometeric and bacilliform plant viruses are known to occur all over the world as pathogens of leguminous crops (Weiss, 1939, 1945; Nariani, 1960; Bos, 1964; Nene, 1968, 1973; Smith, 1972; Singh, 1974; Sen Gupta, 1974; Bird and Maramorosch, 1975; Vanderveken, 1976; Raychaudhuri *et al.*, 1977; Dante and Dolores, 1977; Hampton *et al.*, 1978; Hampton and Braverman, 1979). The important legume viruses reported so far in the literature are: alfalfa mosaic virus, bean leaf roll virus, bean atypical mosaic virus, bean common mosaic virus, bean pod mottle virus, bean southern mosaic virus, bean severe mosaic virus, bean yellow dot virus, bean yellow stipple mosaic virus, bean yellow mosaic virus, broad bean stain mosaic virus, broad bean true mosaic virus, broad bean wilt virus, clover (Red) mosaic virus, clover (Red) mottle virus, clover (Red) vein mosaic virus, clover (White) mosaic virus, clover wound tumour virus, clover yellow mosaic virus, clover yellow vein mosaic virus, cowpea mosaic virus, cowpea aphid borne mosaic virus, cowpea chlorotic mottle virus, double bean yellow mosaic virus, groundnut mottle virus, groundnut stunt virus, leaf crinkle virus of urd, mung little leaf virus, mung yellow mosaic virus, pea early browning virus, pea enation mosaic virus, pea leaf roll virus, pea mosaic virus,

pea necrosis virus, pea seed borne mosaic virus, pea streak virus, pea stunt virus, pea wilt virus, soybean mosaic virus, soybean stunt virus, sunhemp mosaic virus and urd leaf curl virus (C.M.I. /A.A.B. Description of plant viruses, 1970-1978).

Mung bean (V.radiata (L.) Wilczek) and urd bean (V.mungo (L.) Hepper) are also the hosts of several viruses e.g. yellow mosaic virus (Nariani, 1960; Nene, 1972; Raychaudhuri et al., 1977; Prem Chand, 1978; Singh et al., 1979), leaf crinkle virus (Williams et al., 1968; Nene,1969; Grewal, 1977), mosaic (mottle) virus (Sahare and Raychaudhuri, 1963; Nene, 1973; Singh and Varma, 1977), leaf curl virus (Nene, 1972; Raychaudhuri et al., 1977), cucumber mosaic virus (Purivirojkul et al., 1978), little leaf virus (Dante and Dolores, 1977), bean southern mosaic virus (Iwaki and Auzay, 1977), satellite tobacco necrosis virus (Liu, 1969; Yang, 1977), bean (common) mosaic virus (Pierce, 1934; Yarguntiah and Nariani, 1963; Kaiser and Mossahebi, 1974), bean yellow dot virus (Thomas, 1951), cowpea mosaic virus (Dale, 1949), bean severe mosaic virus (Naraini and Kandaswami, 1961), alfalfa mosaic virus (Price, 1940), bean yellow mosaic virus (Pierce, 1934), bean yellow stipple virus (Zaumeyer and Thomas, 1950), double bean yellow mosaic virus (Capoor and Varma, 1948), pea wilt virus (Fry, 1959), pea enation mosaic

virus (Bustrillos, 1965), tobacco ring spot virus (Iwaki and Auzay, 1977) and tobacco mosaic virus (Ramakrishnan et al., 1968, 1973).

Leaf crinkle disease of urd bean was first observed in 1966 in union territory of Delhi and in Uttar Pradesh (Williams et al., 1968). Later it was also observed in Uttar Pradesh (Nene, 1968), Punjab (Bindra, 1971; Khatri et al., 1971), Haryana (Singh, 1974), Himachal Pradesh (Gupta, 1974) and Tamil Nadu (Narayanasamy and Jaganathan, 1973) causing stunting of plants, conspicuous crinkling on upper surface of leaves with rugosity and enlargement of leaf lamina, bushy appearance of inflorescence, flower abortion and supression of pod formation. In Haryana this disease was recorded to be increasing from 1973 through 1975 in different districts (Singh et al., 1979) on mung bean and urd bean crops. This disease was also observed (Poehlman, 1977) in International Mung bean Nurseries grown from seeds collected from India and caused by an unidentified virus. Iwaki and Auzay (1977) reported that the leaf crinkle symptoms were present on some mung bean fields in Indonesia.

Very little work was done on the estimation of losses in yield due to this disease. Nene (1972) recorded the losses in yield from 62 to 100 per cent.

Information on the effect of environment on disease incidence and development is not available.

A perusal of literature indicates that some attempts were made to study the transmission of this disease. Williams *et al.* (1968) attempted sap transmission but failed to transmit the disease. Later, Bindra (1971), Khatri *et al.* (1971) succeeded in the transmission of this disease through sap indicating the viral nature of the disease. Kolte and Nene (1972) achieved 100 per cent infection by sap inoculation when the extraction of the virus was done in 0.1 M potassium phosphate buffer pH 7.6 but Narayanasamy and Jaganathan (1974) recorded the maximum sap transmission at buffer pH 7.2. Chohan and Kaliha (1967) reported the graft transmissibility of the virus associated with similar disease which they described under the name "Curly top". Gupta (1974) also succeeded in transmitting the virus through sap and grafting. The review of literature (Singh, 1974; Raychaudhuri *et al.*, 1977) reveals that there are differences in opinion among different workers on the transmission of the virus causing leaf crinkle disease through insect vector. Bindra (1971) and Khatri *et al.* (1971) reported that the virus was transmissible through the aphid (*Aphis craccivora* Koch.) and leaf hopper (*Circulifer tenellus* Baker) but Kolte and Nene (1972)

found no evidence of transmission of virus by *A.craccivora* Koch. and also by *Bemesia tabaci* Genn. Later, Narayanasamy and Jaganathan (1973) reported the transmission by *B.tabaci* Genn. upto 60 per cent. Dhingra (1975) succeeded in transmitting the virus by *A.craccivora* Koch. and *A.gossypii* Glov. with a short acquisition period preceded by a pre-acquisition fasting in a stylet borne manner.

The role of seed as carrier of this disease was also studied. Kolte and Nene (1972) found that the virus was seed transmissible and about 18.30 per cent seeds from infected plants carried the virus. Gupta (1974) also observed the transmission of this virus through seeds of mung bean, Narayanasamy and Jaganathan (1975 a) reported that the virus occurred in the plumule, radicle and cotyledons of the germinating seed but not in the seed coat and it was found at higher frequency in the plumule. They further recorded that the inoculation of urd bean plants with the virus showed high rate of infection in young plants. The incubation period was positively correlated with plant age at the time of inoculation but showed a negative linear relationship with percentage of seed transmission (Narayanasamy and Jaganathan, 1975 b). They also concluded from sap inoculation tests that the susceptibility of urd bean plants and percentage of seed transmission decreased with increasing plant age. A high degree of infection of young

plants increased seed transmission. The incubation period of the virus was correlated positively with plant age at inoculation and negatively with percentage of seed transmission (Narayanasamy and Jaganathan, 1975 c). Amin et al. (1978) observed that seed inoculation with this virus by vacuum infusion of extract in water soaked seeds was 40 per cent without affecting the seed germination of urd bean. Nene (1972) did not succeed in transmiting this virus through soil and pollen.

The leaf crinkle disease of urd bean and mung bean was found restricted to leguminous plants like mung bean, urd bean, moth bean and cowpea (Nene, 1972; Kolte and Nene, 1975). Gupta (1974) observed that this virus infected bean (Phaseolus vulgaris), mung bean and tobacco. Narayanasamy and Jaganathan (1974) reported that out of 24 plant spp. tested, guar, cowpea, mung bean and pigeon pea were infected.

Nene (1972) recorded the thermal inactivation point of the virus between 60° and 70°C and dilution end point between 10^{-4} and 10^{-5}. The virus in sap stored at room temperature lost its infectivity on fourth day. Infectivity of the virus in sap was found for a period of nine days when stored in refrigerator. Gupta (1974) found the thermal inactivation point between 70° and 75°C, dilution end point between 10^{-3} and 10^{-4} and longevity in vitro

at room temperature for 96 h. Narayanasamy and Jaganathan (1974) reported that the virus was inactivated at > 1:500 dilution, > 60°C and 48 h. at room temperature. Further identification of the virus causing leaf crinkle disease of urd bean and mung bean is unknown, since the virus was not purified. The viruses causing mosaic or mosaic mottle have been identified as a strain of bean common mosaic virus and a strain of cowpea mosaic virus (Sahare and Raychaudhuri, 1963; Nene, 1973; Singh and Varma, 1977). In south India mosaic of mung bean and urd bean has also been found to be caused by tobacco mosaic virus (Ramakrishnan et al., 1968, 1973).

Attempts were not made earlier to purify the virus causing leaf crinkle disease of urd bean and mung bean. However, several other leguminous viruses have been purified using various techniques (Bawden et al., 1951;Schachman, 1959; Steere, 1959; Bancroft, 1962; Hebert, 1963;Leberman, 1966; van Kammen, 1967; Gibbs et al., 1968; van Griensven and van Kammen, 1969; Nariani et al., 1970; Francki,1972; Trautman and Hamilton, 1972; Chowfla and Nariani, 1975).

Though resistant and susceptible varieties and germplasm of mung bean and urd bean have been identified on the basis of symptomatology (Nene, 1972; Singh, 1974; Anonymous, 1977 b, 1978) at various places in India but no work has been done on biochemical evaluation of resistant

and susceptible varieties of mung bean and urd bean to legume viruses. Virus-host interactions lead to metabolic changes pertaining to nitrogen, protein, amino acids, carbohydrates etc. and protein constitution in the host(Brewar *et al*., 1926; Kiraly and Farkas, 1959; Daly *et al*., 1962; Bozarth and Diener, 1963; van Andel, 1966; van Loon and van Kammen, 1970; Zacharius *et al*., 1971; Varma and Poonam, 1978). Very little work seems to have been done in this respect on leguminous crops. van Loon and van Kammen(1970) reported that development of systemic mosaic symptoms on *Nicotiana tabaccum* var. Samsum NN with potato virus S(PVS), potato virus Y (PVY) or cucumber mosaic virus (CMV) were accompanied by differing alternations in protein constituents as detected by electrophoresis in polyacrylamide gel. Barker (1975) described the effect of virus infection on leaf protein and reported that disc electrophoresis of soluble protein in leaf extracts failed to reveal any change induced by infection with raspberry ring spot virus either in inoculated or in systemically infected symptoms bearing leaves of *N. benthamiana*. Evidence was also given that 3 of the 4 proteins previously thought to occur only in virus infected tobacco leaves were probably normal constituents. Varma and Poonam (1978) studied the protein pattern of resistant and susceptible *Lycopersicon* species to tomato leaf curl virus and found that appearance of additional protein in susceptible species acted as tempelate

for virus multiplication. Protein constitution at species and varietal level were also studied in potato, maize and teosinte (Desborough and Peloquin, 1966, 1968, 1969 ; Rana, 1979). Rana (1979) recorded electrophoretic banding pattern on polyacrylamide gel for maize and teosinte and reported that there was a general reduction in the number of protein bands after the germination of seed as the plant age increased. No work has been done for the change in protein constituents in case of leaf crinkle virus infecting mung bean and urd bean plants.

Attempts have also been meager to control the leaf crinkle disease in the field except some work on varietal screening. Nene (1972) reported that out of 13 cultivars of urd bean, none was found free from this disease while in mung bean out of 12 cultivars,screened,cultivars No. 24-3, Pusa Baisakhi, T-2, T-44 and T-51 did not show any symptoms even after artificial inoculation. Singh (1974) reported that under field conditions several varieties of urd bean and mung bean were found free from this disease in Haryana. He further recorded that out of 5 cultivars of urd bean i.e. T-9, UPU-1, G-8, IARI and 26-59, none was free from leaf crinkle. In All India Coordinated Project on Improvement of Pulses at Hissar during Kharif, 1977 and Kharif 1978 out of 469 lines of mung bean and 39 lines of urd bean screened against this disease under natural conditions, 34 lines of mung bean and one line of urd bean were observed free from leaf crinkle symptoms(Anonymous, 1977 b, 1978).

MATERIAL AND METHODS

DISEASE SURVEY

Survey of leaf crinkle disease of mung bean and urd bean in all districts of Haryana was done during summer and kharif seasons of 1975, 1976 and 1979. In each district 4-5 villages were randomly selected and in each village 3-5 fields covering 2-3 hectares were surveyed. In each field an area of 25 sq.meter was selected randomly and the number of plants showing leaf crinkle symptoms along with the total number of plants were counted. The per cent disease was calculated by the formula given below:

$$\text{Per cent disease} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

ESTIMATION OF LOSS IN YIELD

Mung bean cv. Varsha and urd bean cv. T-9 were sown each having 500 sq.meter area of the field at H.A.U. Farm, Hissar during kharif 1975, 1978 and 1979. Plants showing leaf crinkle symptoms were tagged after 24 days of sowing. Thereafter tagging of leaf crinkle diseased plants was done at 10 days interval till the maturity of crop. At the time of harvest the observations on pods per plant, seeds per pod, 1000 grain wt. (g) and yield (g) per plant

were recorded. Per cent yield loss was calculated by the formula as under:

$$\text{Per cent yield loss} = \frac{\text{Yield of healthy plant} - \text{Yield of diseased plant}}{\text{Yield of healthy plant}} \times 100$$

The data were statistically analysed following Snedecor and Cochran (1976).

EFFECT OF TEMPERATURE AND HUMIDITY ON DISEASE INCIDENCE AND DEVELOPMENT

The effect of temperature and humidity on disease incidence and development was studied under natural conditions. Disease incidence was calculated by the formula given earlier under disease survey. Symptom severity grades designated with + sign were given on the basis of visual observations (Table 1). Each symptom severity grade was given arbitrary value and designated as "response value" (Table 1). To quantify the disease severity "coefficient of infection" was calculated by multiplying the per cent disease with "response value" (Table 1). This coefficient combined the amount of infection and severity and was considered as disease development. Per cent disease development is the co-efficient of infection calculated on the basis of 100 as the maximum value (Table 1). Urd bean cv. Krishna was sown in summer and kharif seasons of 1975, 1976 and 1979 at H.A.U. Farm, Hissar. Total number of

Table 1. Determination of resistance/susceptibility of different mung bean and urd bean varieties/germplasm to leaf crinkle virus

Symptom **	Symptom severity grade	Response value	Coefficient* of infection (per cent (disease development)	Over all reactions	Reaction symbol
No symptoms	Nil	0.00	0.00	Highly resistant	HR
Plants showing mild symptoms	+	0.25	1-25	Resistant	R
Plants showing moderate symptoms	++	0.50	26-50	Moderately susceptible	MS
Plants showing severe symptoms in about 50% leaves	+++	0.75	51-75	Susceptible	S
All leaves of plant showing severe symptoms	++++	1.00	76-100	Highly susceptible	HS

$$\text{Per cent disease} = \frac{\text{Number of diseased plants}}{\text{Total number of plant observed}} \times 100$$

* Coefficient of infection (Per cent disease development) = Per cent disease x response value

** Plate 1, Figs. i & ii.

plants and plants showing leaf crinkle symptoms along with severity grade were counted after 24 days of sowing. Further counting of plants showing leaf crinkle symptoms along with severity grade was done at weekly interval till the maturity of crop i.e. upto 65 days after sowing. Data on temperature and humidity were collected from Meteorological Laboratory of the H.A.U. Hissar. This was correlated with disease incidence and disease development of the corresponding period.

TRANSMISSION

Mechanical Transmission : The source of virus inoculum was young infected leaves of urd bean cv. Krishna showing typical leaf crinkle symptoms. The infected fresh leaves were collected, washed with tap water and dried in folds of blotting paper. They were then macerated adding 1 ml of phosphate buffer of desired molarity and pH per g of leaf material in a pestle and mortar at about 4°C. To increase the efficiency of transmission different pH (6.00 to 8.00) and different molarities of phosphate buffer (0.01 - 0.5) were tested. The pulp obtained after crushing the leaves in phosphate buffer was squeezed through two fold muslin cloth to obtain crude extract which was used as virus inoculum. Urd bean cv. Krishna was used as test plant. The test plants were raised in clay pots and maintained

in screen house under insect proof conditions. Inoculations were done on test plants by gently rubbing the upper surface of leaf/leaflet with forefinger dipped in inoculum. Carborandum powder was sprinkled over leaf surface before inoculation to increase the transmission efficiency. After inoculation the leaves were washed with a fine jet of water from a wash bottle to remove the excess inoculum and carborandum powder. To study the effect of plant age on mechanical transmission, leaves were inoculated at the age of 7, 14, 21, 28, 35, 42 and 49 days. Inoculated plants were kept in screen house under insect proof conditions for observation/symptom appearance.

Insect transmission : Transmission of leaf crinkle disease was also studied with insects. Two aphids - bean aphid (*Aphis craccivora* Koch) and cotton aphid (*Aphis gossypii* Glov.), one beetle - red pumpkin beetle (*Aulacophora fevicaulis* Lucas) and white flies (*Bemisia tabaci* Genn.) which were commonly found in pulse crop area and its surrounding fields of cotton and cucurbits in kharif season, were used. These insects were multiplied on healthy plants of cowpea, cotton and cucumber. Whenever needed the required number of insects were carefully removed from the healthy plant on which they were maintained with the help of camel hair brush. White flies

were handled with the help of an aspirator following the technique described by Rathi and Nene (1974). Thereafter, they were given pre-acquisition fasting for 3-4 hours before transferring them to leaf crinkle diseased leaves of urd bean to acquire the virus. The virus acquisition feeding was given for 5 min and 2 hrs. The insects were watched during acquisition feeding and only those individuals which fed were transferred to the test plants. For inoculation feeding the aphids and white flies were transferred as 1,2, 5, 10, 20 and 30 insects per plant whereas A.fevicaulis was transferred as 1, 2, 3, 4 and 5 insects per plant. These insects were allowed for inoculation feeding on test plant urd bean cv. Krishna. Thereafter, test plants were sprayed with 0.1 per cent Thiodan (insecticide) to kill the insects and kept for observations in screen house under insect proof conditions.

Seed transmission : In order to study the transmission of the virus through seed, the plants were raised from the seeds collected from the infected plants of urd bean cv. Krishna, in the screen house under insect proof conditions. Control plants were raised similarly from the seeds collected from healthy plants. To study the effect of plant age at the time of infection on seed transmission, 7, 14, 21, 28, 35, 42 and 49 days old plants of urd bean cv. Krishna were first mechanically inoculated with the virus and

allowed to mature under insect proof conditions. The seeds collected from them were sown and the resulting plants were maintained in screen house under insect proof conditions to observe the disease symptoms. The per cent seed transmission was calculated as below:

$$\text{Per cent seed transmission} = \frac{\text{No. of diseased plants}}{\text{Total number of plants}} \times 100$$

HOST RANGE AND HOST REACTIONS

Host range and host reactions of the virus with special reference to weed plants were studied. Unless otherwise stated, cultivated and weed plants belonging to 19 families were grown in the screen house under insect proof conditions. Apparently healthy seedlings of the weed plants which were not raised from seeds in screen house, were transplanted in the pots from the fields particularly from the areas where pulse crops were grown during kharif season. Ten plants of each species were mechanically inoculated and 5 plants of the same species were kept as uninoculated (control) for observations. Transmission of the virus was confirmed by back inoculation on test plants of urd bean cv. Krishna.

PHYSICAL PROPERTIES

Dilution end point (DEP) : A series of 10 fold dilutions with 0.1 M phosphate buffer pH 7.0 of the standard extract

prepared by crushing 1 g of diseased leaf material with 1 ml of 0.1 M phosphate buffer pH 7.0, were prepared. Infectivity of the virus at each dilution of the sap was tested by mechanically inoculating the plants of urd bean cv. Krishna. At least 10 plants of urd bean cv. Krishna were inoculated with each dilution. Equal number of plants were kept as control (uninoculated).

Thermal inactivation point (TIP) : *Two ml. aliquots of* standard extract from diseased leaves were taken in thin walled Corning glass test tubes of 1 cm diameter. These tubes were heated in a thermostatically controlled water bath for 10 min. at desired temperatures. After heating, the tubes were rapidly cooled by immersing them in ice cold water. Infectivity of the virus of the heated sap was tested on urd bean cv. Krishna as for DEP.

Longevity *in vitro* : The standard extract was stored in test tubes at room temperature (35°C ± 2°C) and in a refrigerator separately. After the desired interval of time the infectivity of the stored extract was determined as it was done for D.E.P. and T.I.P.

PURIFICATION

Preparation of the Clear Extract :

The clear extract was prepared by crushing 100 g

of healthy and leaf crinkle virus infected leaves of urd bean cv.Krishna showing typical leaf crinkle symptoms in 100 ml of 0.1M phosphate buffer pH 7.0 containing 0.3 ml of 2-mercaptoethanol in chilled pestle and mortar at 4°C. The slurry obtained was passed through 4 folds of muslin cloth. The crude extract thus obtained was centrifuged at 10,000 r.p.m. for 15 min. at 4°C in K-24 Refrigerated Centrifuge. The supernatant thus obtained was collected as clear extract and was used as the starting material to purify the virus employing different methods as under:

1. Adjustment of pH
2. Precipitation with salt and treatment with organic solvents
3. Sephadex filteration
4. Ultracentrifugation/differential centrifugation.

1. Adjustment of pH : Thirty ml aliquots of clear extract from healthy and infected leaves were taken in 100 ml beakers. These samples were adjusted to desired pH (5.0 to 3.0) with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide using Digital pH meter (Systronics). After adjustment of the desired pH, the samples were shaken well for 10 minutes under cold condition. The precipitate appeared at different pH was collected by centrifugation at 10,000 r.p.m. for 15 min. using K-24 Refrigerated Centrifuge. The precipitate was dissolved in 0.1 M phosphate buffer pH 7.0 and again

centrifuged at low speed to remove insoluble material. The clear solution obtained after this centrifugation was tested for infectivity on Cholai bean (*V. catjang*) and its ultra violet (U.V.) spectrum (220 - 300 nm) was determined using Beckman UV Spectrophotometer - Model 25.

2. Precipitation with salt and treatment with organic solvents:

To 30 ml of clear extract from healthy and infected leaves, 10 ml of saturated ammonium sulphate was added. The mixture was kept over night in a refrigerator. Precipitate was collected by centrifugation at 10,000 r.p.m. using K-24 Refrigerated Centrifuge for 15 min. and dissolved in 10 ml of 0.1M phosphate buffer, pH 7.0. The material so obtained was again centrifuged at 10,000 r.p.m. for 15 min. at 4°C to remove insoluble material. The supernatant was again precipitated with saturated ammonium sulphate and this cycle was further repeated twice. The final preparation, which was fairly colourless, was tested for infectivity and its U.V. spectrum (220 - 300 nm) was also recorded.

For the purification using organic solvent, 10 ml of chloroform: butanol (1: 1) was added to 30 ml of clear extract from healthy and infected leaves. This mixture was vigorously shaken for 10 min. with hands (under cold), centrifuged at 10,000 r.p.m. for 10 min. and then only watery phase was collected carefully with the help of

Pasteure's pipette. The infectivity of this watery phase was tested and its UV spectrum (220 - 300 nm) was recorded.

3. <u>Sephadex filteration</u> : Sephadex G-25 and Sephadex G-50 columns (20 cm long) were prepared in 25 cm long and 1.5 cm diameter glass tubes with cintered glass filter fitted at the bottom. From bottom end of the tube a capillary was drawn to which a thin silicon tube was fitted which was connected with automatic fraction collector. The flow of the eleuted material was controlled with the help of a stop cork. Ten ml of standard clear extract from leaf crinkle infected leaves of urd bean cv. Krishna was first loaded carefully on the upper surface of the Sephadex G-25 column. Twentysix fractions of 5 ml each were collected with the LKB 17000 MINIRAC AUTOMATIC Fraction Collector at the speed of 2 min./5 ml. The optical density of each fraction was recorded at 260 nm and the infectivity of all the fractions was also tested. The infective fractions were pooled and filled in dialysis bag tied with fine thread at one end. After filling with the above material, the other end of dialysis bag was also tied with fine thread. This leak proof dialysis bag was embeded in polyethylene glycol (Polywax 6000) in a tray. The tray was then kept in a refrigerator till the material inside the dialysis bag was concentrated as desired. This concentrated material was centrifuged at 10,000 r.p.m.

at 4°C to remove the insoluble material. The supernatant thus obtained was again passed through Sephadex G-50 column and 26 fractions of 5 ml each were collected as before. Again each fraction was tested for infectivity and its O.D. at 260 nm was also determined. U.V. spectra (220 - 300 nm) of those fractions which had highest O.D. at 260 nm were also recorded.

4. <u>Ultracentrifugation/differential centrifugation</u> :

The clear extract from leaf crinkle infected and healthy leaves of urd bean cv. Krishna was subjected to differential centrifugation. Low speed (10,000 r.p.m.) centrifugation was done in K-24 Refrigerated Centrifuge for 15 min.at 0-4°C and the high speed centrifugation was done at 30,000 r.p.m. for 2 hr. at 0-4°C using Beckman Ultracentrifuge Model L-5-50. Whenever needed the clear extract was further clarified by 3:1 chloroform:butanol (1:1) treatment. The clarified extract was subjected to differential centrifugation to purify the virus. Also 8 per cent polyethylene glycol (P.E.G.) mol.wt. 6000 and 1.2 per cent sodium chloride (NaCl) was added to the clarified extract and kept in refrigerator over night. The precipitate which appeared after addition of P.E.G. and NaCl was collected by low speed centrifugation (10,000 r.p.m.) for 15 min. This precipitate was dissolved in

desired amount of 0.1M phosphate buffer pH 7.0 and the solution was again centrifuged at low speed (10,000 r.p.m.) for 15 min. to remove insoluble material. This clear solution was then subjected to differential centrifugation to purify the virus. At each stage of purification infectivity was tested by mechanically inoculating the leaves of cholai bean (V.catjang) and U.V. spectrum (220 - 300 nm) of the preparation was determined. The 280 nm/260 nm ratio of the purified preparation was calculated and on the basis of this ratio the approximate per cent nucleic acid content of the virus was determined refering the table given by Layne (1957) for this purpose.

ELECTRON MICROSCOPY

Fine drops of the purified virus solution were placed with Pasteure's pipette on E.M.copper grids coated with 0.2 per cent formvar and carbon. The excess solution was drained off with the help of filter paper. The grids were allowed to dry at room temperature for a few minutes. Thereafter a drop of 2 per cent phosphotungstic acid (PTA) pH 6.0 was put on the grids. The extra PTA was removed from the grids with filter paper. After drying, the grids were put in EM Speciman Gridbox. The preparations were studied in SIEMENS ELMISKOP 102 TRANSMISSION Electron Microscope. This electron microscope was adjusted

to hair pin shaped filament, magnification 8000 and voltage 80 K.V. The apertures used were 200 microns and 50 microns for condenser and object, respectively. The photographs were taken on AGFA GAVERT SCIENTIA cut films of 6.5 x 9.0 cm size having sensitivity of 6. The exposure time was 2.5 seconds. The exposed film was developed in AGFA GAVARET G 7 P developer. Enlarged prints were made on AGFA SPECIAL single weight paper.

IDENTIFICATION OF THE VIRUS

The virus was identified on the basis of particle shape and size determined in electron microscope, per cent nucleic acid in infective virus particles, physical properties, host range, host reactions and transmission following Harrison *et al.* (1971). On the basis of these properties, investigated under this study, cryptogram (Gibbs, 1968) was prepared. Attempts were made to place the virus in a group proposed by Harrison (1969) embodying properties closest to this virus.

PROTEIN CONSTITUTION

Protein constitution of healthy and diseased leaves of some resistant and susceptible varieties of mung bean and urd bean was studied with the polyacrylamide gel electrophoresis technique following Davis (1964).

A brief outline of the procedure followed is given below:

Gel solutions :

(A)	1 N HCl	48.00 ml
	Tris (2-amino-2-hydroxymethyl propane-1, 3-diol)	36.60 g
	TEMED (N. N. N', N'-tetramethyl ethylene diamine)	0.23 ml
	Water to make	100 ml
(B)	Acrylamide	28.00 g
	Bis-acrylamide (N-N'-Methylen Bis-acrylamide)	0.735 g
	Water to make	100 ml
(C)	Ammonium persulphate	0.140 g
	Water to make	100 ml

Solutions (A) and (B) were filtered and stored in dark brown bottles in refrigerator and consumed within a week. Solution (C) was prepared fresh before use.

Running buffer : Standard Tris-glycine buffer pH 8.3

Tris ($NH_2.C.(CH_2.OH)_3$	6.0 g
Glycine ($NH_2.CH_2.COOH$)	28.8 g
Water to make	1000 ml

Tracking dye : 0.05 per cent bromophenol blue in 1 per cent acetic acid.

Preparation of gels : The working solutions were mixed in the following ratio to give standard gel of 7.5 per cent:

Solution (A)	2 ml
Solution (B)	4 ml
Solution (C)	8 ml
Distilled water	2 ml

The mixture of the above solutions was deaerated before preparation. The gels were prepared in 10 cm long plexiglass tubes having internal diameter of 0.5 cm. Leakproof stoppers were fitted on one end of the tube and were kept in vertical position for gel preparation. The running gel solution was filled in these tubes with the help of Pasteure's pipettes upto 7 cm from below. About 3 cm space was left unfilled to accommodate the leaf extract to be electrophoresed. In order to obtain a smooth, flat gel surface, a small amount of distilled water was added gently over the gel solution and then left undisturbed for polymerization. Polymerization of the gels took place in about 40 min.

Preparation of sample solution : Two g of leaf material for each sample was thoroughly washed in distilled water, dried in blotting paper folds and crushed in chilled pestle and mortar at 4°C adding 2 ml of 0.1M phosphate buffer pH 7.0 and 0.01 ml of 2-mercaptoethanol. The crude extract thus prepared was centrifuged at 10,000 r.p.m. for 15 min. at 4°C in K-24 Refrigerated Centrifuge to obtain the clear

extract. One ml of 0.05 per cent bromophenol blue (tracking dye as prepared above) was added per 5 ml of clear extract. Ten per cent sucrose was also added in the extracts to increase the density for displacing the buffer solution in the gel tubes. This also prevented the sample solution from mixing with the buffer solution over the gel tube.

Electrophoresis : The standard tris-glycine buffer(pH 8.3) was filled up to 3/4 of the lower chamber (10 cm in diameter, 8 cm in height) of the acrylophore. The rubber stoppers were carefully removed and the gel tubes were pushed in position in the holes of the upper chamber (10 cm in diameter, 6 cm in height) of the acrylophore. With the help of λ pipettes 200 ul aliquot of sample solution was added at the top of each gel. Over the sample solution the buffer was layered to fill the gel tubes completely. The tubes at this stage were lowered and submerged in the buffer solution in the upper chamber.

Electrophoresis was done under cold conditions by keeping cathode at top and anode at bottom. This was started with 2 mA current per tube and when the tracking dye entered the gels, the current supply was increased to 3 mA per tube. Electrophoresis was continued till the tracking dye migrated to the other end of the gel.

Removal of the gel : After the completion of electrophoresis

the gel columns were loosened from their tubes by gently rimming them at their lower ends with a blunted long B-D 23 hypodermic needle through which dilute glycerine was allowed to flow. The gels were forced from one end of the tubes by putting water pressure through metallic syringe. The gels coming out of the tubes were gently allowed to rest in a tray containing distilled water and then transferred to staining solution:

Staining of gels : The gels were stained in 0.5 per cent amido black solution in 5 per cent acetic acid for 25-30 min. The destaining was done by repeated washings in 7 per cent acetic acid. Destained gels showing stained protein bands were preserved in 7 per cent acetic acid.

Photographs and scanning of gels : The gels were put in glass tubes containing 7 per cent acetic acid. These tubes were placed on white back ground and photographed. Densitogram curves of each gel with automatic recorder at 630 nm were also prepared with gel speed 5 cm./min. and chart speed 2 inches/min. using Beckman Spectrophotometer Model-25, having gel scanning adjustment.

DISEASE CONTROL

SCREENING OF MUNG BEAN AND URD BEAN VARIETIES/ GERMPLASM AGAINST LEAF CRINKLE VIRUS :

Selected and promising varieties/germplasm lines, 390 of mung bean and 138 of urd bean, were sown in the

field at the H.A.U. Farm, Hissar during kharif 1975, 1976, 1978 and 1979 for screening against leaf crinkle virus. On the basis as shown in Table 1, they were categorized as highly resistant (HR), resistant (R), moderately resistant(MR), susceptible (S) and highly susceptible (HS). The varieties/ germplasm which were found highly resistant under field conditions were further screened under insect proof screen house conditions after mechanical inoculation with the virus. One hundred plants of each variety/germplasm were grown under insect proof conditions. After 10 days of sowing these plants were sap inoculated. The varieties/germplasm lines after artificial (mechanical) inoculation were categorised for their reaction in the same way as was done under field conditions.

SEED TREATMENT

HEAT THERAPY :

Wet heat therapy, dry heat therapy and solar heat therapy were tried for curing the leaf crinkle infected seeds of urd bean cv. Krishna.

a) Wet heat therapy: Samples of one hundred seeds of urd bean cv. Krishna were taken in 250 ml conical flasks containing sufficient water to cover the seeds. The flasks were immersed in a thermostatically controlled water bath at desired temperatures for desired period of time. During

heating, the seeds immersed in water were continuously stirred. After heating for the required time the flasks were rapidly cooled and seeds were sown in clay pots under insect proof conditions in the screen house. Observations on seed germination and per cent diseased plants were recorded till 30 days after sowing.

(b) Dry heat therapy : Samples of 100 seeds were kept at different temperatures for required period in an oven and sown after treatment in clay pots. Observations for seed germination and per cent diseased plants were recorded till 30 days after sowing.

(c) Solar heat therapy : In order to know the effect of solar heat on inactivation of leaf crinkle virus, the seeds of urd bean cv. Krishna were collected from leaf crinkle virus infected plants. These seeds were soaked in water for 1, 2, 3 and 4 hr. in the forenoon and then spread on cemented surface for exposure to sun light (Solar heat) between 12.oo noon to 4.oo P.M. on hot and clear days in the month of May and June. Thereafter, these solar heat treated seeds were bagged and kept at room temperature till they were sown in clay pots under insect proof conditions in the screen house at the time of normal kharif sowing. For each treatment 3 replications were kept. Observations on seed germination and per cent diseased plants were recorded till 30 days after sowing. Data were statistically analysed (Fischer and Yates, 1963).

CHEMOTHERAPY :

For chemotherapy the following six antiviral chemicals, four systemic insecticides and four systemic fungicides were tried as seed treatment as given below:

NAME OF THE CHEMICAL	SEED TREATMENT
Antiviral chemicals	**Seed dip for 30 min. in**
1. Validomycin-A	0.05% and 0.1% in water
2. 2-Thiouracil	0.05% and 0.1% in 0.1% sodium carbonate
3. 8-Azaguanine	0.05% and 0.1% in 0.1% sodium carbonate
4. 8-Azaadenine	0.05% and 0.1% in 0.1% sodium carbonate
5. Guanidine hydrochloride	0.05% and 0.1% in water
6. Guanidine carbonate	0.05% and 0.1% in water
Systemic insecticides	
7. Phosphomedon(Dimecron) 100 S	0.025 % and 0.05% in water
8. Dimethoate(Rogor) 30 EC	0.05 % and 0.1% in water
9. *Oxydemeton-methyl* (Matasystox) 25 EC	*0.05 % and 0.1% in water*
10. Monocrotophos(Nuvacron)40EC	0.05% and 0.1% in water
Systemic fungicides	**Seed dressing**
11. Bavistin	2.5 g/kg seed
12. Benlate	2.5 g/kg seed
13. Thiophonate-M	2.5 g/kg seed
14. Vitavax	2.5 g/kg seed

3. Temic (Aldicarb) 10 G	1 kg/ha
4. No insecticide(control)	Nil
Liquid	Foliar spray,10 and 25 days after sowing
5. Phosphomedon(Dimecron) 100 S	0.025% and 0.035 % in water
6. Dimethoate(Rogar) 30 EC	0.05% and 0.1% in water
7. Oxydemton-methyl (Metasystox) 25 EC	0.05% and 0.1% in water
8. Monocrotophos(Nuvacron) 40 EC	0.05% and 0.1% in water
9. No insecticide(control)	Water.

The effect of these insecticides were determined on the basis of per cent disease and grain yield. Data was statistically analysed (Snedecor and Cochrane, 1976). The additional grain yield due to insecticidal treatment was calculated by substracting the yield obtained in control from the yield of the plants treated with insecticides. The income in cash and cost of insecticides were calculated on the three years average market price at Hissar i.e. 1975, 1976 and 1979. The net income or net loss due to treatment was compared by substracting the cost for treatment from the additional cash income due to treatment.

COMBINATIONS OF SEED TREATMENT AND INSECTICIDAL SPRAYS

Experiments were also conducted during kharif 1978

and 1979 in the field in randomized block design using leaf crinkle virus infected seeds of urd bean cv.Krishna with different combinations of seed treatment and foliar spray of insecticides which were found most effective as seed treatment to control the seed borne infection and as foliar sprays found most effective in controlling the spread of virus by killing its insect vectors. The plot size was 5 x 4 m with three replications for each treatment. To control seed borne infection and to check its further spread by insect vector, the following combinations of seed treatment and foliar sprays were tried.

1. No seed treatment + No foliar spray of insecticides
2. No seed treatment + Foliar spray of insecticides
3. Seed treatment with heat therapy + Foliar spray of insecticides
4. Seed treatment with chemotherapy + Foliar spray of insecticides.

Observations were recorded on seed germination and per cent diseased plants at 25 days and 50 days after sowing. The data was statistically analysed (Fischer and Yates,1963).

RESULTS

DISEASE SURVEY

The extent of occurrence of leaf crinkle disease of urd bean and mung bean in summer and kharif seasons of 1975, 1976 and 1979 in different districts of Haryana is presented in Table 2, 3 and 4, Figs. 1 and 2, which indicate that the per cent disease was less in summer season than kharif season. Per cent disease was less in 1975. It slightly increased in 1976. During 1979 it was still more than 1976 (Table 2) except in districts Bhiwani and Mohindergarh in mung bean crop (Table 3, Fig. 1) and in districts Jind, Mohindergarh and Rohtak in urd bean crop (Table 4, Fig. 2).

During summer season the maximum disease incidence on mung bean crop was in Kurukshetra district being 3.48 per cent, 3.81 per cent and 4.65 per cent during 1975, 1976 and 1979 respectively followed by Sonepat, Ambala, Karnal, Jind, Rohtak, Sirsa, Gurgaon/Faridabad. There was no disease in Mohindergarh, Bhiwani and Hissar districts in 1975, 1976 and 1979 (Table 2) during summer season. Since urd bean crop was not available in summer season there is no data of disease incidence in urd bean crop for these years (Fig. 2). Data also indicate that disease incidence during kharif was maximum in Rohtak district i. e. upto 34.63 per cent in mung bean (Table 3, Fig. 1) and upto 37.13 per cent

Table 2. Occurrence of leaf crinkle disease in different districts of Haryana during summer season of 1975, 1976 and 1979 on mung bean cv.Pusa Baisaki

Name of the district	Per cent disease*		
	1975	1976	1979
Ambala	2.36	3.24	4.62
Bhiwani	0.00	0.00	-
Faridabad	x	x	-
Gurgaon	1.39	2.23	0.00
Hissar	0.00	0.00	0.00
Jind	2.08	3.05	2.90
Karnal	2.29	3.13	2.80
Kurukshetra	3.48	3.81	4.65
Mohindergarh	0.00	0.00	-
Rohtak	1.54	2.68	2.87
Sirsa	1.07	2.45	-
Sonepat	2.61	3.47	4.56

$$* = \text{Per cent disease} = \frac{\text{Number of diseased plants}}{\text{Total No. of plant counted}} \times 100$$

- = Variety not found

x = District did not exist.

Table 3. Occurrence of leaf crinkle disease in different districts of Haryana during kharif season of 1975, 1976 and 1979 on mung bean cvs. Pusa Baisakhi, J-45 and Varsha.

Name of the district	Per cent disease*								
	1975			1976			1979		
	Pusa Baisakhi	J-45	Varsha	Pusa Baisakhi	J-45	Varsha	Pusa Baisakhi	J-45	Varsha
Ambala	4.35	13.12	18.35	6.66	17.20	18.16	6.95	-	19.35
Bhiwani	1.91	4.15	8.45	2.18	5.72	9.76	-	-	9.60
Faridabad	x	x	x	x	x	x	-	12.25	15.50
Gurgaon	3.89	8.47	11.95	3.94	11.85	14.88	-	11.00	16.43
Hissar	2.65	9.47	12.62	4.33	12.96	14.67	4.28	18.93	17.50
Jind	8.13	21.39	27.75	8.10	25.51	29.30	10.23	-	29.00
Karnal	7.15	17.64	22.97	7.91	23.46	24.69	8.82	25.25	-
Kurukshetra	5.88	11.72	16.66	6.14	18.00	19.81	-	20.00	22.70
Mohindergarh	1.12	3.78	6.23	1.43	5.80	8.67	-	-	6.50
Rohtak	8.84	22.95	29.01	9.43	25.44	31.67	10.18	26.88	34.63
Sirsa	3.17	6.33	13.18	4.45	13.62	13.56	5.00	20.00	16.00
Sonepat	6.76	18.27	23.18	8.20	18.10	24.18	10.15	22.56	26.24

$* = \text{Per cent disease} = \frac{\text{Number of diseased plants}}{\text{Total No. of plants counted}} \times 100$.

\- = Variety not found.

x = District did not exist.

Table 4. Occurrence of leaf crinkle disease in different districts of Haryana during kharif season of 1975, 1976 and 1979 on urd bean cvs. H 70-3, Mash 1-1 and T-9

Name of the district	Per cent disease *								
	1975			1976			1979		
	H 70-3	Mash 1-1	T-9	H 70-3	Mash 1-1	T-9	H 70-3	Mash 1-1	T-9
Ambala	1.31	12.38	17.95	1.82	17.42	19.70	-	-	20.25
Bhiwani	-	4.45	8.85	-	5.15	9.95	-	-	10.18
Faridabad	x	x	x	x	x	x	-	-	17.00
Gurgaon	0.68	7.65	11.46	1.38	9.83	14.90	-	-	16.50
Hissar	0.85	9.29	13.50	1.16	9.14	14.80	2.0	14.66	18.00
Jind	2.18	21.13	29.53	2.59	26.19	30.91	-	-	30.00
Karnal	1.87	17.87	22.63	1.84	21.17	24.42	2.02	24.73	26.25
Kurukshetra	1.41	13.77	18.44	1.40	16.20	19.22	3.50	-	23.50
Mohendergarh	-	3.40	7.66	-	4.87	8.82	-	-	8.00
Rohtak	2.31	21.56	34.05	2.94	27.42	37.13	3.26	26.35	35.78
Sirsa	0.72	6.14	12.15	1.35	6.00	13.66	-	-	18.00
Sonepat	1.64	16.77	23.68	2.36	22.47	24.21	4.21	22.25	25.95

$$* = \text{Per cent disease} = \frac{\text{Number of diseased plants}}{\text{Total number of plant counted}} \times 100.$$

- = Variety not found.

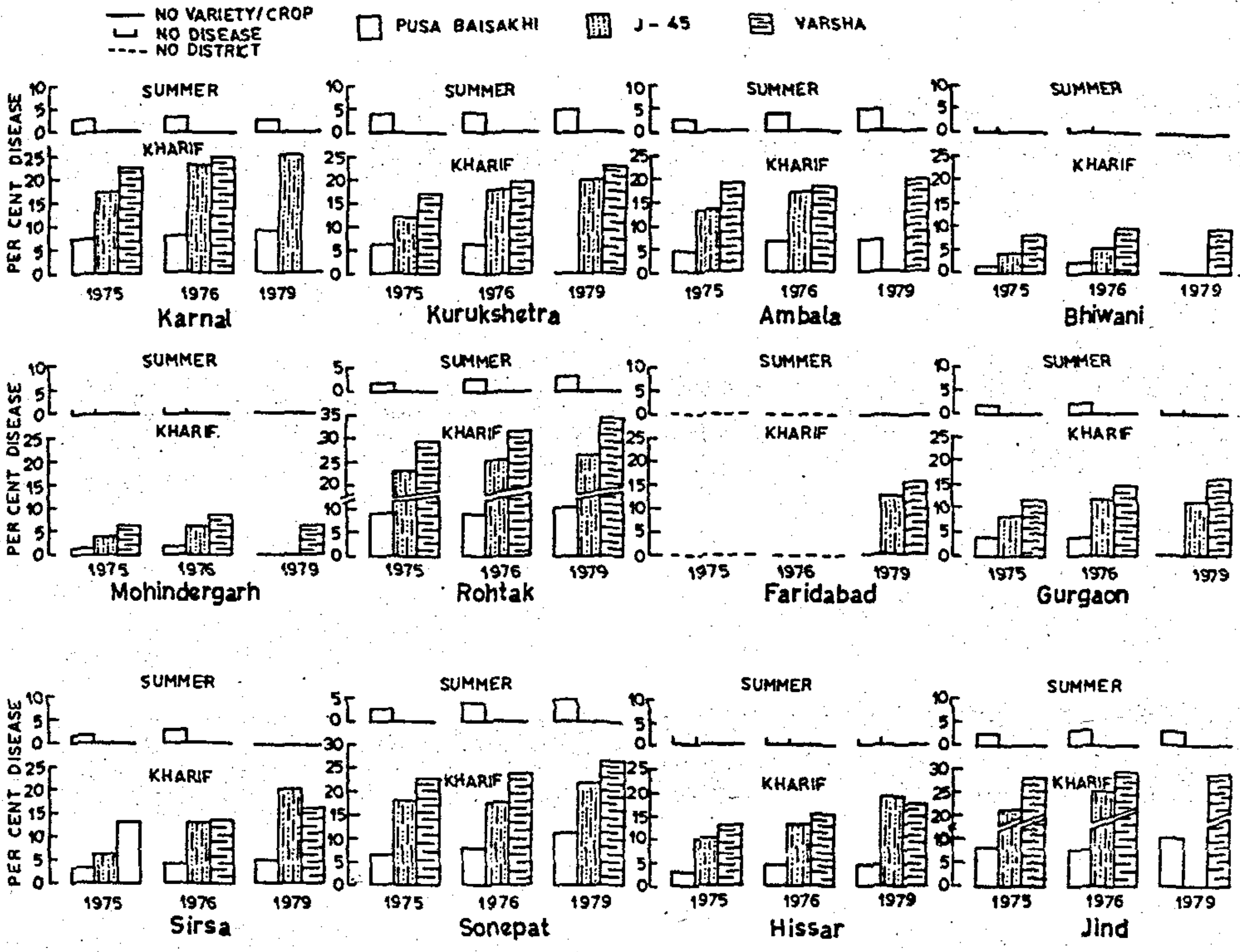
x = District did not exist.

EXPLANATION OF FIG. 1

(on back side)

FIG. 1 Bar diagrams indicating the occurrence of leaf crinkle disease in different districts of Haryana during summer and kharif seasons of 1975, 1976 and 1979 on mung bean cvs. Pusa Baisakhi, J-45 and Varsha.

FIG.1.

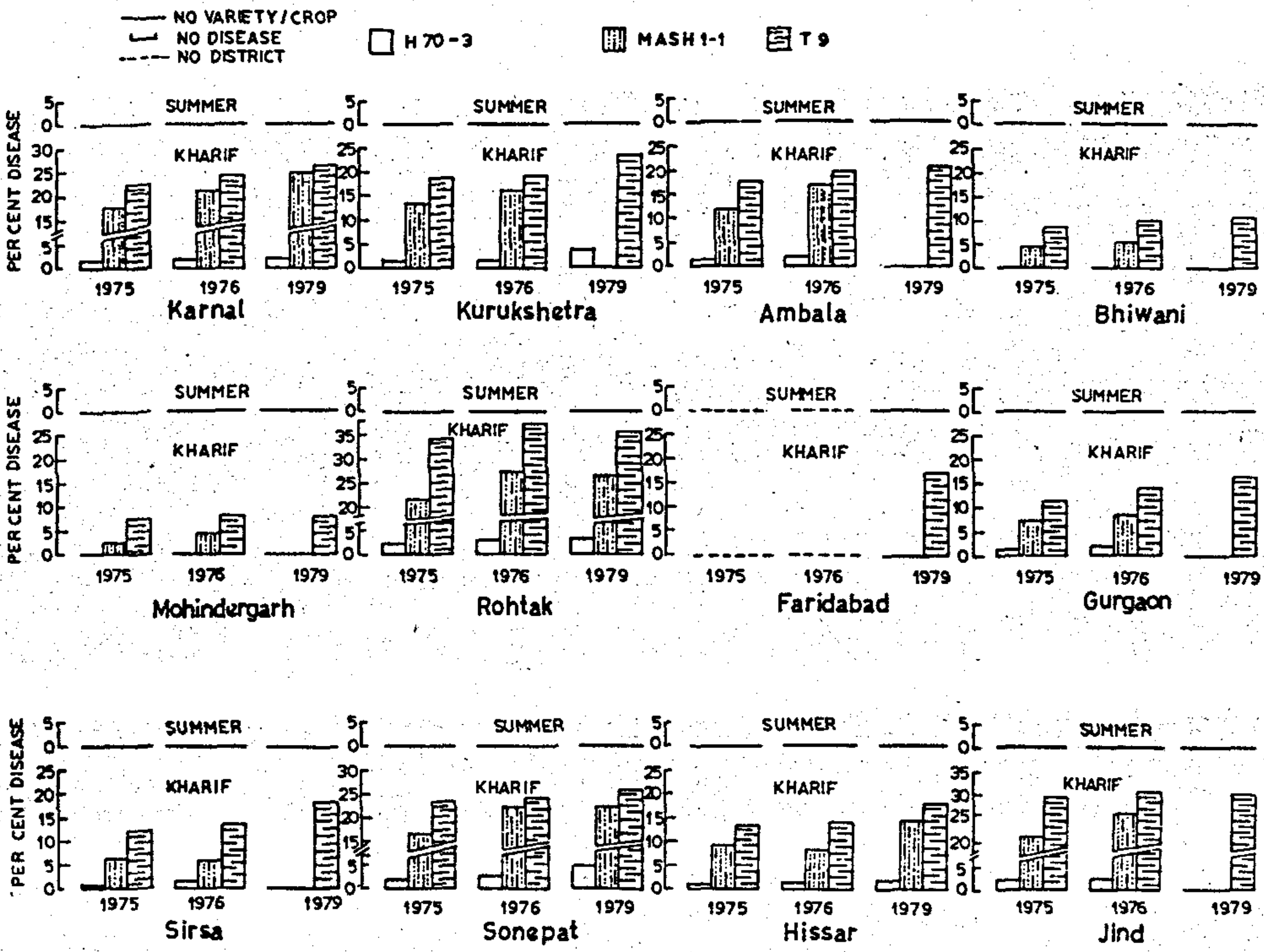


EXPLANATION OF FIG. 2

(on back side)

FIG. 2 Bar diagrams indicating the occurrence of leaf crinkle disease in different districts of Haryana during summer and kharif seasons of 1975, 1976 and 1979 on urd bean cvs.H 70-3, Mash 1-1 and T-9..

FIG. 2.



in urd bean (Table 4, Fig. 2), followed by Jind(29.3 and 30.9 per cent), Sonepat (26.24 and 25.95 per cent), Karnal (25.25 and 26.25 per cent), Kurukshetra (22.70 and 23.50 per cent), Ambala (19.35 and 20.25 per cent), Sirsa (20.0 and 18.0 per cent), Hissar (18.93 and 18.0 per cent), Faridabad (15.50 and 17.00 per cent), Gurgaon (16.43 and 8.82 per cent), Bhiwani (9.76 and 10.18 per cent) and Mohindergarh (8.67 and 18.0 per cent) districts. However, occurrence of the disease was more in urd bean crop than mung bean in all the districts of Haryana (Tables 3 and 4, Figs 2 and 3). Among mung bean cvs. under survey mung bean cv. Varsha had maximum disease incidence followed by J-45 and Pusa Baisakhi (Table 3, Fig.1). Among urd bean cvs. under survey urd bean cv. T-9 had more disease incidence than Mash 1-1 and H 70-3 (Table 4, Fig. 2).

LOSS IN YIELD

The results on loss in yield of mung bean cv. Varsha and urd bean cv. T-9 are presented in Tables 5 and 6. It is evident from data that the yield loss varied from 2.12 per cent to 93.98 per cent in mung bean cv. Varsha (Table 5) and from 2.82 per cent to 95.17 per cent in urd bean cv. T-9 (Table 6) depending upon the stage of plant growth at which infection occurred. Data also indicate that if the plant got infected early, the loss was more. The yield loss was very little and statistically non-significant in plants in which leaf crinkle symptoms appeared at later stage i.e. about 34 days after sowing in mung bean cv. Varsha and about 44 days after sowing in urd bean cv. T-9. Table 5 indicates that in cv. Varsha when disease appeared within 34 days after sowing, there was statistically significant reduction in yield components i.e. pods/plant, seeds/pod, 1000 grain weight and yield per plant resulting 77.88 per cent to 93.98 per cent loss in yield. When disease appeared after 34 days of sowing its effect on pods/plant, seeds/pod, 1000 grain weight and yield per plant was statistically nonsignificant. Table 6 indicates that there was statistically significant reduction in pods/plant, seeds/pod, 1000 grain weight and yield per plant in urd bean cv. T-9 if the disease appeared within 44 days after sowing resulting 49.73 per cent to 95.17 per cent loss in yield. When disease

Table 5. Loss in yield components (pods/plant, seeds/pod and 1000 grain weight) and grain yield in mung bean cv. Varsha due to leaf crinkle disease

Interval (days) between sowing and symptoms appearance	Number of pods/plant	Number of seeds/pod	1000 grain weight (g)	Yield(g)/ plant	Per cent yield loss*
24	1.91	5.42	44.75	0.34	93.98
34	3.41	7.15	52.10	1.25	77.88
44	9.85	8.91	61.00	5.35	5.31
54	10.04	9.00	61.20	5.53	2.12
Control(No disease)	10.10	9.13	61.55	5.65	-
C.D. at 5% level	0.42	0.34	0.51	0.3	

Figures are the average of 3 years i.e. 1975, 1978 and 1979 based on 200 - 997 plants each year.

$$\text{Per cent yield loss} = \frac{\text{Yield of healthy plant} - \text{Yield of diseased plants}}{\text{Yield of healthy plant}} \times 100.$$

Table 6. Loss in yield components (pods/plant, seeds/pod and 1000 grain weight) and grain yield in urd bean cv. T-9 due to leaf crinkle disease

Interval(days) between sowing and symptoms appearance	Number of pods/plant	Number of seeds/pod	1000 grains weight (g)	Yield (g) per plant	Per cent yield *
24	3.25	3.10	35.65	0.36	95.17
34	5.20	4.00	36.25	0.75	89.95
44	20.67	4.85	37.50	3.75	49.73
54	34.25	5.28	38.05	6.90	7.50
64	34.95	5.45	38.20	7.25	2.82
Control(no disease till maturity)	35.23	5.53	38.30	7.46	-
C.D. at 5% level	1.38	0.29	0.40	0.62	

Figures are the average of three years i.e. 1975, 1978 and 1979 based on 204 - 1003 plants each year.

$$* \text{ Per cent yield loss} = \frac{\text{Yield of healthy plants} - \text{yield of diseased plants}}{\text{Yield of healthy plants}} \times 100.$$

appeared after 44 days of sowing its effect was statistically non-significant on pods/plant, seeds/pod, 1000 grain weight and yield/plant.

EFFECT OF TEMPERATURE AND HUMIDITY ON DISEASE INCIDENCE AND DEVELOPMENT

Meteorological data on temperature and humidity and the observations on disease incidence and development during summer and kharif seasons on urd bean cv. Krishna during 1975, 1976 and 1979 under natural conditions at H.A.U. Farm,Hissar are presented in Tables 7, 8 and 9, respectively.

The data indicate that when there was average maximum temperature around 35°C, average minimum temperature around 25°C, average morning relative humidity more than 70 per cent and average evening relative humidity more than 50 per cent, the leaf crinkle symptoms were more conspicuous. Data also indicate that when the plants remained exposed for a week to 38°- 45°C in morning relative humidity around 60 per cent and evening relative humidity around 40 per cent, the leaf crinkle symptoms did not appear. However,disease symptoms were observed when maximum temperature was not more than 37°C for about a week with slight more humidity. The disease symptoms were not observed even if the temperature came down below 37°C with slight more humidity for

Table 7. Effect of temperature and humidity on disease incidence and development of leaf crinkle disease of urd bean cv. Krishna during 1975 at H.A.U. Farm, Hissar.

Dates of observation	Temperature and humidity						Disease Observations	
	Max. tem. (^{o}C)		Min. tem. (^{o}C)		Average per cent relative humidity		Per cent disease	Per cent disease development
	Range	Average	Range	Average	Morning	Evening		
			Summer season (date of sowing 25.4.1975)					
25.4.75 - 18.5.75	36.4 - 44.4	40.0	16.1 - 31.7	21.6	61.4	37.8	0.00	0.00
19.5.75 - 25.5.75	38.7 - 42.5	41.1	18.7 - 26.6	22.3	50.6	32.6	0.00	0.00
26.5.75 - 1.6.75	38.7 - 44.3	41.9	21.8 - 27.5	25.0	52.7	35.6	0.00	0.00
2.6.75 - 8.6.75	36.0 - 36.9	36.6	21.1 - 24.2	23.2	63.7	37.6	3.10	0.78
9.6.75 - 15.6.75	34.7 - 44.7	41.7	20.9 - 29.9	24.4	54.1	34.6	0.00	0.00
16.6.75 - 22.6.75	35.9 - 44.9	40.1	24.2 - 32.6	27.7	58.4	39.0	0.00	0.00
23.6.75 - 29.6.75	33.5 - 45.6	39.7	22.6 - 27.8	26.0	72.1	47.3	0.00	0.00
			Kharif season (date of sowing 11.7.1975)					
11.7.75 - 3.8.75	25.1 - 41.9	35.9	21.3 - 28.9	27.0	79.3	65.9	3.30	0.82
4.8.75 - 10.8.75	28.0 - 37.3	32.3	23.7 - 27.0	25.0	91.6	84.4	5.60	1.40
11.8.75 - 17.8.75	30.5 - 37.1	35.1	25.5 - 27.7	26.3	85.4	68.1	9.80	7.40
18.8.75 - 24.8.75	31.9 - 35.7	33.6	22.8 - 26.1	24.9	87.7	77.3	12.80	12.80
25.8.75 - 31.8.75	32.3 - 34.5	33.5	22.5 - 26.0	24.4	85.0	81.6	14.20	14.20
1.9.75 - 7.9.75	26.6 - 34.3	32.4	22.5 - 25.4	24.4	92.0	77.6	14.50	14.50
8.9.75 - 14.9.75	28.8 - 32.4	30.5	21.6 - 24.1	23.3	87.0	79.0	14.50	14.50

crinkle disease of urd bean cv. Krishna during 1976 at H.A.U. Farm, Hissar.

Dates of observation	Temperature and humidity						Disease Observations	
	Max. tem. (°C)		Min. tem. (°C)		Average per cent relative humidity		Per cent disease	Per cent disease developmen
	Range	Average	Range	Average	Morning	Evening		
			Summer season (date of sowing 28.4.1976)					
28.4.76 - 21.5.76	36.2 - 43.3	38.0	16.4 - 26.7	21.2	72.6	24.8	0.00	0.00
22.5.76 - 28.5.76	35.2 - 42.1	39.5	20.5 - 26.5	23.4	69.3	38.3	0.00	0.00
29.5.76 - 4.6.76	42.6 - 45.2	43.6	23.8 - 28.7	22.3	47.9	20.0	0.00	0.00
5.6.76 - 11.6.76	32.8 - 37.1	36.3	22.3 - 30.8	26.4	69.4	45.6	0.00	0.00
12.6.76 - 18.6.76	27.6 - 35.1	32.6	20.9 - 30.2	23.8	77.1	61.6	0.00	0.00
19.6.76 - 25.6.76	30.0 - 40.0	34.8	20.7 - 24.2	22.8	74.6	52.1	0.00	0.00
26.6.76 - 2.7.76	41.3 - 44.5	42.7	25.0 - 31.0	28.9	69.1	32.3	0.00	0.00
			Kharif season (date of sowing 12.7.1976)					
12.7.76 - 4.8.76	30.3 - 40.1	34.3	24.1 - 27.6	26.9	84.7	75.0	19.40	0.97
5.8.76 - 11.8.76	27.8 - 34.5	31.1	23.8 - 25.4	24.7	88.3	85.3	34.90	17.45
12.8.76 - 18.8.76	30.3 - 35.1	32.4	23.7 - 25.5	24.4	86.6	81.3	64.40	48.30
19.8.76 - 25.8.76	30.4 - 36.3	33.9	22.6 - 26.1	24.6	86.4	77.1	85.60	85.60
26.8.76 - 1.9.76	32.3 - 34.1	33.0	24.2 - 25.0	24.6	88.7	81.6	95.00	95.00
2.9.76 - 8.9.76	26.2 - 34.0	32.1	22.5 - 25.6	24.5	92.4	84.7	97.00	97.00
9.9.76 - 15.9.76	30.4 - 35.4	33.7	22.0 - 23.7	23.2	88.0	72.0	97.00	97.00

Table 9. Effect of temperature and humidity on disease incidence and development of leaf crinkle disease of urd bean cv. Krishna during 1979 at H.A.U. Farm, Hissar.

Dates of observation	Temperature and humidity						Disease observations	
	Max. tem.(°C)		Min. tem.(°C)		Average per cent relative humidity		Per cent disease	Per cent disease development
	Range	Average	Range	Average	Morning	Evening		
			Summer season (date of sowing 1.5. 1979)					
1.5.79 - 24.5.79	32.6 - 43.2	38.9	15.4 - 25.6	21.5	50.3	23.1	0.00	0.00
25.5.79 - 31.5.79	34.6 - 36.6	35.80	20.5 - 23.3	21.5	50.7	29.3	19.60	4.90
1.6.79 - 7.6.79	38.0 - 42.7	38.7	19.4 - 25.4	22.1	44.3	16.1	0.00	0.00
8.6.79 - 14.6.79	33.6 - 47.4	42.6	22.8 - 30.6	25.7	52.4	26.9	0.00	0.00
15.6.79 - 21.6.79	38.6 - 44.8	42.4	23.0 - 28.5	25.8	51.9	20.1	0.00	0.00
22.6.79 - 28.6.79	39.7 - 43.4	41.7	26.8 - 31.0	28.6	56.9	34.9	0.00	0.00
29.6.79 - 5.7.79	34.7 - 36.2	35.4	24.6 - 27.8	25.1	65.0	54.0	0.00	0.00
			Kharif season(date of sowing 3.9.1979)					
3.7.79 - 26.7.79	31.0 - 44.1	37.8	19.6 - 31.0	26.5	75.1	57.4	6.5	1.62
27.7.79 - 2.8.79	35.3 - 40.3	37.7	23.0 - 27.5	26.2	75.4	42.6	19.8	4.95
3.8.79 - 9.8.79	28.6 - 37.5	34.4	25.1 - 28.0	26.7	87.0	62.9	32.2	8.05
10.8.79 - 16.8.79	31.4 - 38.0	35.2	24.0 - 27.5	25.6	79.4	54.1	56.0	42.00
17.8.79 - 23.8.79	35.4 - 37.0	36.2	24.8 - 27.2	25.5	77.1	47.9	73.2	55.20
24.8.79 - 30.8.79	34.6 - 37.8	36.8	22.0 - 30.2	24.9	70.7	38.6	81.2	60.90
31.8.79 - 6.9.79	33.4 - 40.1	38.1	22.0 - 31.0	25.8	68.0	37.1	83.0	61.25

about a week if the plants were already exposed to 41°C - 45°C continuously for a week (Table 8) or to more than 47°C even for a day (Table 9), in morning per cent relative humidity around 45 and evening per cent relative humidity around 20.

TRANSMISSION

Mechanical transmission :

The virus was easily transmissible by mechanical means. The mechanical transmission was more efficient when inoculum (sap) was prepared in phosphate buffer rather than in water and when carborandum powder was used as abrasive (Tables 10 and 11). To see the effect of molarity and pH of phosphate buffer and the carborandum powder as abrasive on the efficiency of meçhanical transmission, the disease leaves of urd bean cv. Krishna were crushed in 0.01 - 0.5 molar phosphate buffer pH 6.0 - 8.00 separately. Thereafter inoculum prepared as above was rubbed with forefinger with or without carborandum powder (400 mesh) sprinkled over the leaves before inoculation. The results as presented in Table 10 and 11 indicate that the efficiency of mechanical transmission was maximum when the inoculum (sap) was prepared in 0.1M phosphate buffer, pH 7.0 and when carborandum powder was used as an abrasive.

Table 10. Effect of milar concentrations of phosphate buffer pH 7.0 and carborandum powder on mechanical transmission of leaf crinkle virus

Phosphate buffer (molar)	Without carborandum powder Plants inoculated/infected	With carborandum powder Plants inoculated/infected
0.01	15/0	15/5
0.05	15/1	15/9
0.1	15/2	15/11
0.15	15/1	15/10
0.2	15/1	15/10
0.3	15/0	15/8
0.4	15/0	15/7
0.5	15/0	15/5
Water	15/0	15/2

Table 11. Effect of different pH of 0.1M phosphate buffer and carborandum powder on mechanical transmission of leaf crinkle virus

Buffer pH	Without carborandum powder Plants inoculated/ infected	With carborandum powder Plants inoculated/ infected
6.0	15/2	15/3
6.2	15/5	15/7
6.4	15/6	15/7
6.6	15/8	15/9
6.8	15/10	15/12
7.0	15/11	15/14
7.2	15/10	15/12
7.4	15/10	15/13
7.6	15/10	15/11
7.8	15/8	15/10
8.0	15/5	15/6

Table 12. Effect of age of urd bean cv. Krishna on susceptibility to leaf crinkle virus

Experiment No.	Age(days) of plants at the time of inoculation						
	7	14	21	28	35	42	49
I	10/10	10/10	10/9	10/7	10/4	10/1	10/0
II	10/9	10/9	10/8	10/6	10/5	10/0	10/0
III	10/10	10/9	10/7	10/5	10/4	10/2	10/0
IV	10/9	10/9	10/8	10/5	10/3	10/0	10/0
V	10/9	10/10	10/8	10/6	10/4	10/1	10/0
Total:	50/47	50/47	50/40	50/29	50/20	50/4	50/0

Figures indicate plants inoculated/infected.

The effect of plant age on the efficiency of mechanical (sap) transmission or on susceptibility of plants was studied by inoculating the plants of urd bean cv. Krishna at different stages of plant growth. The results are presented in Table 12 which indicate that all the stages of plant growth are not appropriate for mechanical transmission or the plants were not equally susceptible to leaf crinkle virus at different ages. Younger the plant more the efficiency of mechanical transmission or the younger the plant more it is susceptible. The highest transmission or susceptibility was recorded when the plants were inoculated at the age of 7-14 days (Table 12). The plants were not infected when mechanically inoculated at the age of 49 days.

Insect transmission :

Two aphid species i.e. *Aphis craccivora* Koch., *Aphis gossypii* Golv., one beetle i.e. *Aulacophora fevicaulis* Lucas and one white fly i.e. *Bemesia tabaci* Genn. were used to transmit the leaf crinkle virus. The results obtained are presented in Table 13 which indicate that the *A.craccivora* and *A.gossypii* transmitted the virus after 5 min.acquisition feeding but when these aphids were given 2 h acquisition feeding no evidence of transmission of the virus was obtained. *A.fevicaulis* and *B.tabaci* neither transmit the virus after 5 min.acquisition feeding nor after 2 h acquisition feeding. *A.craccivora* and *A.gossypii* transmitted the virus

Table 13. Insect transmission of leaf crinkle virus of urd bean

Insect	Acquisition feeding period	No. of insects/ plant	Plants inf./inocu- lated	Per cent trans- mission
Aphis craccivora	5 min.	1	0/20	0.00
		2	0/20	0.00
		5	5/20	25.00
		10	10/20	50.00
		20	14/20	70.00
		30	14/20	70.00
	2 h	No transmission		
	Control(No virus acquisition feeding)	No transmission		
Aphis gossypii	5 min.	1	0/20	0.00
		2	0/20	0.00
		5	4/20	20.00
		10	10/20	50.00
		20	12/20	60.00
		30	12/20	60.00
	2 h	No transmission		
	Control(No virus acquisition feeding)	No transmission		
Aulacophora fevicaulis	5 min.	No transmission		
	2 h	No transmission		
Bemisia tabaci	5 min.	No transmission		
	2 h	No transmission		
No insect (control)		No transmission.		

upto 70 per cent plants and upto 60 per cent plants after 5 min.acquisition feeding using 20 insects per plant. Data also indicate that the virus is non-persistent.

Seed transmission :

The results of seed transmission are presented in Tables14 and 15 which indicate that the leaf crinkle virus was seed transmissible. It is evident from Table 14 that average seed transmission was 21.32 per cent being 20.03, 22.20 and 21.73 per cent during 1976, 1978 and 1979 respectively. Table 15 indicates that the per cent seed transmission decreased as the age of the plants at the time of inoculation/infection increased. The maximum transmission of the virus through seed was observed when the plants were inoculated at 7 days age being 58.97, 63.33 and 63.04 per cent during 1976, 1978 and 1979, respectively.

Table 14. Transmission of leaf crinkle virus through infected seeds collected from field diseased plants of urd bean cv.Krishna

Year	Seeds obtained from	Number of seeds sown	Number of seeds germinated	Diseased plants	Per cent trans-mission
1976	Diseased plants	1500	1428	286	20.03
	Healthy plants	1500	1476	0.00	0.00
1978	Diseased plants	1400	1267	279	22.20
	Healthy plants	1400	1358	0.00	0.00
1979	Diseased plants	1600	1482	322	21.73
	Healthy plants	1600	1544	0.00	0.00

Table 15. Effect of age of plant of urd bean cv. Krishna at the time of mechanical inoculation with leaf crinkle virus, on seed transmission

Year	Observation	Age(days) of plant at the time of inoculation from which diseased seeds were collected after maturity							Seeds collected from healthy plants
		7	14	21	28	35	42	49	
1976	No. of seed sown	51	58	65	118	107	110	105	101
	No. of seed germinated	39	47	56	114	103	107	103	100
	No. of diseased plants	23	26	27	34	5	0	0	0.0
	Per cent seed transmission	58.97	55.32	48.2	29.82	4.85	0	0	0.0
1978	No. of seed sown	42	61	66	101	94	95	102	105
	No. of seed germinated	30	46	52	95	89	92	100	103
	No. of diseased plants	19	28	26	31	5	1	0	0.0
	Per cent seed transmission	63.33	60.60	50.0	32.63	5.62	1.09	0	0.0
1979	No. of seed sown	56	64	70	103	105	92	110	100
	No. of seed germinated	46	56	63	98	102	91	109	100
	No. of diseased plants	29	33	31	31	4	0	0.0	0.0
	Per cent seed transmission	63.04	58.89	49.2	31.63	3.92	0	0.0	0.0

HOST RANGE AND HOST REACTIONS

Following 10 cultivated and 5 weed plant species were found susceptible to the virus after mechanical inoculation :

Family	Plant species
Amaranthaceae	*Gomphrena globosa* L.
Convolvulaceae	**Convolvulus arvensis* L.
Euphorbiaceae	*Ricinus communus* L.var.Aruna.
Leguminosae	*Dolichus lablab* L., *Glycine max* var.Bragg, *Phaseolus aconitifolius* Jacq. var.Ph.68-04 and Ph 222, *P.vulgaris* L.vars.Pinto and Sexa. *Vigna catjang* Endl. (Cholai bean), *V.radiata* (L.) Wilczek, *V.mungo*(L.)Hepper, *V.sinensis* Savi vars.PT 194207 and HFC 42-1.
Solanaceae	**Datura stramonium* L., **D.metel* L. **D.metaloides* L. and **D.incrmis* L.

The symptoms appeared on susceptible hosts are described in Table 16.

Following 39 cultivated and 18 weed plant species were not infected with the virus after mechanical inoculation:

Family	Plant species
Acanthaceae	*Justicia quinqueangularis Koen.
Amaranthaceae	*Achyranthes aspera L.
	*Amaranthus spp.,*Celosia argentia L.
	*Digerira arvensis Forsk.
Cannabinaceae	*Cannabis sativa L.
Chenopodiaceae	*Chenopodium amaranticolor Coste and Reyn., *C.quinoa L., Spinaceae oleraceae L.
Compositae	*Xanthium strumarium L.
Cruciferae	Brassica oleraceae L.var Hissar 1.
Cucurbitaceae	Cucumis sativus L., Momordica charantia L., Citrullus vulgaris Schred, Cucumis melo L. var. Momordica, Benincasa hispida(Thums) Cogn. Citrullus vulgaris Duthie and Fuller var.Fistulosus, Luffa acutangula (L.)Roxb Lagenaria vulgaris L.
Cyperaceae	*Cyperus rotundus L.
Euphorbiaceae	*Euphorbia hirta L.,
	*E.microphylla Heyne,
	*Phyllanthus niruri L.

Graminae	*Sorghum vulgare* Pers.var.JS 20, *Avena sativa* L.vars.Weston-11 and HFO 114, *Pennisetum typhoideum* Staff and Hubb. var.NHB 3, *Oryza sativa* L., *Hordeum vulgare* L., *Triticum sativum* L., vars.C 306, K 227 and S 308.
Leguminosae	*Arachis hypogea* L. var. MH-1, *Cajanus cajan* Milsp var.Prabhat *Cicer arietinum* L., *Cyamopsis tetragonoloba* (L.) Taub. vars.FS2, FS 277, Long poded and Pusa Alibahar, *Lathyrus odoratus* L.,*Phaseolus coccineus* L.var.Kentucky wonder, *P.vulgaris* L.vars. Bountiful,Stripped bountiful, EC 19037, Dutch costeknife, Top crop, and contender *P.lunatus* L., *Pisum sativum* var.Multifreezer, *Vicia faba* L., *Vigna unguiculata*(L.) Walp var. Blackeye 5.
Malvaceae	*Hibiscus esculentus* Moench, *Gossypium hirsutum* L.var. H14, *G.indicum* L. var. G 27, *Trianthema monogyna* L*.

Nyctaginaceae	*Boerhaavia diffusa L.
Pedaliaceae	Sesamum indicum L.
Polygalaceae	*Polygala chinensis L.
Portulaceae	*Portulaca oleraceae L.
Solanaceae	Lycopersicon esculentum Mill vars. S 12, HS 101, HS 102 and Marglobe, Capsicum frutescens L., C.pendulum L., Nicotiana tabacum L. vars.Xanthi, White Burley and Samsun, N.glutinosa L., N.rustica L., *N.plumbaginifolia Viv., Petunia hybrida Wilm., Solanum molongena L.

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* = Weed plants.

Table 16. Reactions/symptoms on susceptible hosts after infection with leaf crinkle virus

Plant species	Symptoms
V. mungo (L.)Hepper	Under natural conditions the first symptoms usually appeared about 22-25 days after sowing. On sap inoculation the symptoms appeared about 15-18 days after inoculation. Generally the third trifoliate leaf showed the symptoms first by an increase in size and lighter green colour followed by conspicuous crinkling on the upper surface, rugosity and malformation. Leaf tip curved downward and plant stunted. Inflorescence looked bushy, flowering and pod formation suppressed. (Plate 1 Fig.ii, Plate 2 Figs.i, ii & iii).
V. radiata (L.)Wilczek	-do- (Plate 1 Figs. i & iii).
V. sinensis Savi	
var. PT 194207 :	Systemically infected young leaves became dark green in colour followed

EXPLANATION OF PLATE 1

(on back side)

PLATE 1

Fig. i = *Mung bean leaf lets: (healthy) showing* no symptoms (a), mild symptoms (b), moderate symptoms (c), and severe symptoms (d) of leaf crinkle disease.

Fig.ii = Urd bean leaf lets: (healthy) showing no symptoms (a), mild symptoms (b), moderate symptoms (c), and severe symptoms (d) of leaf crinkle disease.

Fig.iii = A twig from healthy plant (a) and a twig from leaf crinkle disease plant(b) of mung bean. The twig from disease plant (b) showing leaf crinkling, rugosity and malformation of leaf lamina, bushy appearance of inflore-scence and no pod formation.

PLATE 1.

Fig.i

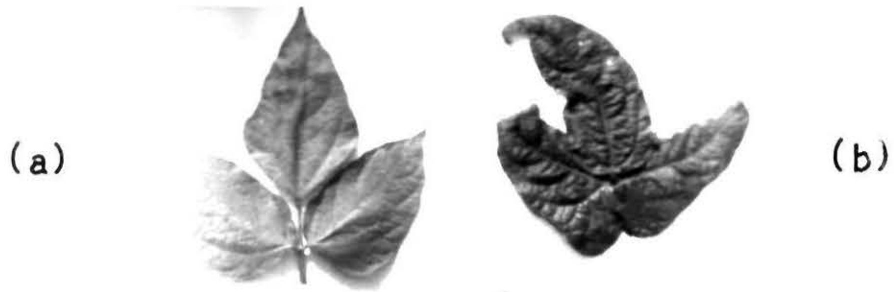
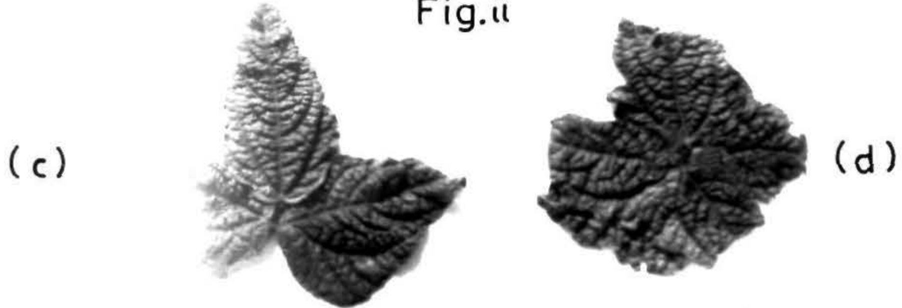


Fig.ii



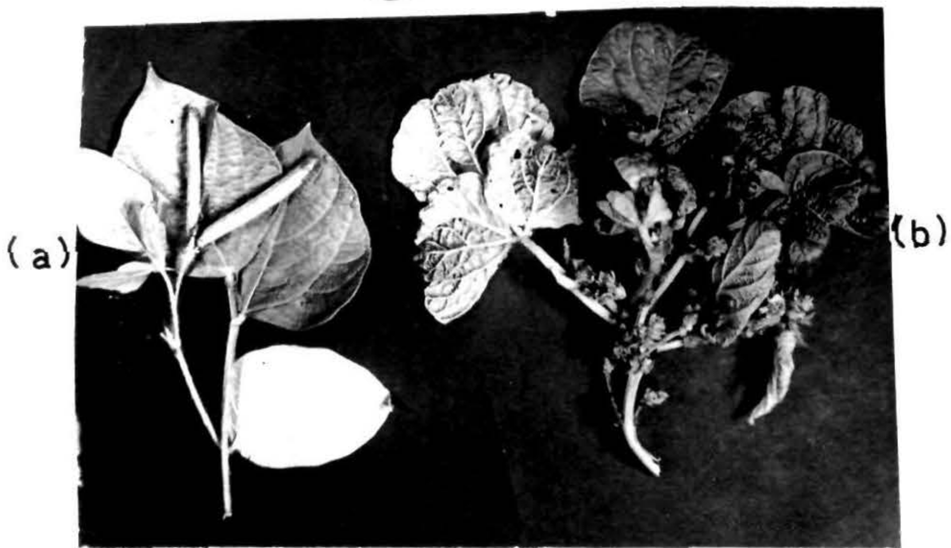


Fig. iii

PLATE 2

Fig. i = Urd bean leaf showing characteristics leaf crinkle symptoms.

Fig. ii = Urd bean pods from healthy plants (upper row) and from leaf crinkle disease plants (lower row). The pods from disease plants are distored and reduced in size.

Fig.iii = Urd bean seeds from pods of healthy plants (a) and from pods of leaf crinkle disease plants (b). The seeds from disease pods are small in size.

PLATE 2.

Fig. i

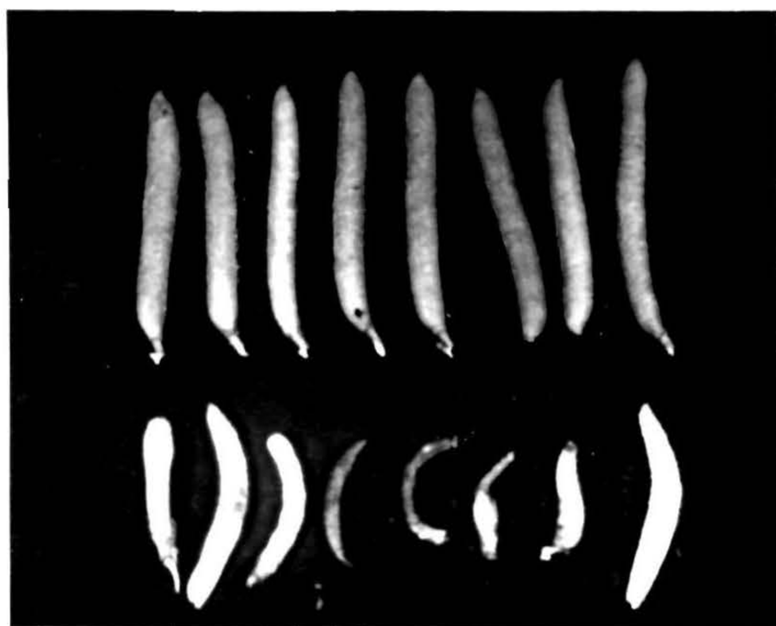


Fig. ii

Fig. iii

(a) (b)

by severe mosaic, leaf curling and reduction in leaf size. Symptoms appeared 2-3 weeks after sap inoculation. (Plate 3 Fig. i).

var.HFC 42-1: The sap inoculated primary leaves developed small necrotic lesions about 9-10 days after inoculation followed by typical severe systemic mosaic in uninoculated trifoliate leaves after 15 days of local lesion appearance. (Plate 3 Fig. ii).

V.catjang Endl. When leaves were inoculated with crude extract from infected leaves, only systemic mosaic symptom on young leaves appeared about 14-18 days after inoculation. (Plate 3 Fig. iii). When leaves were infected with purified virus, necrotic local lesions appeared on primary and trifoliate leaves about a week after inoculation followed by systemic mosaic on uninoculated trifoliate leaves about 1-2 weeks after appearance of local lesions. (Plate 7 Figs. i & iii).

EXPLANATION OF PLATE 3

(on back side)

PLATE 3

Fig. i = Plants of *Vigna sinensis* var. PT 194207 showing severe mosaic, curling and reduction in size of young leaves after systemic infection with leaf crinkle virus.

Fig. ii = Primary leaves of *V.sinensis* var. HFC 42-1 (lowest two leaves) showing distinct small necrotic local lesions 10 days after inoculation with leaf crinkle virus. The trifoliate leaves showing severe systemic mosaic on the same plant after 15 days of local lesion appearance on primary leaves.

Fig.iii = Cholai bean (*V.catjang*) plants showing systemic mosaic on young leaves after infection with leaf crinkle virus.

PLATE 3.

Fig. i

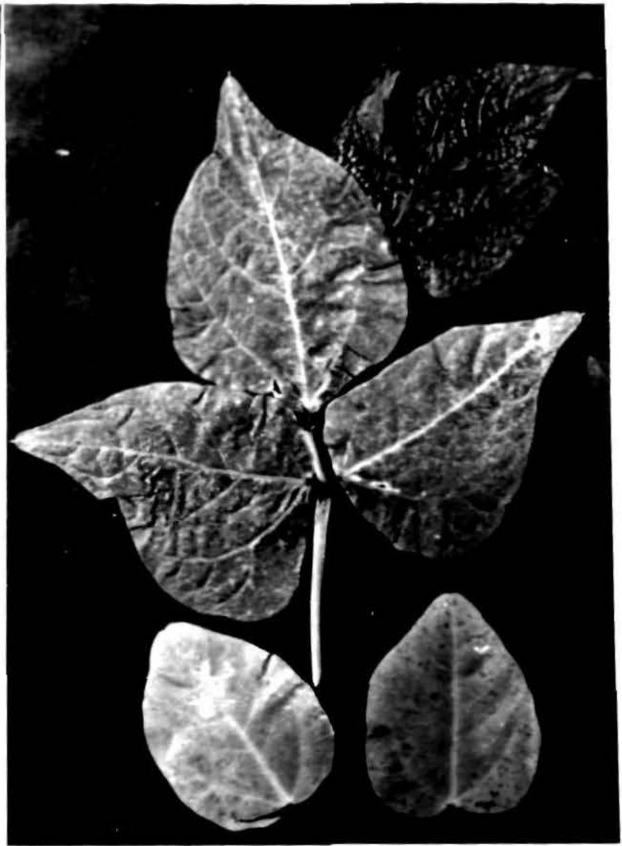


Fig. ii

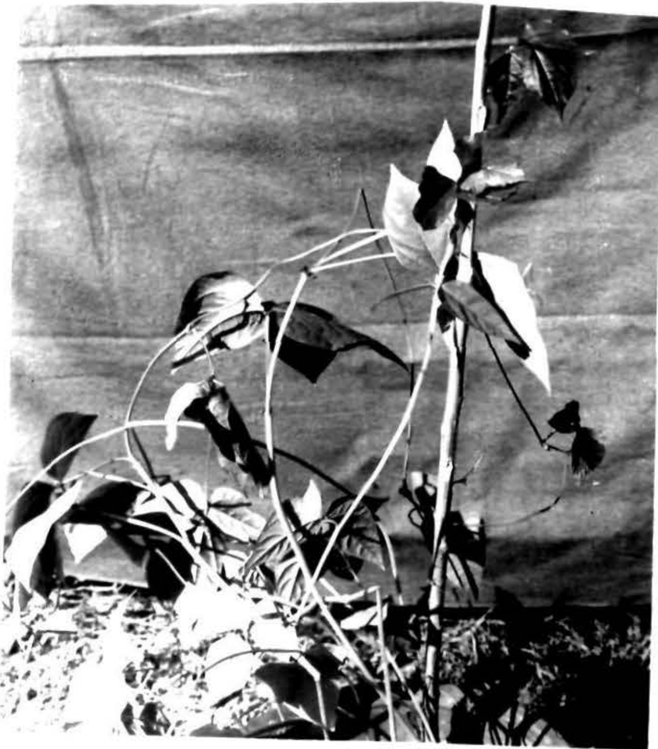


Fig. iii

P. aconitifolius Jacq.	Crinkling and mosaic mottling in systemically infected leaves,25-30 days after sap inoculation of the plants. Plants became stunted, inflorescence became bushy,flower and pod setting suppressed. (Plate 4 Fig. i).
P. vulgaris L.	
var.Pinto	Necrotic lesions on inoculated primary leaves 10 days after inoculation. No systemic infection. (Plate 5 Fig. ii).
var.Sexa	Chlorotic mosaic about 22-25 days after inoculation,leaf size reduced and occasional rolling of leaves was also seen. (Plate 5 Fig. i).
D. lablab L.	Small pin point necrotic local lesions on inoculated leaves about 10-12 days after sap inoculation. No systemic symptoms.
R. communis L.	Necrotic lesions on inoculated leaves about 9-10 days after sap inoculation. No systemic symptoms. (Plate 4 Fig. ii).

EXPLANATION OF PLATE 4

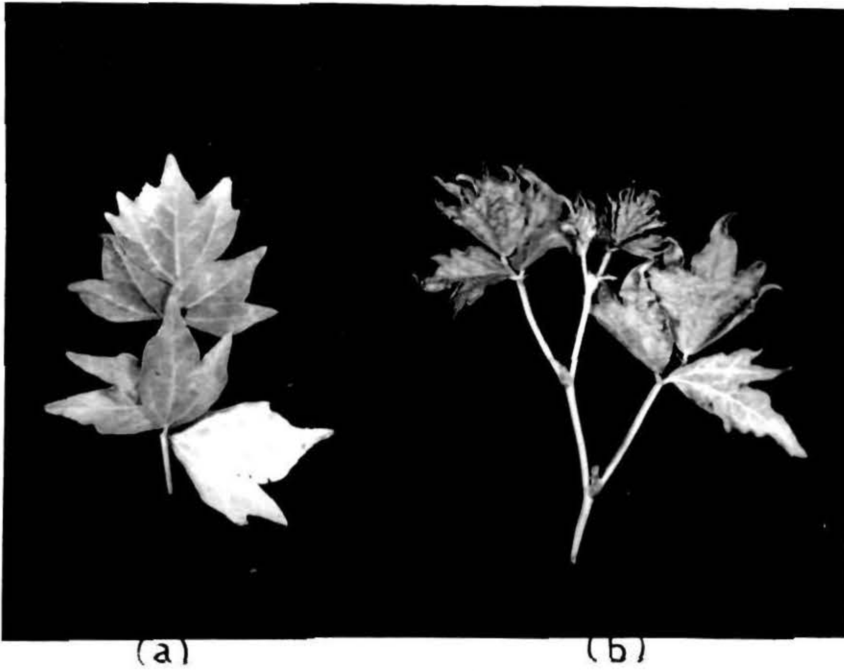
(on back side)

PLATE 4

Fig. i = Leaves from healthy plant (a) and from leaf crinkle disease plant(b) of *Phaseolus aconitifolius*. Crinkling and mosaic mottling are clearly visible in diseased leaves (b).

Fig. ii = Leaves of *Ricinus communis* showing distinct necrotic local lesions 10 days after sap inoculation with leaf crinkle virus.

PLATE 4.



(a) (b)

Fig. i

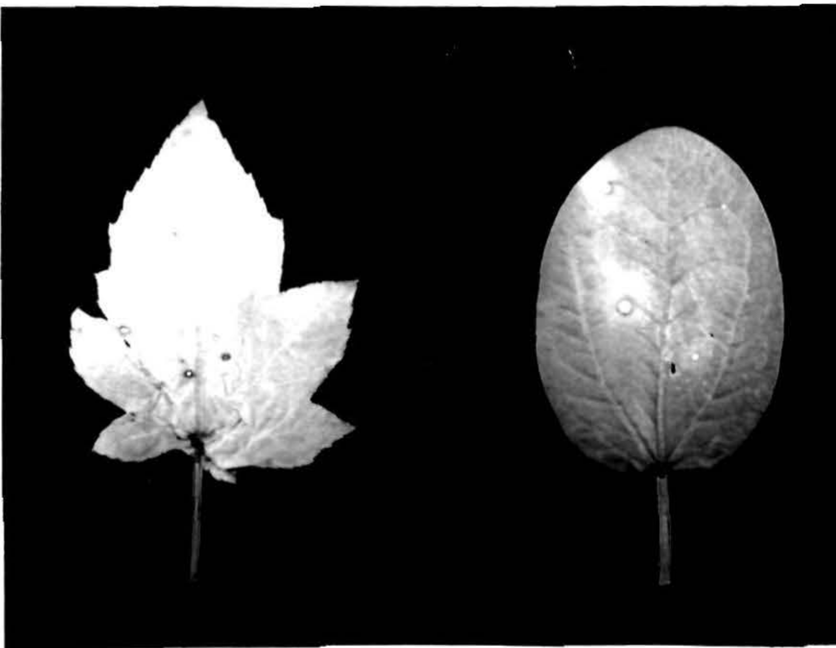


Fig. ii

G.globosa L.	Chlorotic spots on inoculated leaves after two to three weeks of sap inoculation. No systemic symptoms. (Plate 5 Fig. iii).
G.max (L.) Merr. var. Bragg	Two to three weeks after sap inoculation,the infected plants showed malformation and reduction in size of the leaves. Length of internodes drastically reduced and the plants became stunted.
D.stramonium L.	Necrotic lesions about 20 days after sap inoculation. No systemic symptoms. (Plate 6 Fig. i).
D.metel L.	-do-
D.metaloides L.	-do-
D.incrimis L.	-do-
C.arvensis L.	After two to three weeks of sap inoculation,the infected plants showed reduction in size of leaves exhibiting crinkling and mosaic mottling. Inflorescence became bushy,internodes increased in length and reduced in thickness. (Plate 6 Figs. ii & iii).

EXPLANATION OF PLATE 5

(on back side)

PLATE 5

Fig. i = Plants of P.vulgaris var.Sexa showing chlorotic mosaic,leaf size reduced and downward rolling of leaves after systemic infection with leaf crinkle virus.

Fig. ii = A primary leaf of P.vulgaris var. Pinto showing distinct necrotic local lesions 10 days after sap inoculation with leaf crinkle virus.

Fig. iii = A leaf of G.globosa showing chlorotic spots 3 weeks after sap inoculation with leaf crinkle virus.

PLATE 5.

Fig. i

Fig. ii

Fig. iii

EXPLANATION OF PLATE 6

(on back side)

PLATE 6

Fig. i = Leaves of *Datura metel* showing necrotic local lesions 20 days after sap inoculation with leaf crinkle virus.

Fig. ii = *Convolvulus arvensis* healthy plants(a) and leaf crinkle infected plants (b). The infected plants showing reduction in leaf size, leaf crinkling and mosaic mottling.

Fig. iii = A part of healthy plant (a) and leaf crinkle infected plant (b) of *C.arvensis*. The leaves of infected plant are showing crinkling, mosaic mottling and reduction in size.

PLATE 6.

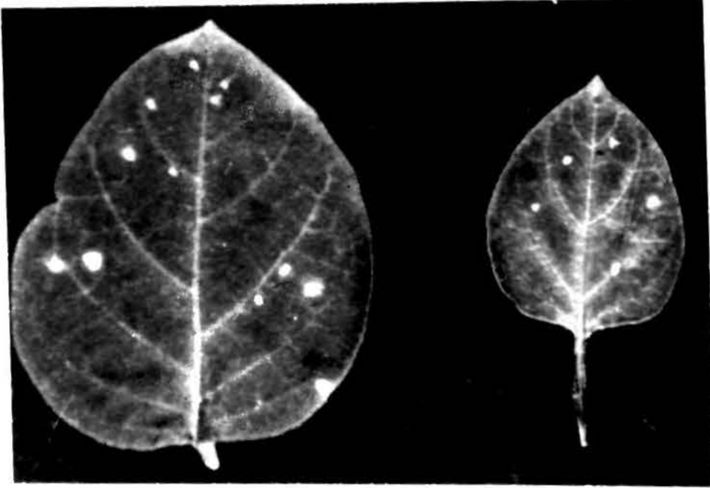
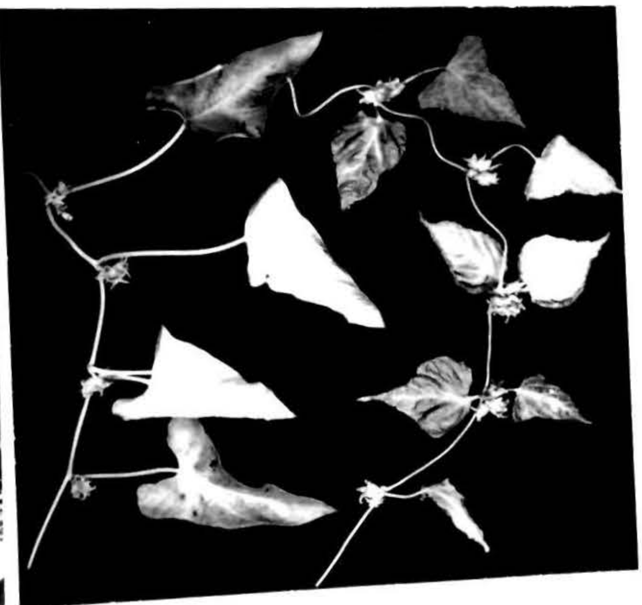


Fig. i



(a) (b) (a) (b)

Fig. ii Fig. iii

PHYSICAL PROPERTIES OF THE VIRUS

Dilution end point : The results obtained are presented in Table 17 which indicate that leaf crinkle virus remained infectious upto the dilution of 1:1,000.

Thermal inactivation point : Leaf crinkle virus in leaf extract with stood heating for 10 min.at 64°C (Table 18) and not beyond.

Longevity *in vitro* : The results obtained are presented in Table 19 which indicate that leaf crinkle virus remained infective upto 3 days at room temperature (35°C ± 2°C) and 9 days when stored in a refrigerator.

PURIFICATION OF THE VIRUS

Attempts were made to purify the virus employing different methods such as adjustment of pH, precipitation with salt (ammonium sulphate) and treatment with organic solvent i.e. chloroform:butanol (1:1), Sephadex (G-25 and G-50) filteration and differential centrifugation. The results obtained are as under :

a) Adjustment of pH :

When clarified extracts from healthy and diseased leaves were adjusted to pH 5.0, 4.5, 4.0, 3.5 and 3.0 separately, there was precipitation in the solution. The resulting precipitate from healthy and diseased leaves after

Table 17. Dilution end point of leaf crinkle virus of urd bean

Dilution	Number of plants inoculated	Number of plants infected
1:1	25	24
1:10	25	24
1:100	25	23
1:1000	25	7
1:10000	25	0
1:100000	25	0
1:1000000	25	0

Table 18. Thermal inactivation point of leaf crinkle virus of urd bean

Temperature °C	Number of plants inoculated	Number of plants infected
40	25	21
45	25	19
50	25	18
52	25	18
54	25	14
56	35	12
58	25	8
60	25	7
62	25	5
64	25	5
66	25	0
68	25	0
70	25	0
72	25	0
Control (unheated sap)	25	23

Table 19. Longevity *in vitro* of leaf crinkle virus of urd bean

Storage period (h)	Temperature	
	Room temperature ($35^{o}C \pm 2^{o}C$)	Refrigerator
	No. of plants inoculated/infected	No. of plants inoculated/infected
0 (Control)	20/20	20/20
12	20/13	20/19
24	20/8	20/19
36	20/6	20/18
48	20/2	20/18
60	20/1	20/16
72	20/1	20/15
84	20/0	20/14
96	20/0	20/12
108	20/0	20/11
120	20/0	20/10
132	20/0	20/8
144	20/0	20/8
156	20/0	20/8
168	20/0	20/5
180	20/0	20/3
192	20/0	20/2
204	20/0	20/2
216	20/0	20/1
228	20/0	20/0
240	20/0	20/0
252	20/0	20/0
264	20/0	20/0
276	20/0	20/0
288	20/0	20/0
300	20/0	20/0

being dissolved in 0.1M phosphate buffer pH 7.0 did not show any infectivity. The UV spectrum (220 - 300 nm) of these solutions did not indicate highest absorption at 260 nm. The colour of these solutions was greenish yellow.

b) Precipitation with salt and treatment with organic solvent:

The final preparations obtained from healthy and diseased leaves after repeated precipitation of the clear extract with ammonium sulphate were greenish yellow. It was neither infective and nor showed highest absorption at 260 nm when UV spectrum (220 - 300 nm) was recorded.

The clarified extracts obtained from healthy and diseased leaves after chloroform:butanol (1:1) treatment were tested for infectivity and their UV spectrum was determined. Some infectivity (Plate 7 Fig. iib) was observed in clarified extract from diseased leaves which was not found in case of clarified extract from healthy leaves (Plate 7 Fig. iia). None of the preparation indicated highest absorption at 260 nm.

c) Sephadex (G-25 and G-50) filteration :

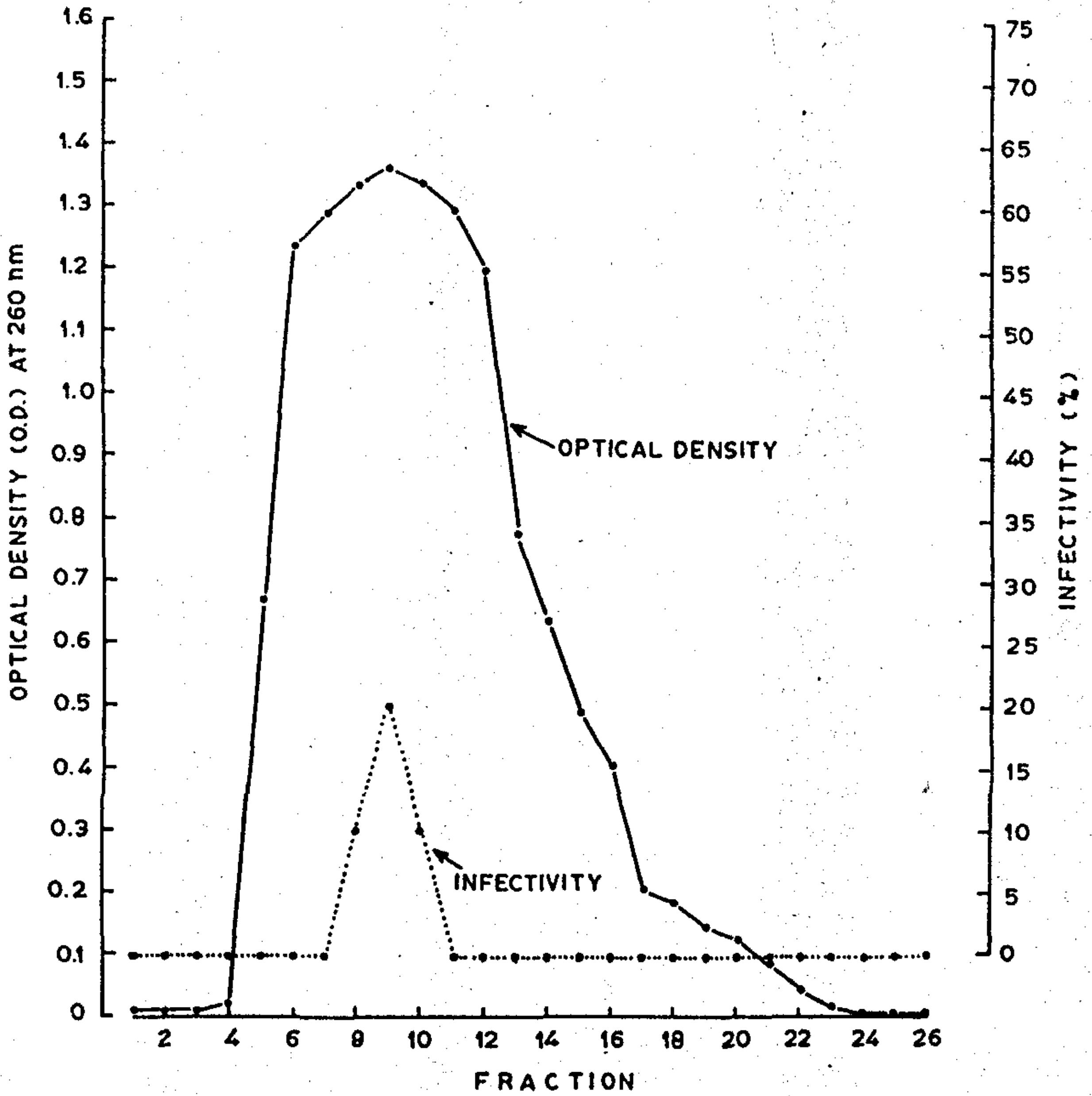
The results of virus purification by Sephadex G-25 and by Sephadex G-50 are presented in Figs. 3 and 4. Results with Sephadex G-25 (Fig. 3) indicated that the highest optical density at 260 nm was in fractions between 6 to 12 but fractions 8, 9 and 10 only were infective (Plate 7 Fig.ii b) which were greenish yellow in colour. Further purification

EXPLANATION OF FIG. 3

(on back side)

FIG. 3 Curves showing optical density(O.D.) at 260 nm and infectivity of the fractions collected after filteration of standard extract from diseased leaves cv. Krishna through Sephadex G-25 column.

FIG. 3.

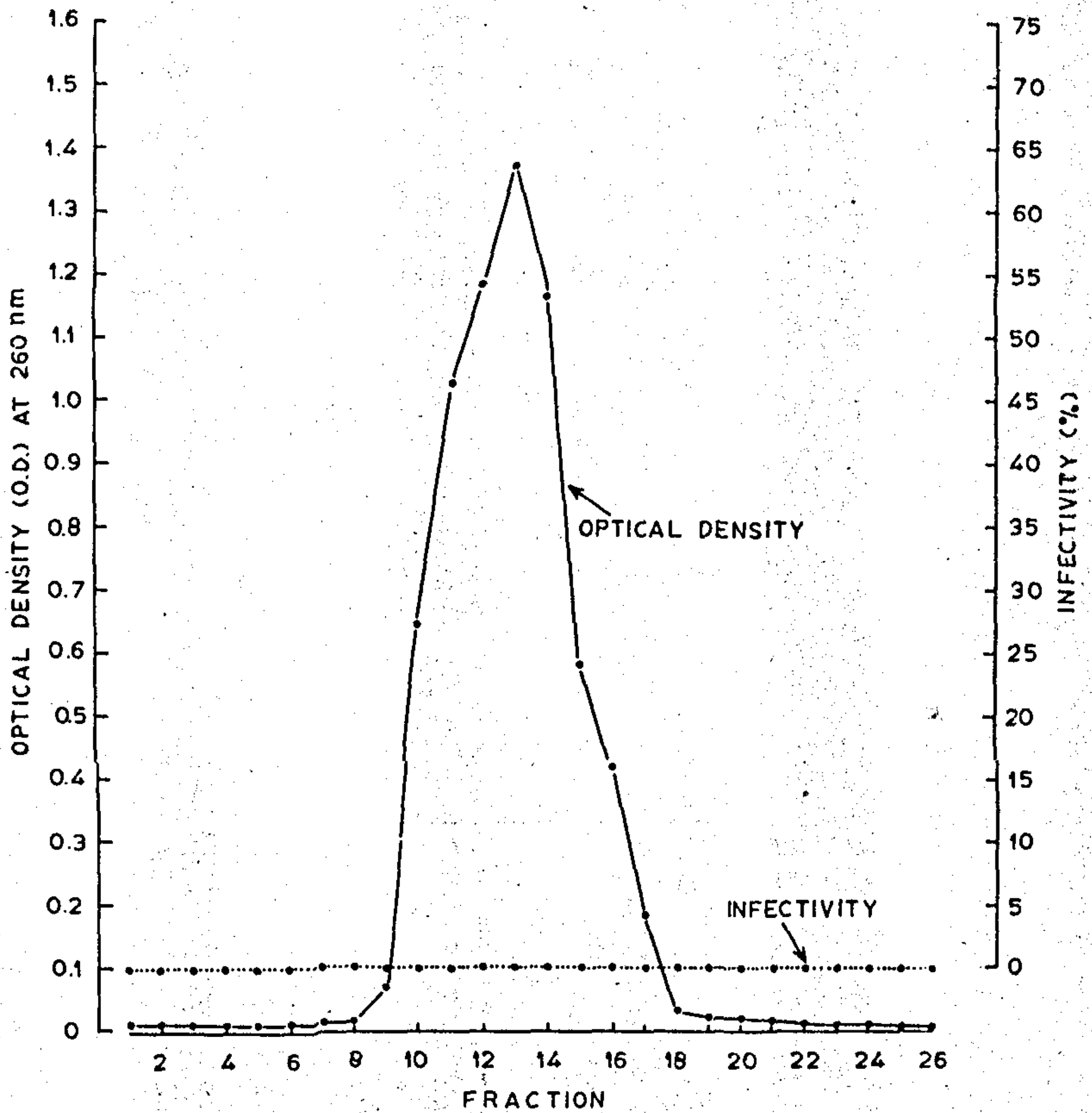


EXPLANATION OF FIG. 4

(on back side)

FIG. 4 Curves showing optical density (O.D.) at 260 nm and infectivity of the fractions obtained through Sephadex G-50 column.

FIG. 4.



of infective fractions (8 + 9 + 10) by Sephadex G-50 indicated (Fig. 4) that none of the fractions obtained after filteration with Sephadex G-50 was infective though the highest descending optical density value at 260 nm was in fractious 13, 12, 14 and 11. The UV spectrum (220 - 300 nm) of these four fractions was recorded. It was found that none of these four fractions showed highest absorption at 260 nm indicating absence of the virus.

d) Ultracentrifugation/differential centrifugation :

Purification by ultracentrifugation/differential centrifugation was done in different stages as under:

STAGE 1 :

Standard extract from healthy(H)/ diseased (D) leaves.

(200 ml)

Centrifuged at 30,000 r.p.m. for 2 h at 0 - 4°C (High speed centrifugation)

Pellet collected | Superntant discarded

Suspended in 20 ml of 0.1M phosphate buffer pH 7.0 and centrifuged at 10,000 r.p.m.(Low speed centrifugation) for 15 min at 0 - 4°C.

Pellet(insoluble material) discarded | Superntant collected

Tested for infectivity (Plate 7 Fig. ii) and UV spectrum determined (Fig.5 H1 & D1).

Diluted 4 times with
0.1 M phosphate buffer
pH 7.0 and centrifuged
at 30,000 r.p.m. for 2 h
at 0 - 4°C.

Pellet collected | Superntant discarded

Suspended in 20 ml of
0.1 M phosphate buffer
pH 7.0 and centrifuged
at 10,000 r.p.m. for
15 min.at 0-4°C.

Pellet(insoluble material)
discarded

Superntant collected

Tested for infectivity
(Plate 7 Fig. ii) and
UV spectrum was deter-
mined (Fig. 5 H_2 & D_2).

Diluted 4 times with 0.1M
phosphate buffer pH 7.0
and centrifuged at 30,000
r.p.m. for 2 h at 0-4°C.

Pellet collected | Superntant discarded

Suspended in 2 ml of
0.1M phosphate buffer
pH 7.0 and centrifuged
at low speed 10,000 r.p.m.
for 15 min.at 0-4°C.

Pellet(insoluble material)
discarded

Superntant collected

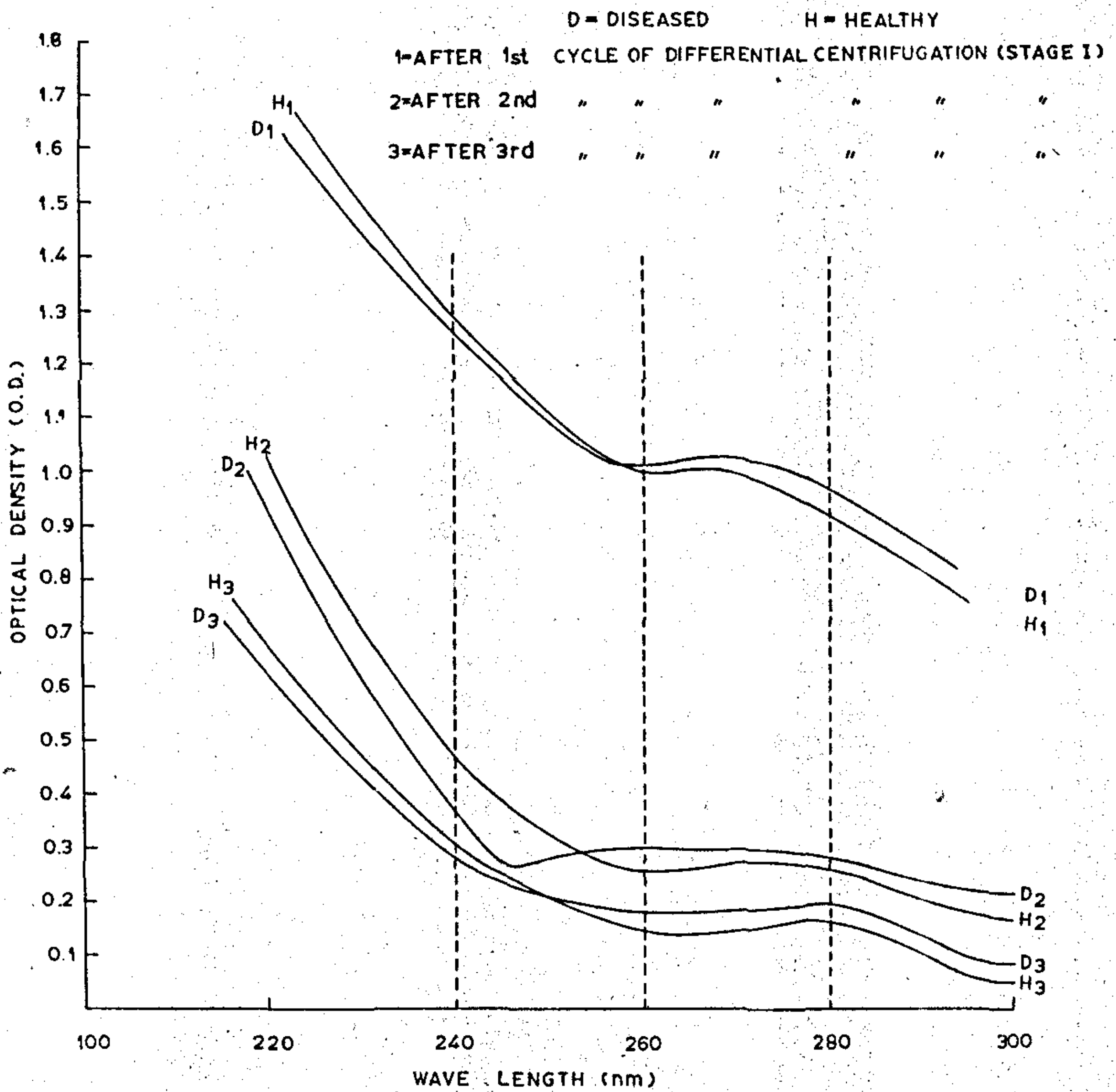
Tested for infectivity(Plate 7
Fig. ii). UV spectrum was deter-
mined after diluting 1 ml of these
preparations to 10 times with
0.1M phosphate buffer pH 7.
(Fig. 5 H3 & D3).

EXPLANATION OF FIG. 5

(on back side)

FIG. 5 Curves showing UV spectra (200 - 300 nm) of preparations from healthy (H) and leaf crinkle diseased (D) leaves of urd bean cv. Krishna after Ist(H1 & D1), 2nd (H2 & D2) and 3rd (H3 & D3) cycle of differential centrifugation STAGE 1.

FIG. 5.



Infectivity of the preparation H1, D1, H2, D2, H3 & D3 of differential centrifugation was tested on cholai bean (*V.catjang*). D1, D2 and D3 did not produce local lesions on inoculated leaves but systemic symptoms were seen (Plate 7 Fig. ii b) in 60, 65 and 45 per cent plants respectively. Preparations from healthy leaves (H1, H2 and H3) did not show infectivity (Plate 7 Figs. ia & ii a). The above results indicated that there was loss of infectivity of the virus after 3rd cycle of differential centrifugation. None of the preparation from either healthy (H1, H2 and H3) or diseased leaves (D1, D2 and D3) showed highest UV absorption at 260 nm (Fig. 5). The preparations were not considered satisfactory and further treatments were done as shown in stages 2, 3 and 4.

STAGE 2 :

To the standard extract from healthy and diseased leaves chloroform:butanol (1:1) was added in the ratio of 3:1. The mixture was shaken well for 10 min under cold condition and then centrifuged at 10,000 r.p.m. for 15 min. The watery phase was collected. This watery phase was subjected to 2 cycles of differential centrifugation as in Stage 1 above. The preparations thus obtained from diseased leaves (DA) and healthy leaves (HA) were tested for infectivity using cholai bean as test plant. Preparation from diseased leaves (DA) produced 35 local lesions per inoculated

leaf of cholai bean (Plate 7 Fig. i d) followed by systemic infection of uninoculated leaves (Plate 7 Fig. ii b). The infectivity results were negative in case of preparation obtained from healthy leaves (HA), Plate 7 Figs. ia & ii a). Preparation from diseased leaves (DA) but not from healthy leaves (HA) showed highest absorption at 260 nm when their UV spectrum was determined (Fig. 7). The colour of these preparations from healthy and diseased leaves was slightly yellowish. The preparations were not considered satisfactory and further treatments were done as shown in Stage 4.

STAGE 3 :

To the standard extract 8 per cent polyethyletic glycol (mol. wt. 6000) and 1.2 per cent sodium chloride was added, shaken well and the mixture was kept in a refrigerator over night. The precipitate appeared was collected by low speed centrifugation and dissolved in 0.1M phosphate buffer pH 7.0. Insoluble material from the solution was then removed by low speed centrifugation (10,000 r.p.m.) for 15 min. The clear supernatant was subjected to 2 cycles of differential centrifugation as in stage 1 and 2 above. The preparations thus obtained from healthy (HB), and diseased (DB) leaves were greenish yellow in colour. The preparation from diseased leaves (DB) when inoculated on cholai bean produced about 15 local lesions per leaf (Plate 7 Fig. i b) followed by systemic infection of uninoculated

leaves(Plate 7, Fig. ii b). The preparation from healthy leaves was not infective (Plate 7 Figs. i a & ii a). However, the preparation from healthy (HB) and diseased (DB) leaves did not show highest absorption at 260 nm when their UV spectrum were determined (Fig. 7). Further purification, therefore, was done as shown in Stage 4.

STAGE 4 :

The procedure adopted for further purification of virus stage 4 is described in Fig.6. The purified preparations thus obtained from diseased (DC) and healthy (HC) leaves were colourless. These colourless preparations from diseased (DC) and healthy (HC) leaves were turbid and transparent, respectively. These preparations were tested for infectivity and their UV spectrum was also determined(Fig.7). In the infectivity test on cholai bean the purified preparation from diseased leaves (DC) produced 125 local lesions per inoculated leaf (Plate 7 Fig. i c) followed by systemic infection of uninoculated leaves (Plate 7 Fig. ii b) in all the inoculated plants. The purified preparations from healthy (HC) leaves was not infective (Plate 7 Figs. i a and ii a). This purified preparation from diseased leaves(DC) also showed highest absorption at 260 nm but not the one (HC) from healthy leaves (Fig. 7). The preparation obtained after following the procedure in Stage 4 (Fig.6) was the purified virus.

Standard extract from diseased/healthy leaves
200 ml

Added 3:1 chloroform butanol (1:1) shaken well
for 10 min. under cold conditions

Centrifuged at 10,000 r.p.m. for 15 min.

Pellet and chloroform phase discarded | Watery phase collected

Added 8% PEG + 1.2% NaCl, shaken well, kept over night in a refrigerator and then centrifuged at 10,000 r.p.m. for 15 min.

Pellet collected | Supernatant discarded

Pellet resuspended in 0.1M phosphate buffer pH 7.0 and then the suspension/solution was centrifuged at 10,000 r.p.m. for 15 min.

Pellet (insoluble material) discarded | Supernatant collected

Centrifuged at 30,000 r.p.m. for 2 h

Pellet collected | Supernatant discarded

Pellet resuspended in 20 ml of 0.1M phosphate buffer pH 7.0. The suspension/solution was centrifuged at 10,000 r.p.m. for 15 min.

Pellet discarded | Supernatant collected

Centrifuged at 30,000 r.p.m. for 2 h

Pellet collected | Supernatant discarded

Pellet resuspended in 2 ml of 0.1M phosphate buffer pH 7.0

Centrifuged at 10,000 r.p.m. for 15 min.

Pellet(insoluble material) discarded | Supernatant collected (<u>Purified virus preparation</u>)

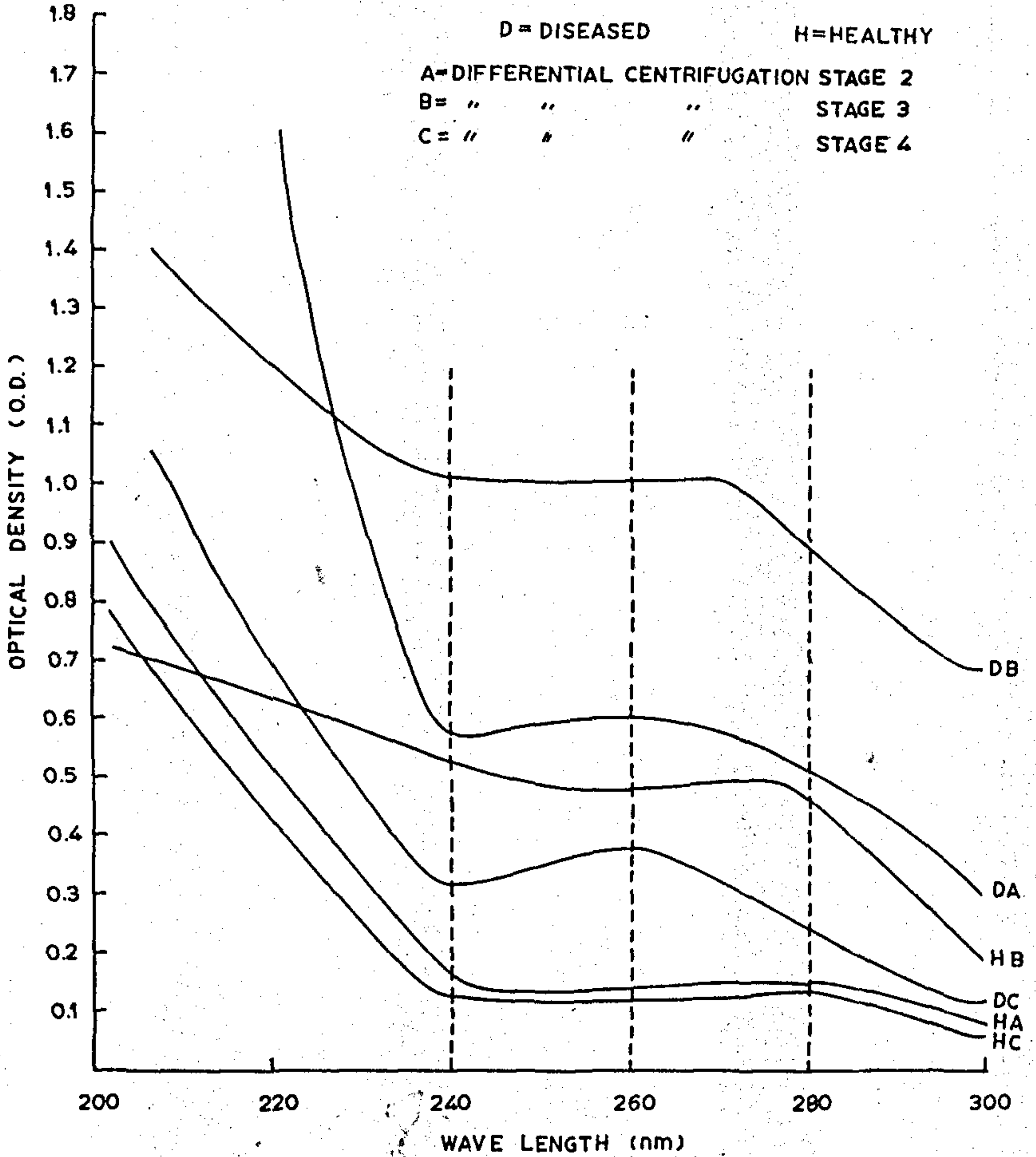
Fig. 6. Flow diagram indicating the procedure followed for the purification of leaf crinkle virus of urd bean and mung bean.

EXPLANATION OF FIG. 7

(on back side)

FIG. 7 Curves showing UV spectra (200-300 nm) of preparations from healthy (H) and leaf crinkle diseased (D) leaves of urd bean cv. Krishna obtained with techniques STAGE 2 (HA & DA), STAGE 3 (HB & DB) and STAGE 4 (HC & DC) of differential centrifugation. Among all the UV spectrum curves the curve DC showed highest peak at 260 nm.

FIG. 7.



EXPLANATION OF PLATE 7

(on back side)

PLATE 7

Fig. i = Leaflets of Cholai bean (V.catjang) showing necrotic local lesions one week after inoculation with purified virus (b, c and d). There are no lesions on the leaflet (a) inoculated with purified preparation from healthy leaves.

Fig. ii = Healthy leaf of Cholai bean (a) showing no symptom,& systemic mosaic (b). Diseased leaf (b) showing systemic mosaic symptoms 10 days after local lesion appearance on inoculated leaves.

PLATE. 7.

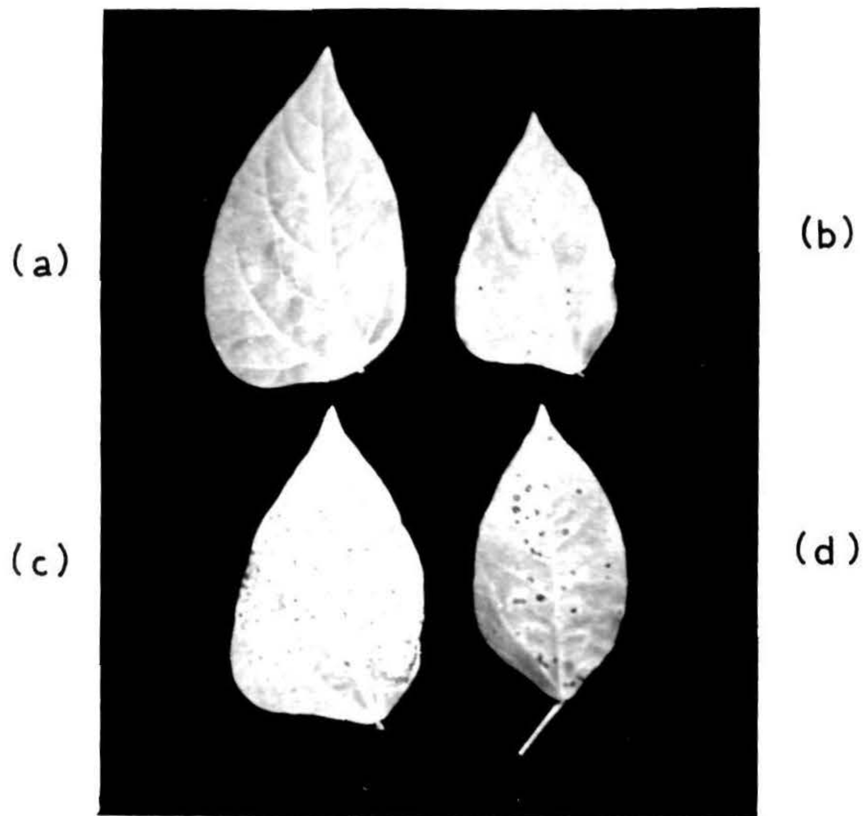


Fig. i

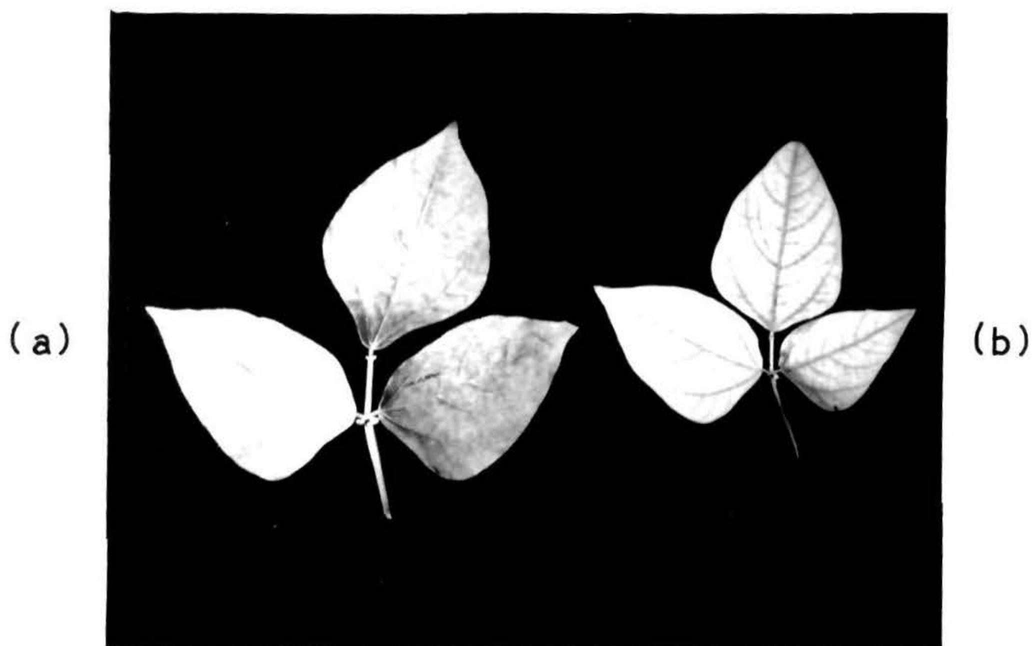


Fig. ii

PLATE 8

Fig. i = Electron micrograph of purified preparation of the leaf crinkle virus of urd bean/mung bean at 20,000 magnification showing spherical virus particles negatively stained with 2 % PTA pH 6.

Fig. ii = Electron micrograph of purified preparation of leaf crinkle virus of urd bean/mung bean at 40,000 magnification showing spherical virus particles negatively stained with 2 % PTA pH 6.

PLATE 8.

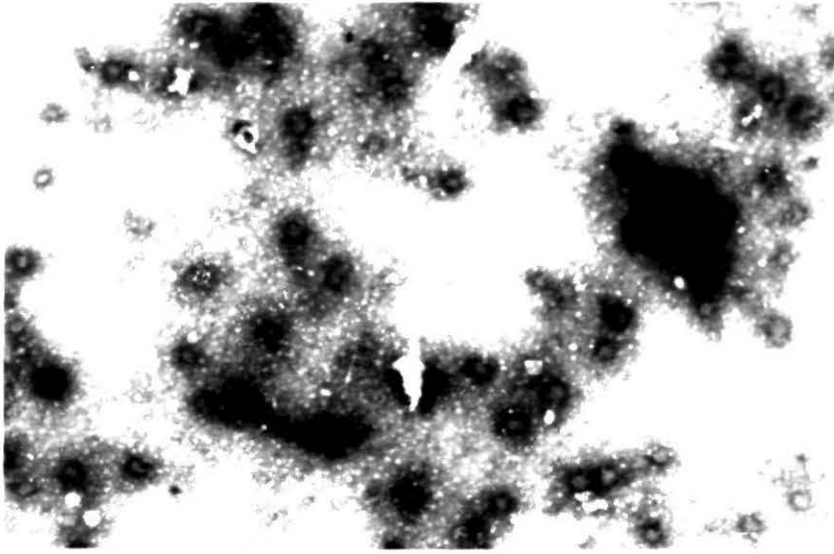


Fig. i

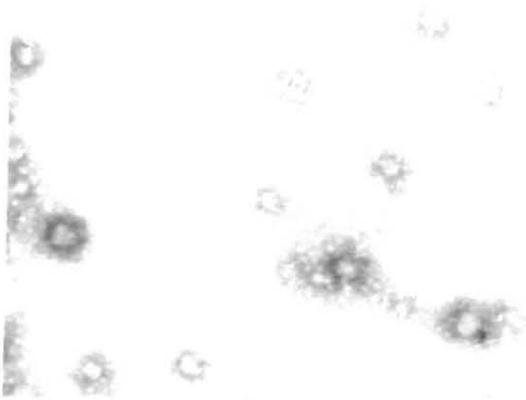


Fig. ii

Thermal inactivation point : Between 64° - 66°C.

Dilution end point : 1:1,000- 1: 10,000

Longevity in vitro: 3 days (room temperature 35°C ± 2°C)
9 days in refrigerator.

Cryptogram : */* : */15.24 : S/S : S/Ap.

When the above mentioned properties of the leaf crinkle virus of urd bean and mung bean were compared in the literature it was observed that this may be a new virus and when this was compared with some closely related isometric plant viruses as shown in Table 20, it was concluded that the leaf crinkle virus as described in this dissertation has greater similarities with pea enation mosaic virus (Harrison, 1969) and therefore, may be grouped with it.

PROTEIN CONSTITUTION

The results on protein constitution of leaves of resistant and susceptible varieties of mung bean (resistant: L-24-2 and 15227 ; susceptible: PS-16 and Green mung) and urd bean (resistant: DLU 90 and DLU 487; susceptible Krishna and PLS 364) are presented in Figs. 8 and 9, Plate 9. Protein constitution of mung bean and urd bean varieties was determined in 7 days old healthy seedlings and in leaves of 27 days old healthy and diseased plants. The diseased leaves were those which were sap inoculated with the leaf crinkle

Table 20. Comparison of the leaf crinkle virus of urd bean and mung bean with some closely related isometeric plant viruses

Virus with cryptogram	Particle size	Per cent nucleic acid in infective particles	Host range	Reaction on Mung bean	Reaction on Urd bean	Physical properties TIP	Physical properties DEP	Physical properties LIV	Transmissions	Reference
Leaf crinkle virus of urd bean and mung bean (as described in this dissertation). */*:*/15.24:S/S/Ap.	50 nm	15.24	Mostly leguminous plants	L.C.	L.C.	64°- $66^{o}C$	1:1000- 1:10000	3 days	Sap, seed Insect (Aphid)	Described in this dissertation.
Cauliflower mosaic virus D/2:4.5/16:S/S:S/Ap.	50 nm	16.00	Mostly Cruciferous plants	N.I.	N.I.	70°- $75^{o}C$	1:2000	14-15 days	Sap, Insect (Aphid)	Tompkins (1937), Pirone et al. (1960), Day and Venables (1961).
Cucumber mosaic virus R/1:1.3,+0.8/19:S/S:S/Ap.	28-30 nm	19.00	Wide host range	M	-	55°- $66^{o}C$	1:500 1:1000	48 h	Sap, seed, Insect (Aphid)	Ainsworth (1940), Tomilinson et al. (1959), Scott 1963), Smith (1972), Purivirojkul et al. (1978).
Clover wound tumour virus R/2:E16/22:S/S:S,I/Au	70 nm	22.0	Wide host range	N.I.	N.I.	-	-	-	Insect (Leaf hopper) only	Black (1945), Black and Brakke (1952), Streissle and Granados (1968).

contd.....

Table 20 (contd.)....

Pea enation mosaic virus R/1:1.6/28+1.3/28:S:S:S/Ap.	30 nm	28.0	Mostly leguminous plants	M	-	55° - 65°C	1:3000	3 days	Sap and insect (Aphid)	Pierce(1935), Chaudhuri(1950), Bustrillos (1965).
Cowpea mosaic virus R/1:2.3/34+1.5/28:S/S:S/Cl	24-27 nm	28.0 34.0	Mostly leguminous plants	L.L., M.M.	-	65°- 70°C	1:10000 1:100000	20 days	Sap, seed and insect (Beetles)	Dale(1949), vanKammen (1967), vanGriensvan and vanKammen (1969).

L.C. = Leaf crinkling, M = Mosaic, M.M. = Mosaic mottling, L.L. = Local lesion,
N.I. = Not infected, * and - = Not known, TIP = Thermal inactivation point,
DEP = Dilution end point, LIV = Longevity *in vitro* at room temperature.

virus on 8th day after sowing.

Results indicated (Fig. 8, Plate 9) that resistant (L-24-2 and 15227) and susceptible (PS-16 and Green mung) varieties of mung bean had slow mobile 4 proteins except in variety 15227 in 7 days old healthy seedlings. In variety 15227 one more protein was present in 7 days old healthy seedlings. This could be due to varietal characteristic. With increasing age i.e. till 27 days after sowing there was no change in number of proteins in healthy leaves of resistant varieties but in case of susceptible varieties there was a reduction in two proteins. After virus inoculation (done on 8th day after sowing) the number of proteins also did not change in leaves of resistant varieties though the concentration of slow mobile proteins decreased and of fast mobile proteins increased. In case of susceptible varieties the original 4 proteins either disappeared or changed into fast mobile proteins which did not distinctly separate following the technique as described in material and methods (Fig. 8, Plate 9).

In case of urd bean 7 days old seedlings of resistant (DLU 90 and DLU 487) and susceptible (Krishna and PLS 364) varieties revealed 9 and 8 protein bands, respectively. This indicated that one additional protein was present in resistant varieties as compared to susceptible ones. In healthy leaves of 27 days old plants of resistant varieties

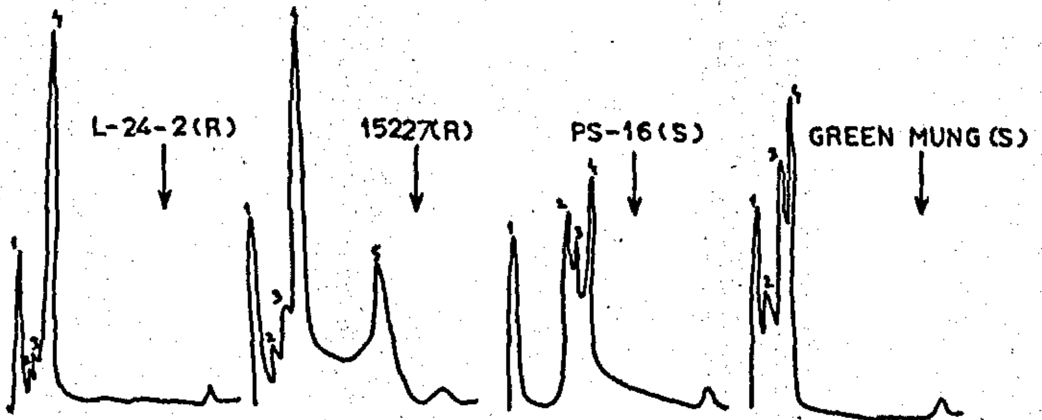
EXPLANATION OF FIG. 8

(on back side)

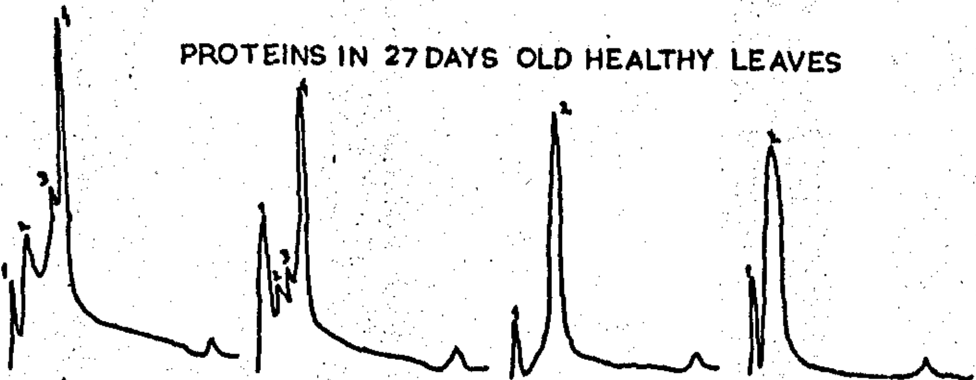
FIG. 8 Densitogram curves of gels showing protein pattern in healthy leaves of 7 days and 27 days old plants and in diseased leaves of 27 days old plants of resistant (R) and susceptible (S) varieties of mung bean to leaf crinkle virus.

FIG. 8.

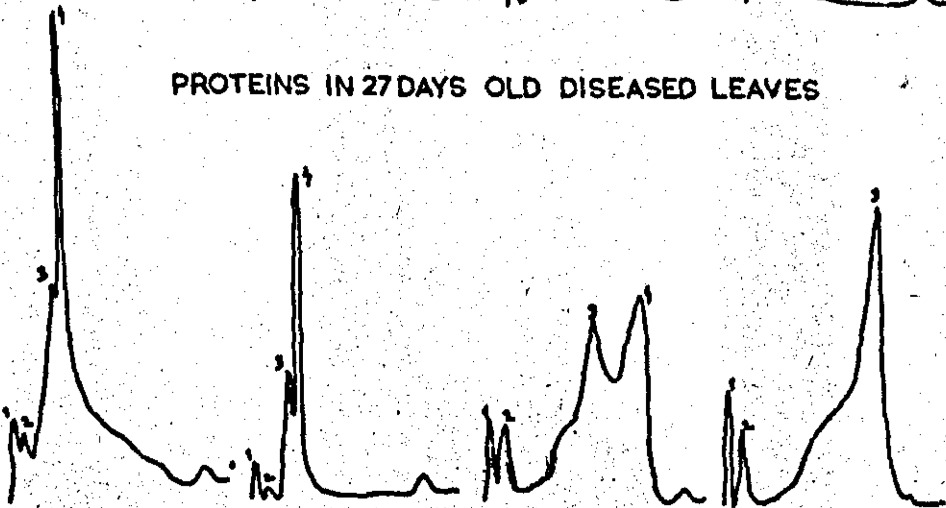
PROTEINS IN 7 DAYS OLD HEALTHY SEEDLINGS



PROTEINS IN 27 DAYS OLD HEALTHY LEAVES



PROTEINS IN 27 DAYS OLD DISEASED LEAVES



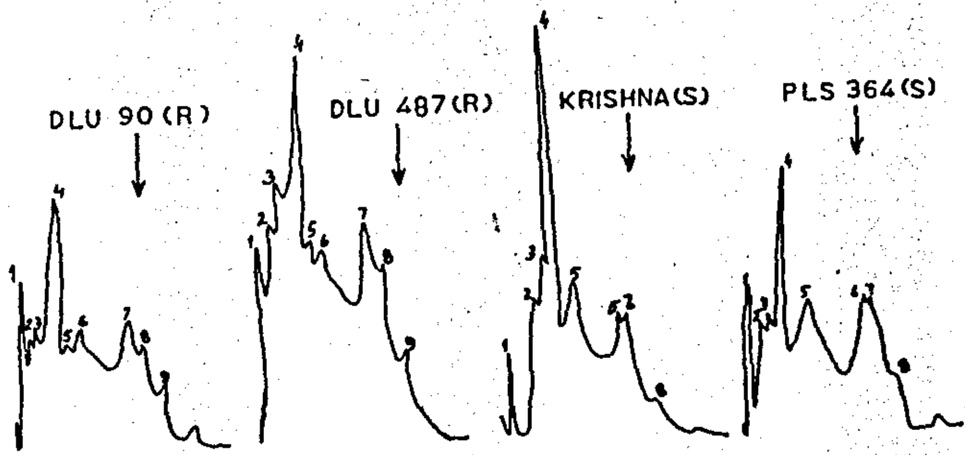
EXPLANATION OF FIG. 9

(on back side)

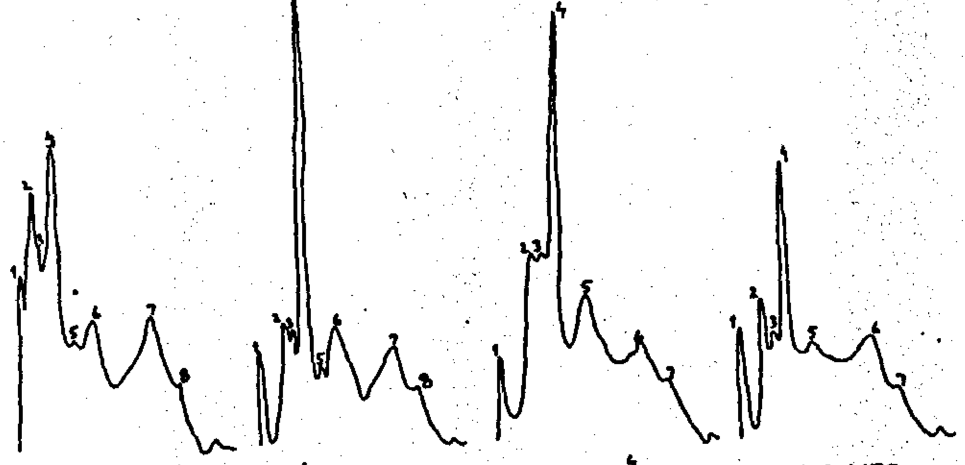
FIG. 9 Densitogram curves of gels showing protein pattern in healthy leaves of 7 days and 27 days old plants and in diseased leaves of 27 days old plants of resistant (R) and susceptible (S) varieties of urd bean to leaf crinkle virus.

FIG. 9.

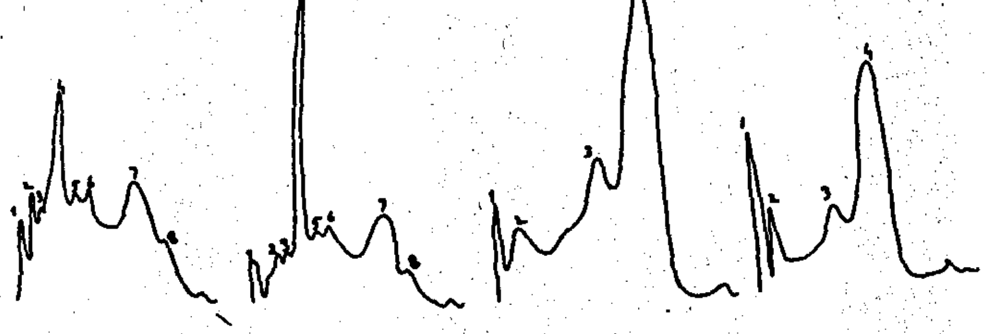
PROTEINS IN 7 DAYS OLD HEALTHY SEEDLINGS



PROTEINS IN 27 DAYS OLD HEALTHY LEAVES



PROTEINS IN 27 DAYS OLD DISEASED LEAVES



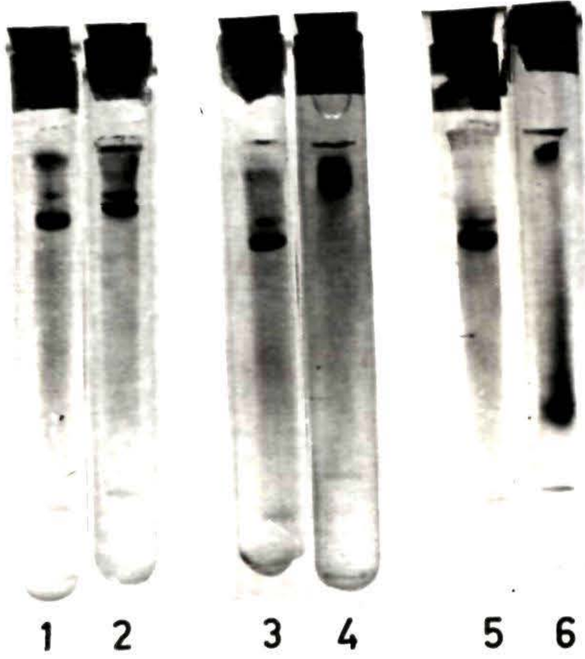
EXPLANATION OF PLATE 9

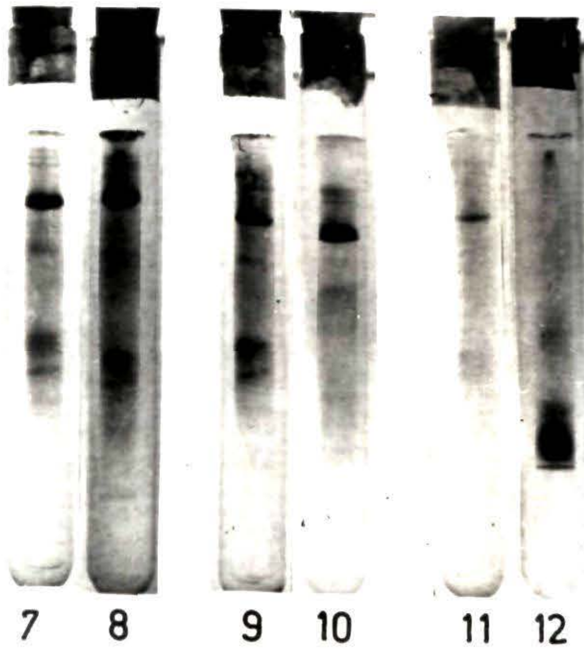
(on back side)

PLATE 9

1. Gel showing 4 protein bands in 7 days old healthy seedlings of mung bean var.L-24-2 resistant to leaf crinkle virus.
2. Gel showing 4 protein bands in 7 days old healthy seedlings of mung bean var. Green mung susceptible to leaf crinkle virus
3. Gel showing 4 protein bands in 27 days old healthy leaves of mung bean var. L-24-2 resistant to leaf crinkle virus.
4. Gel showing 2 protein bands in 27 days old healthy leaves of mung bean var. Green mung susceptible to leaf crinkle virus.
5. Gel showing 4 protein bands in 27 days old diseased leaves of mung bean var. L-24-2 resistant to leaf crinkle virus.
6. Gel showing protein 2 or 3 bands in 27 days old diseased leaves of mung bean var. Green mung susceptible to leaf crinkle virus. It is clear that the proteins were fast mobile which did not distinctly separate.
7. Gel showing 9 protein bands in 7 days old healthy seedlings of urd bean var. DLU 90 resistant to leaf crinkle virus.
8. Gel showing 8 protein bands in 7 days old healthy seedlings of urd bean var. PLS 364 susceptible to leaf crinkle virus.
9. Gel showing 8 protein bands in 27 days old healthy leaves of urd bean var. DLU 90 resistant to leaf crinkle virus.
10. Gel showing 7 protein bands in 27 days old healthy leaves of urd bean var. PLS 364 susceptible to leaf crinkle virus.
11. Gel showing 8 protein bands in 27 days old diseased leaves of urd bean var. DLU 90 resistant to leaf crinkle virus.
12. Gel showing 3 or 4 protein bands in 27 days old diseased leaves of urd bean var. PLS 364 susceptible to leaf crinkle virus. It is clear that proteins were fast mobile which did not distinctly separate.

PLATE 9.





(DLU 90 and DLU 487) there were 8 protein bands and of susceptible varieties (Krishna and PLS 364) there were 7 protein bands (Fig. 9, Plate 9). Thus there was decrease in one protein with the increase in age in leaves of both resistant and susceptible varieties of urd bean plants. The number of proteins did not alter after virus inoculation (done on 8th day after sowing) in leaves of resistant varieties of 27 days old urd bean plants. In diseased leaves of susceptible varieties, the characteristics 7 proteins as exhibited in healthy leaves of 27 days old plants, either disappeared or changed into high concentration of fast mobile proteins as is clear from intense coloured bands (Fig. 9, plate 9) which did not distinctly separate following the present technique. The susceptibility/ resistance of urd bean and mung bean therefore seemed to depend upon the synthesis of fast mobile proteins or on how much slow mobile proteins changed into fast mobile proteins during virus multiplication.

CONTROL MEASURES

RESISTANCE/SUSCEPTIBILITY OF MUNG BEAN AND URD BEAN VARIETIES/GERMPLASM

Results on varietal/germplasm screening of mung bean and urd bean to leaf crinkle virus under natural conditions and after artificial inoculation i.e., after sap inoculation in the screen house under insect proof conditions, are

presented in Tables 21, 22 and 23. Highly resistant (HR), resistant (R), moderately susceptible (MS), susceptible(S) and highly susceptible (HS) varieties/germplasm lines were identified on the basis as presented in Table 1.

Out of 390 mung bean varieties/germplasm lines screened under field conditions, 9 were highly resistant, 71 resistant, 216 moderately susceptible, 89 susceptible and 5 highly susceptible (Table 21).

The results of field screening of urd bean varieties/germplasm lines (Table 22) indicated that out of 138 varieties/germplasm lines screened, 11 were highly resistant, 28 resistant, 70 moderately susceptible, 27 susceptible and 2 highly susceptible.

Varieties/germplasm lines of mung bean and urd bean found highly resistant under field condition were selected and further screened after sap inoculation in the screen house under insect proof condition. The results (Table 23) indicated that none of the selected varieties/germplasm lines of mung bean and urd bean were found highly resistant. Two varieties (L-24-2 and 15227) of mung bean and two varieties (DLU 90 and DLU 487) of urd bean were found resistant. Rest of the varieties were moderately susceptible, susceptible and highly susceptible (Table 23).

Table 21. Screening of mung bean varieties/germplasm lines for resistance/susceptibility to leaf crinkle virus under field conditions

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Total varieties/germ plasm = 390

Highly resistant (9) : 15176, 15225, 15227, 15229, 15277, L-24-2, ML-5, T-44 and T-51.

Resistant (71): 52-2, 56-2, 57-3, 60-1, 64-2, 67-2, 73-2, 75-5, 77-1, 77-4, 77-6, 84-4, 84-6, 86-1, 92-2, 147, 15127, 60081, Culture 2, H 70-101, HyH-12-14, K-141, Khella-10, L-696-1, LC-1177-9-6-2B, LC-15225-1, LM-6, LM 10, LM 30, LM 35, LM 42, LM 45, LM 52, LM 56, LM 62, LM 64, LM 68, LM 71,LM 90, LM 109, LM 139, LM 147, LM 149, LM 153, LM 154, LM 204, LM 209, LM 226, LM 360, LM 371, LM 374, LM 424, LM 427, LM 431, MG 40, MH 2, MH 58, MH 61, MH 120, MH 128, MH 129, MH 143, ML-31, No. 127, P 43-67, PIM S-3, PTMS-4, PTMS-5, PM-146, Pusa Baisakhi and T 2.

Moderately susceptible (216) : 4-1, 5-3-8, 10/133, 11/99, 11/135, 12/133, 40-1, 54-1, 54-5, 56-1, 56-4, 57-2, 58-1, 58-2, 58-3, 59-2, 59-3, 59-4, 63-3, 64-6, 67-1, 67-4, 77-3, 84-5, 138, 138-1, 140, 142-1, 189-4-1, 237, 616, 1476, 11157, 11165, 14701, B-1, B-11, BR-2, CE S 87, Cot I, Cot -16, Cot 70, Cot 71, D-2-15, D-45-6, EC 27515, EC 98451, G-40, GC 192, GL 182, Gujrat-I, Gujrat-2, H 12-7, H 70-3, H 70-5, H 70-7, H 70-10, H 70-14, H 70-18, H 70-21, H 70-68, Hanumangarh, HYH-4-3A, HP-6, i/c-1008-13-2-5A, i/c-1008-13-2-5B, i/c-1160-1-12B, i/c-1109-1-5B, i/c-1204-3-2-2B, i/c-2030-5, i/c-15277, i/c-16401, i/c-2184, J-2, JO-70,K-851, Kardhar, Kishangarh, Krishna-11, LamGG 122, Lam GG 127, LM-1, LM-2, LM-4, LM-7, LM-9, LM-12, LM-14, LM 15, LM 16, LM 18, LM 20, LM 23, LM 34, LM 44, LM 50, LM 51, LM 59, LM 82, LM 95, LM 105, LM 126, LM 130, LM 133, LM 142, LM 144, LM 162, LM 142, LM 151, LM 162, LM 167, LM 174, LM 194, LM 201, LM 219, LM 221, LM 227, LM 232, LM 233, LM 235, LM 241, LM 242, LM 243, LM 245, LM 248, LM 250, LM 251, LM 253, LM 260, LM 297, LM 303, LM 304, LM 308, LM 309, LM 312, LM 318, LM 326, LM 329, LM 333, LM 339, LM 343, LM 346, LM 350, LM 357, LM 372, LM 387, LM 397, LM 403, LM 406, LM 408, LM 417, LM 420, LM 426, LM 434, LM 444, LM 448, LM 464, LM 472, LM 481, LM 484, LM 486, LM 488, LM 495, LM 696, MAELIVA, MC 37, MH 74, MH 79,

contd....

Table 21 (contd.).....

MH 99, MH 103, MH 113, MH 123, MH 130, MH 136, MH 140, MH 299, ML-1, ML-3, ML-4, ML-6, ML-8, ML-9, ML-10, MP-6, No-299, P 246, P 426, P 493-B-2, P 2006-1, PH 11, PIMS-I, PIMS-2, PLM 44, PLM 84, PLM 171, PLM 242-1, PLM 440, PLM 446-1, PLM 449-1, PLM 537, PLM 956, PLM 1037, PS 2/1 PS-10, PS-17, PS-32, PS-51, PV-4, R-38, R-192-1, R-288-8, RHSSIAH, S9, SHINING MUNG, SSM-1, T-1, and YELLOW MUNG.

<u>Susceptible</u> (89) : 1-170-68, 37/2, 40-2, 42-1, 44-7, 45-1, 49-1, 56-3, 57-1, 60-3, 64-5, 73-3, 75-3, 84-1, 84-7, 85, 86, 142, 235, 237-1, 247, 294-1, 331, 365, B 105, CES-55, CES-55-1, EC 1528, G-1, G-65, H 70-11, HSG, i/c-1000-7-1-5B, i/c-1007-1-5B, i/c-1089-B-29-5B, i/c 15276, J 45, J781, K6040, KP6, LM-26, LM28, LM29, LM53, LM87, LM97, LM-106, LM 143, LM 168, LM 170, LM 189, LM 228, LM 246, LM 252, LM 276, LM 316, LM 322, LM 328, LM 344, LM 356, LM 364, LM 370, LM 402, LM 412, LM 429, LM 438, LM 451, Madira, MH 101, MH 127, ML 24, NP 23, P-66-26, P-646, PLM 24, PM 106, PM 124, PLM 215, PLM 1030, PM 2, PS 7, Rm-1, Rm-21, RS-4, Russian, S-8, Sheela, TT9E and Varsha.

<u>Highly susceptible</u> (5) : Green Mung, LM 55, LM 200, LM 373 and PS-16.

Table 22. Screening of urd bean varieties/germ plasm lines for resistance/susceptibility to leaf crinkle virus under field condition

Total varieties/germ plasm: 138

Highly resistant (11): DLU 90, DLU 487, H 70-3, LU 201, Pant 4-26, Pant U 20, Pant U 26, UG-152, UG-153, UG-157 and UPU - I.

Resistant (28) : B-12-4-4, Bulandsher Local, G8, H76-1, H76-18, H76-19, H76-22, H76-23, H76-24, H76-31, H76-32, H76-38, H76-39, H76-40, H76-49, H76-51, H76-54, H76-61, H76-72, H76-73, H76-92, H76-115, JUI, KLO, Lain BG 296, SEL I, UG117 and UPU-2.

Moderately Susceptible (70): BG 295, G-104, H10-31, H-31, H75-2-7, H76-2, H76-4, H76-6, H76-7, H76-8, H76-10, H76-12, H76-13, H76-14, H76-59, H76-88, H76-101, HU-I, K78, LM 296, LU 207, LU 238, No. 12/8, No. 338/3, Pant 430, Pant U.30, PH-15, PH 23, PLU 118A, PU 73-4, PUSA I, S-311, UG-135, UG-170, UH-I, UH 17, UH 18, UH 22, UH 30, UH 33, UH 36, UH 37, UH 38, UH 41, UH 42, UH 45, UH 52, UH 53, UH 55, UH 56, UH 58, UH 60, UH 61, UH 62, UH 72, UH 82, UH 101, UH 107, UH 108, UH 109, UH 110, UH 111, UH 112, UH 113, UH 114, UH 115, UH 116, UH 117, UH 118, and UPU 3.

Susceptible (27): 4-5-2, BP-3, CULTURE-I, H76-3, H76-5, H76-11, H76-15, H76-16, T-9, UAH2, UAH-2A, UAH-2B, UH-2, UH-3, UH-14, UH-15, UH-20, UH-27, UH-28, UH-29, UH-32, UH-34, UH-102, UH-103, UH-104, UH-105, and UH 106.

Highly Susceptible (2): Krishna and PLS-364.

Table 23. Screening of selected mung bean and urd bean varieties/germ plasm lines (found highly resistant under field condition) to leaf crinkle virus after sap inoculation in screen house

<u>MUNG BEAN</u> (9) :		
Highly resistant	=	None
Resistant (2)	=	L-24-2 and 15227
Moderately susceptible(5)	=	T.44, T-51, 15176, 15225 and 15229
Susceptible (1)	=	15277
Highly susceptible(1)	=	ML-5.
<u>URD BEAN</u> (11):		
Highly resistant	=	None
Resistant (2)	=	DLU 90 and DLU 487
Moderately susceptible (5)	=	H70-3, LU-201, Pant U-26, UG-153, and UG-157
Susceptible (1)	=	UPU - 1.
Highly susceptible (3)	=	UG 152, Pant 4-26 and Pant U-20.

SEED TREATMENT

HEAT THERAPY

Results on wet heat therapy, dry heat therapy and solar heat therapy are presented in Tables 24, 25 and 26 respectively.

Wet heat therapy : The results of wet heat therapy of infected seeds (Table 24) indicated that none of the plants showed leaf crinkle symptoms raised from treated seeds without affecting seed germination when seeds were given this treatment for 30 min.at 65°C or for 20 min. at 70°C. Absence of seed transmission of the virus was also observed when the seeds were treated at 75°C for 10 minutes but at this temperature germination of seed was also affected.

Dry heat therapy : It is evident from Table 25 that dry heat therapy of infected seeds did not check the seed transmission of the virus without adversely affecting the seed germination at any temperature from 50° - 75°C.

Solar heat therapy : Data in Table 26 indicate that the exposure of infected seeds for 4 h (12.oo noon to 4.oo p.m.) after 3 or 4 h pre-soaking (in forenoon) in water appreciably checked the virus transmission through seeds without affecting seed viability. Exposure to solar heat for 4 h without pre-soaking in water was not found statistically significant in reducing the virus transmission through seed.

Table 24. Effect of wet heattherapy on seed germination and transmission of the *virus through* seeds *collected* from leaf crinkle virus infected plants of urd bean cv. Krishna

Temperature °C	Time of exposure (min)					
	10	20	30	40	50	60
50	20(100)	18(100)	15(100)	12(100)	8(100)	7(100)
55	18(100)	17(100)	13(99)	11(100)	6(100)	4(100)
60	18(100)	14(98)	10(100)	4(100)	3(67)	2(49)
65	5(100)	3(100)	0(100)	0(77)	0(45)	0(25)
70	1(100)	0(100)	0(76)	0(47)	0(21)	0(8)
75	0(94)	0(89)	0(43)	-(0)	-(0)	-(0)
Control (Room temperature)	19*(100)	20(100)	19(100)	20(100)	20(100)	20(100)

*Figures in parenthesis indicate number of plants germinated out of 100.

*Figures without parenthesis indicate per cent plantsshowing leaf crinkle symptoms after germination.

Table 25. Effect of dry heat therapy on seed germination and transmission of the virus through seeds collected from leaf crinkle virus infected plants of urd bean cv. Krishna

Temperature °C	Time of exposure (min)					
	10	20	30	40	50	60
50	21(100)	19(99)	19(96)	17(95)	12(93)	9(92)
55	20(94)	18(82)	18(80)	15(72)	8(48)	7(45)
60	19(85)	16(73)	8(48)	4(26)	2(12)	0(10)
65	8(62)	6(51)	6(35)	4(25)	0(10)	0(7)
70	6(34)	5(21)	0(6)	0(1)	-(0)	-(0)
75	0(6)	0(5)	-(0)	-(0)	-(0)	-(0)
Control (Room temperature)	*21(100)	20(100)	19(99)	19(100)	19(98)	19(100)

* Figures in parenthesis indicate number of plants germinated out of 100.

* Figures without parenthesis indicate per cent plants showing leaf crinkle symptoms.

Table 26. Effect of solar heat therapy on seed germination and transmission of the virus through seed collected from leaf crinkle virus infected plants of urd bean cv. Krishna

Seed treatment	Exposure to solar heat	Per cent seeds germinated	Per cent diseased plants
No soaking in water	Nil	90 (71.68)*	21.40(27.58)
No soaking in water	4 h (12.oo noon to 4 PM)	85 (67.27)	17.80(25.04)
Soaking in water - 1 h (11 AM - 12 noon)	-do-	92 (73.69)	12.40(20.71)
Soaking in water - 2 h (10 AM - 12 noon)	-do-	95 (77.25)	3.30(10.60)
Soaking in water - 3 h (9 AM - 12 noon)	-do-	96 (78.59)	0.10(1.95)
Soaking in water - 4 h (8 AM - 12 noon)	-do-	94 (75.90)	0.10(1.95)
C.D. at 5% level		- (7.53)	- (2.81)

* Figures in parenthesis are the transformed values.

CHEMOTHERAPY

The data (Table 27) on the effect of different chemicals (antiviral chemicals, systemic insecticides and systemic fungicides) as seed treatment (seed dip and seed dressing) against leaf crinkle virus indicate that among all the chemicals tested none was found completely checking the virus transmission through seed. However, 2-thiouracil (0.1 per cent) was found most effective among all the chemicals and there was 70 per cent reduction of seed transmission of the virus as compared to control in which no chemical treatment was given to infected seeds. Data also indicate that out of six antiviral chemicals tested the effect of 2-thiouracil (0.05 per cent and 0.1 per cent) and 8-azaguanidine (0.05 per cent and 0.1 per cent) was found only statistically significant in reducing the virus transmission through seed. Among the four systemic insecticides and four systemic fungicides tested as seed treatment none was found statistically significant in reducing the virus transmission through seed. The seed treatment with the above mentioned chemicals (Table 27) did not show statistically significant adverse effect on the seed viability.

Table 27. Effect of chemicals as seed treatment (seed dip and seed dressing) on seed germination and transmission of the virus through seeds collected from leaf crinkle virus infected plants of urd bean, cv. Krishna

Chemical	Concentration	Per cent reduction in seed germination	Per cent reduction in seed transmission of the virus
	Antiviral chemicals(seed dip)		
Validomycin-A	0.05%	11.3(19.74)*	5.0(12.94)
Validomycin-A	0.1 %	10.5(18.97)	5.5(13.68)
2-Thiouracil**	0.05%	2.5(9.17)	62.5(52.35)
2-Thiouracil**	0.1 %	1.0(5.82)	70.0(56.86)
8-Azaguanine**	0.05%	7.5(16.04)	36.5(37.24)
8-Azaguanine**	0.1 %	7.1(15.50)	43.0(41.00)
8-Azaadenine**	0.05%	7.4(15.90)	15.5(23.27)
8-Azaadenine**	0.1 %	6.5(14.88)	13.0(22.08)
Guanidine hydrochloride	0.05%	12.2(20.50)	6.0(14.35)
Guanidine hydrochloride	0.1 %	13.5(21.65)	6.5(14.88)
Guanidine carbonate	0.05%	13.4(21.54)	4.5(12.35)
Guanidine carbonate	0.1 %	14.0(22.15)	6.0(14.21)
	Systemic insecticides		
Metasystox 25 EC	0.05%(a.i.)	7.1(15.55)	5.5(13.62)
Metasystox 25 EC	0.1 %(a.i.)	14.3(22.32)	6.0(14.37)
Rogor 30 EC	0.05%(a.i.)	7.1(15.48)	5.0(12.95)
Rogor 30 EC	0.1 %(a.i.)	14.3(22.31)	5.5(13.60)
Nuvacron 40 EC	0.05%(a.i.)	1.5(7.25)	0.5(4.45)
Nuvacron 40 EC	0.1 %(a.i.)	10.7(19.15)	1.0(5.75)
Dimecron 100 S	0.025%(a.i.)	3.6(10.95)	1.5(7.18)
Dimecron 100 S	0.035%(a.i.)	4.5(12.35)	2.5(9.21)
	Systemic fungicides(seed dressing)		
Bavistin	2.5 g/kg	3.3(10.64)	2.5(9.21)
Benlate	2.5 g/kg	1.1(6.31)	1.0(5.82)
Thiphanate-M	2.5 g/kg	1.6(7.50)	2.0(8.34)
Vitavax	2.5 g/kg	2.8(9.75)	1.5(7.10)
Sodium carbonate	0.1 %	7.5(15.91)	5.4(13.55)
Water	-	1.7(7.70)	-
Dry seed	-	3.3(10.61)	-
C.D. at 5% level	-	- (16.31)	-(14.97)

*=Figures in parenthesis are transferred values

** = Concentration prepared in 0.1% sodium carbonate.

- = Nil.

DISEASE CONTROL BY SOIL AND FOLIAR APPLICATION OF INSECTICIDES

Results on the effect of soil and foliar application granular and liquide insecticides, respectively on leaf crinkle disease incidence (per cent disease) and yield of urd bean cv. Krishna along with the economics on cost and benefit are presented in Table 28. Data on disease incidence indicate that all the insecticides were found statistically significant in reducing the disease incidence. When the disease was 34.0 per cent and 36.5 per cent in control plant (no insecticide) it was reduced to 17.5 per cent - 20.5 per cent in plants treated with granular insecticides as soil application and liquid insecticide as foliar application, respectively. It is also evident from Table 28 that the effects of all insecticides were statistically significant in increasing the grain yield from 4.645 - 4.70 quintals/h in control (no insecticide) to 5.358 - 7.125 quintals/h in plants treated with insecticides. Data also indicate that granular insecticides applied in soil were less effective in increasing the yield as compared to liquid insecticides used as foliar sprays. The maximum yield (7.125 q/h) with the minimum disease incidence (17.50 per cent) was recorded in treatment of two foliar sprays of Dimecron 100 S (0.035 per cent a.i.) at 10 and 25 days after sowing. None of the insecticidal treatment had adverse effect on yield and the maximum net income (Rs. 833.25/h) was found in case of the

Table 28. Leaf crinkle disease and grain yield of urd bean cv. Krishna after soil and foliar application of insecticides and net income/loss due to treatments

Treatment	Per cent disease	Yield (q/h)	Excess yield (q/h) due to treatment over control	Excess income due to treatment (Rs.)	Cost due to treatment (Rs.)	Net income or loss due to treatment (Rs.)
Soil application						
1. Phorate 10 G @ 1 kg a.i./h	20.50(26.98)	5.375	0.675	236.25	250.00	- 13.75
2. Disulfoton 5 G @ 1 kg a.i./h	20.00(26.58)	5.850	1.150	402.50	360.00	+ 38.50
3. Aldicarb 10 G @ 1 kg/h	20.50(26.97)	5.358	0.658	230.30	300.00	- 69.70
Foliar application at 10 and 25 days after sowing						
4. Dimecron 100 S 0.025% a.i.	18.60(25.69)	6.634	1.989	697.17	22.60	674.57
5. Dimecron 100 S 0.035% a.i.	17.50(25.48)	7.125	2.480	868.00	34.75	833.25
6. Roger 30 EC 0.05% a.i.	19.00(25.94)	6.050	1.405	491.75	90.00	401.75
7. Roger 30 EC 0.1 % a.i.	18.50(25.51)	6.665	2.020	707.00	135.00	562.00
8. Metasystox 25 EC 0.05 % a.i.	19.00(26.01)	6.225	1.580	553.00	113.75	439.25
9. Metasystex 25 EC 0.1 % a.i.	18.00(25.19)	6.818	2.173	760.55	142.25	618.30
10. Nuvacron40 EC 0.05% a.i.	20.00(26.55)	5.875	1.230	430.50	268.50	162.00
11. Navacron40 EC 0.1 % a.i.	19.50(26.24)	5.950	1.305	456.75	330.00	126.00
12. Control water	36.50(37.24)	4.645	-	-	-	-
13. Control No treatment	34.00(35.75)	4.70	-	-	-	-
C.D. at 5%	- (2.62)	0.41	-	-	-	-

Figures in parenthesis are transferred values.

treatment of Dimecron (0.035 per cent a.i.) used as foliar spray twice i.e. at 10 and 25 days after sowing. There was net loss of Rs. 13.75 and Rs. 69.70 per hectare when Phorate 10G and Aldicarb 10G were applied in the soil as granules because of their high cost and being less effective. There was net income of Rs.38.50 per hectare with Disulfoton 5G. All the insecticidal spray treatments were found financially beneficial giving net income between Rs.126.00 to Rs.833.25 per hectare (Table 28).

FIELD CONTROL OF LEAF CRINKLE DISEASE THROUGH COMBINATIONS OF SEED TREATMENT AND FOLIAR APPLICATION OF INSECTICIDES

Results (Table 29) on seed treatment followed by foliar application of insecticides twice i.e. at 10 and 25 days after sowing indicate that there was no significant disease incidence in the treatments in which infected seeds were given wet heat therapy or solar heat therapy along with foliar application of insecticides. Seed treatment with 2-thiouracil was not much effective to check the transmission of virus through seed however its effect was statistically significant. None of the above treatment (Table 29) significantly affected the seed germination. In the treatment in which seed was not treated but only insecticidal sprays were given the transmission through seed was not reduced significantly but the further spread of the disease in the field was negligible (Table 29).

Table 29. Occurrence of leaf crinkle disease of urd bean cv. Krishna after different combinations of seed treatment and spray of insecticides

Treatment		Seed germination (%)	% disease	
			After 25 days of sowing	After 60 days of sowing
Wet heat therapy of seeds (65^oC for 30 min)	+ Dimecron 100 S @ 0.035 % a.i. two sprays at 10 and 25 days after sowing.	95.00*(77.10)	0.05(0.15)	0.15(2.50)
Wet heat therapy of seeds (70^oC for 20 min)	+ -do-	94.50 (76.52)	0.10(1.90)	0.20(2.84)
Solar heat therapy of seeds	+ -do-	95.50 (77.80)	0.10(1.90)	0.25(3.05)
Seed dip in 0.2 % 2-thiouracil for 30 min	+ -do-	90.00 (71.67)	7.5 (16.05)	9.2(17.80)
No seed treatment	+ -do-	94.50 (76.50)	21.00(27.32)	22.80(28.57)
No seed treatment	+ No insecticidal spray	93.00 (74.79)	22.00(27.99)	78.50(62.43)
C.D. at 5% level		- (4.96)	- (2.37)	- (3.12)

* Figures in parenthesis indicate transformed values.

DISCUSSION

In view of the heavy losses caused by leaf crinkle virus disease to mung bean and urd bean in Haryana, detailed investigations on various aspects of leaf crinkle disease of urd bean and mung bean were carried out. Due to lacunae in information about the virus and the disease it was felt desirable to undertake the following studies:

1. Survey and epidemiology
2. Purification and identification of the virus
3. Protein constitution of resistant and susceptible varieties of mung bean and urd bean
4. Control measures.

Survey of all districts of Haryana during summer and kharif seasons revealed that during summer season the leaf crinkle disease occurred from zero to 4.62 per cent on mung bean crop. Urd bean crop was not found in any district of Haryana during 1975, 1976 and 1979 summer season. During kharif season the disease was to the extent of 34.63 per cent on mung bean and 37.13 per cent on urd bean. The low disease incidence during summer could be due to high temperature and low humidity. The disease incidence was more on urd bean crop than mung bean crop in all districts of Haryana which could be due to comparative resistance/susceptibility at species level. Among mung bean cultivars commonly grown in Haryana, Pusa Baisakhi had least incidence followed by

J-45 and Varsha and among urd bean cultivars H 70-3 had least disease incidence followed by Mash 1-1 and T9. This could be due to comparative resistance/susceptibility at varietal level. The disease incidence was less in 1975. It slightly increased during 1976 and during 1979 it was still more than 1976. Singh et al. (1979) reported continuous increase in leaf crinkle disease in Haryana from 1973 through 1975. This increase in disease incidence could be due to the spread of the virus through infected seed(Kolte and Nene, 1972) and further spread through the insect vectors (Bindra, 1971; Khatri et al., 1971; Dhingra, 1975). Seed and aphid transmission of the leaf crinkle virus has also been confirmed in the present studies. The observations on disease incidence also indicated that the Haryana state has two different regions i.e. north region and south region where intensity of leaf crinkle disease was different in the same cultivar. The districts located in north region i.e. Ambala, Kurukshetra, Karnal, Sonepat, Jind and Rohtak showed more disease as compared to the districts situated in south region i.e. Mohindergarh, Bhiwani, Hissar, Sirsa, Faridabad and Gurgaon. The high intensity of the disease in north region may mainly be attributed to comparatively low temperature and high humidity during summer and in kharif seasons as compared to the districts in south region (Anonymous, 1976, 1977 a).

The loss in yield due to leaf crinkle disease in mung bean cv. Varsha and urd bean cv. T-9 was found ranging from 2.12 to 93.18 per cent in mung bean and from 2.82 to 95.17 per cent in urd bean at Hissar depending upon the stage of plant growth at which infection occurred. Earlier the infection greater was the loss. In Uttar Pradesh at Pantnagar the loss due to this disease was estimated in urd bean cv. T-9 from 62 to 100 per cent (Nene, 1972). The variation in the range of loss could be due to the variation of environmental factors prevailing at Hissar and Pantnagar.

During summer season the leaf crinkle symptoms on mung bean and urd bean crops at Hissar either did not appear at all or were mild on some plants only when the temperature came down to about 37°C or below for about a week during the crop season. This could be due to the reason that virus multiplication might have been suppressed and the symptoms masked at high temperature (38° - 45°C) and low humidity (morning relative humidity around 60 per cent and evening relative humidity around 40 per cent). Appearance of symptoms when the temperature came down below 37°C with slight increase in humidity for about a week further confirm masking of symptoms at higher temperature than 38°C. When the crop remained at high temperature (41° - 45°C) and low humidity (morning relative humidity around 45 per cent and evening relative humidity around 20 per cent) for a week or

at $47^{o}C$ for a day after infection, the leaf crinkle symptom did not appear even though the temperature came down below $37^{o}C$ for about a week. The possible reason could be that the virus in the plants was either inactivated or suppressed to the extent that it could not multiply upto the concentration of symptom expression when the plants after being exposed to 41° - $45^{o}C$ continuously for a week or more or at $47^{o}C$ for a day, got the chance to grow below $37^{o}C$. The effect might be either directly on the virus in the host plant or indirectly by altering the host plant physiology.

The efficiency of sap transmission of leaf crinkle virus was greatly increased using carborandum powder as an abressive and when the sap was prepared in 0.1M phosphate buffer pH 7.0. Changing the molarity and pH of the phosphate buffer did not enhance the efficiency of mechanical transmission. Younger plants (1 - 2 weeks after sowing) of urd bean cv. Krishna were more sensitive/susceptible to mechanical inoculation than the older ones. This confirmed the findings of Narayanasamy and Jaganathan (1975 b).

Out of the two aphid species viz. <u>A</u>.<u>craccivora</u> <u>A</u>.<u>gossypii</u>, one species of beetle viz. <u>A</u>.<u>fevicaulis</u> and one species of white fly viz. <u>B</u>.<u>tabaci</u> used for virus transmission, <u>A</u>.<u>craccivora</u> and <u>A</u>.<u>gossypii</u> transmitted the virus after a short virus acquisition feeding of 5 min. This confirmed the transmission of the virus in stylet-borne manner. The

transmission of the leaf crinkle virus in stylet-borne manner has been reported earlier also through A.craccivora and A.gossypii (Dhingra, 1975). No evidence of transmission through A.fevicaulis and B.tabaci was obtained during the present studies.

The percentage of transmission through seed was found to be 21.13 and the transmission was observed highest in the seeds collected from plants which were sap inoculated at early stages of growth i.e. about 7 days after sowing. The transmission of the virus through seed decreased with the increase in plant age at the time of sap inoculation. Similar observations have been reported by Narayanasamy and Jaganathan (1975 a, b) also.

Host range was not strictly restricted to leguminous plants. The virus also infected 5 weed species namely C.arvensis, D.stramonium, D.metel, D.metaloides and D.incremis and some other plants namely G.globosa and R.communis var. Aruna belonging to families other than leguminosae. Among leguminous plants, 8 cultivated plant species viz. D.lablab, G.max var.Bragg, P.aconitifolius vars. Ph 68-04 and Ph 222, P.vulgaris vars. Pinto and Sexa, V.sinensis vars. PT 194207 and HFC 42-1 and V.catjang (Cholai bean), V.mungo and V.radiata were susceptible.When leaves of V.catjang (Cholai bean) were mechanically inoculated with infective crude sap they did not show any local lesions but were systemically infected

showing mosaic mottling symptoms. However, when the leaves of _V.catjang_ were mechanically inoculated with purified preparations of the leaf crinkle virus, they produced local lesions followed by systemic mosaic. Possible reason could be the presence of some inhibitors in the crude sap which interferred with the host metabolism at the infective sites inhibiting the production of local lesions and resulting in only systemic infection. During the process of virus purification the local lesion inhibiting substances might have been removed. Therefore, the purified virus preparation produced local lesions on inoculated leaves followed by systemic mosaic. The production of local lesions followed by systemic mosaic has also been reported in case of several other viruses such as alfalfa mosaic virus on _N.tabacum_, _D.stramonium_ and _Petunia_ spp. (Hull, 1969), Barley stripe mosaic virus on _Spinacia_ (Hollings, 1959), necrotic lesion strain of bean yellow mosaic virus on _P.lunatus_, _V.sesquipedalis_ and _V.sinensis_ (Zaumeyer and Fisher, 1953); sweet pea streak virus on _P.vulgaris_ (Ainsworth, 1940), Spinach strain of cucumber mosaic virus on _Cucumis sativus_ var. Every day (Bhargava, 1951) and cucumber strain 6 on Turkish tobacco, cucumber, spinach, tomato, _N.glutinosa_ and _N.langsdorfii_ (Price, 1934).

The virus had a dilution end point (D.E.P.) between 1:1,000 to 1:10,000 , thermal inactivation point (TIP)

between 64° to 66°C and longevity in vitro of 3 days at room temperature (35°C $\pm$ 2°C) and 9 days at refrigerator temperature. D.E.P., T.I.P. and longevity in vitro of some closely related viruses have been given in Table 20 for comparison.

Attempts were made to purify the virus employing different methods. Precipitation of the virus at different pH and with ammonium sulphate, fractionation on Sephadex G-25 and Sephadex G-50 coloumns failed to purify the virus. The virus was purified by chloroform:butanol (1:1) treatment and precipitation with PEG (8 per cent) + NaCl (1.2 per cent) followed by differential centrifugation using 0.1M phosphate buffer pH 7.0 as suspension medium. Several leguminous viruses have been purified following such a procedure e.g. alfalfa mosaic virus, cowpea mosaic virus and bean pod mottle virus (Steere, 1956, 1959; Bancroft, 1962; vanKammen,1967; Clark, 1968). 0.1M phosphate buffer pH 7.0 has also been found satisfactory as suspension medium in case of several other leguminous viruses (Markham, 1959; Steere, 1964). Failure of purification of the virus by precipitation at different pH, with ammonium sulphate and Sephadex filteration suggested close association of the virus nucleoprotein with other host proteins. Failure of purification for carnation ring spot virus and significant loss of tobacco mosaic virus in purification by precipitation at different

pH below 5 and failure of purification for tobacco ring spot virus and Rothmsted culture of tobacco mosaic virus by ammonium sulphate precipitation has also been reported due to the close association of the virus nucleo protein with host proteins (Pirie, 1950; Kassanis, 1955; Steere, 1956; Francki, 1966). The inactivation or loss of virus during the process of Sephadex filteration might be the cause of failure in purification by this technique and this needs further investigation.

The leaf crinkle virus described here had spherical particles with an average diameter of 50 nm and 15.24 per cent nucleic acid in infective virus particles. The above information helped in preparing the following cryptogram :

/: */15.24: S/S : S/Ap.

After going rhrough the literature on leguminous viruses (Gibbs, 1968; Smith, 1972; Gibbs and Harrison,1976; Hampton *et al.*, 1978; C.M.I./A.A.B. Description of plant viruses 1970-78) it was found that none of the legume virus reported so far produced the characteristic leaf crinkle symptoms on mung bean and urd bean as is in the present case and also none of the legume virus transmitted by aphids has spherical particles with an average diameter of about 50 nm. Based on the studies presented in this dissertation it can be concluded that the leaf crinkle virus of urd bean and mung bean occurring in India is a

new virus. Further, the following spherical plant viruses may be selected having one or the other similarity with the leaf crinkle virus of urd bean and mung bean (Table 20):

1. Cauliflower mosaic virus(Tompkins, 1937)
2. Cucumber mosaic virus(Purivirojkul et al.,1978)
3. Clover wound turmour virus(Black, 1945)
4. Pea enation mosaic (Bustrillos, 1965)
5. Cowpea mosaic virus (Dale, 1949).

The comparison of properties of the above viruses (Table 20) indicates that the leaf crinkle of urd bean and mung bean has greater similarities with the pea enation mosaic virus (Harrison, 1969) and may be grouped along with it.

Protein constitution of mung bean and urd bean varieties resistant and susceptible to leaf crinkle virus has not been studied earlier. Protein constitution determined by polyacrylamide gel electrophoresis (Davis, 1964) was different in mung bean and urd bean. Present studies indicated that 7 days old healthy seedlings of resistant and susceptible varieties of mung bean revealed 4 protein bands except in variety 15227 which showed 5 protein bands perhaps because of its slightly different genetic make up. Resistant varieties of urd bean showed 9 protein bands and susceptible varieties 8 protein bands in 7 days old seedlings. These results indicated that proteins were perhaps

related to susceptibility/resistance to leaf crinkle virus. Four proteins of slow mobility were found common both in resistant and susceptible varieties of mung bean (*V.radiata*) and urd bean (*V.mungo*). This gives an indication that these four common proteins of slow mobility were perhaps the characteristics of genus *Vigna*. The presence of 4 or 5 more fast mobile proteins in urd bean and their absence or if present then in very low concentration so that they could not be detected by technique followed in mung bean indicate the difference at species level. In general, urd bean is more severely infected than mung bean to leaf crinkle virus. These additional 4-5 fast mobile proteins in fairly good concentration in urd bean and their absence or if present than in very low concentration in mung bean suggest their relationship with susceptibility and symptom severity. In other words, the greater the number or higher the concentration of fast mobile proteins, higher was the susceptibility to leaf crinkle virus as far as mung bean and urd bean is concerned. Desborough and Peloquin (1966) studied protein pattern of 26 species of *Solanum* and suggested that the proteins may be species specific.

Though the number of proteins in 7 days old healthy seedlings of resistant and susceptible varieties of mung bean was 4 or 5 and of urd bean it was 9 in resistant varieties and 8 in susceptible varieties but with the pro-

gressing age i.e. at 27 days after sowing the faster mobile 2 proteins disappeared in susceptible varieties but one fast mobile protein may or may not disappeared in resistant varieties of mung bean. In urd bean one faster mobile protein disappeared in both resistant and susceptible varieties with progressing age. Rana (1979) studied protein constitution of maize and teostine by polyacrylamide gel electrophoresis and also found that there is general reduction in number of protein as the age progressed after germination.

The comparison of proteins in healthy and diseased leaves of resistant and susceptible varieties of mung bean and urd bean of same age (27 days) indicated that the number of proteins did not change after leaf crinkle virus inoculation in leaves of resistant varieties L-24-2, 15227, DLU 90 and DLU 487 of mung bean and urd bean though the concentration of slow mobile protein decreased and of the fast mobile protein increased. This indicated slight alteration in protein synthesis after virus inoculation of resistant varieties. After leaf crinkle virus infection in susceptible varieties Green mung, PS-16, Krishna and PLS 364 of mung bean and urd bean, all proteins which were detected in the healthy leaves of corresponding age either disappeared or changed into very fast mobile proteins. The susceptibility/resistance, therefore, of the mung bean and urd bean

plants after inoculation with leaf crinkle virus perhaps depended upon the synthesis of fast mobile protein or on how much slow mobile proteins changed into fast mobile proteins during virus multiplication. Greater the change into fast mobile proteins higher was the susceptibility to leaf crinkle virus. Varma and Poonam (1978) also studied protein patterns in the leaves of resistant and susceptible tomato plants to leaf curl virus and found that some low molecular weight proteins in susceptible plants acted as templated for virus multiplication. Perhaps similar situation exists in case of mung bean and urd bean also vis-a-vis leaf crinkle virus infection.

Out of the 390 varieties/germplasm lines of mung bean and 138 varieties/germplasm lines of urd bean screened under field conditions for susceptibility/resistance to leaf crinkle virus, 9 varieties/germplasm of mung bean viz. 15176, 15225, 15227, 15229, 15277, L-24-2, ML-5, T-44 and T 51 and 11 varieties/germplasm of urd bean viz. DLU 90, DLU 487, H 70-3, LU 201, Pant 4-26, Pant U-20, Pant U-26, UG-152, UG-153, UG-157 and UPU-1 were found highly resistant. The highly resistant material under field condition when further screened after artificial inoculation (sap inoculation), none of the varieties/germplasm of mungbean and urd bean was found highly resistant. Mung bean varieties L-24-2 and 15227 and urd bean varieties DLU 90 and

DLU 487 proved resistant and not highly resistant to this virus after artificial inoculation in the screen house. Mung bean cvs. Pusa Baisakhi, T1, T44 and T51 were found susceptible to leaf crinkle virus in Haryana though these varieties were reported as resistant to leaf crinkle virus (Nene, 1972) in Uttar Pradesh.

There was appreciable reduction in transmission of virus through seed when seeds were treated with wet heat for 30 min. at 65°C or 20 min.at 70°C without affecting the seed viability. The transmission of the virus through infected seeds was also appreciably checked but the germination was also adversely affected when the seeds were treated with dry heat for 60 min. at 60°C or 50 min.at 65°C. When the infected seeds were exposed for 4 h (12.oo noon to 4.oo p.m.) to solar heat after 3 or 4 h pre-soaking in water in the month of May/June there was appreciable reduction of the virus transmission through seed without affecting seed germination. No work was done on heat therapy against seed borne viruses of mung bean and urd bean but some work has been reported on the control of seed borne legume virus of cowpea, whereby Sharma and Varma (1975) reported that hot water treatment of 30 min.at 45°C or for 10 min. at 50°C, dry heat treatment for 50 min. at 55°C or 30 min. at 60°C or 20 min. at 65°C reduced the seed transmission of cowpea

banding mosaic virus appreciably. Eradication of seed borne viruses by heat therapy has also been observed in other cases such as seed borne viruses of sugarcane by hot air treatment at 54°C for 8 h or by hot water treatment at 50°C for 2 - 3 h (Martin, 1930; Lauden, 1953; Hughes, 1953; Knust, 1953; Singh, 1967; Singh et al.,1973) and eradication of potato leaf roll virus in the potato tubers by keeping them at 37°C in a moist atmosphere for 10 to 20 days (Kassanis, 1949; Thirmulachar, 1954). Among six antiviral chemicals, four systemic insecticides and four systemic fungicides tested as seed treatment to control the seed transmission of the virus, only 2-thiouracil 0.1 per cent and 0.5 per cent and 8-azaguanine 0.1 per cent and 0.5 per cent were found effective. 2-thiouracil and 8-azaguanine are well known antiviral chemicals though largely used as foliar application rather than for seed treatment (Commoner and Mercer, 1951; Matthews, 1953, 1970; Matthews and Smith, 1953; Holmes, 1955; Linder et al., 1960; Francki, 1962; Chiu and Sill, 1962; Raychaudhuri, 1963; Levin and Litt, 1965; Varma and Poonam, 1977). Guanidine hydrochloride was effective for inhibition of tobacco mosaic virus in tomato plants (Varma and Kamlesh Kumari, 1970) and guanidine carbonate was found an important inhibitor of tobacco necrosis virus in bean leaves (Varma,1968)

but guanidine hydrochloride and guanidine carbonate were not found effective as seed treatment in the present study against leaf crinkle virus.

The spread of virus diseases can be checked by controlling their insect vectors through the application of insecticides either as soil or foliar application (Gates, 1958; Broadbent et al., 1960; Broadbent, 1964; Watson and Plumb, 1972). Amongst the insecticides tested in this study, Dimecron (0.035 per cent a.i.) when sprayed twice at 10 days and 25 days old plants was found most effective with the maximum control of disease spread with minimum cost and maximum net benefit. Chaudhari and Bhanot (1974) also found Dimecron as most effective against aphids out of 10 insecticides tested.

Those treatments which checked the seed transmission effectively but did not control the field spread through aphid vector and those insecticidal sprays which only controlled the vectored spread of the virus in the field but not virus transmission through infected seeds, were combined. Among the combinations of seed treatment and insecticidal sprays, seed treatment either with wet heat (70°C for 20 min. or 65°C for 30 min) or with solar heat (3 h soaking in water than 4 h exposure i.e. 12.oo noon

to 4.co p.m. to solar heat) followed by two sprays of Dimecron (0.035 per cent a.i.) at 10 days and 25 days after sowing were found most effective and significantly controlled the disease being 78.5 per cent in control (untreated crop) and 0.25 per cent in treated crop.

SUMMARY

Studies on leaf crinkle disease of urd bean (*Vigna mungo* (L.) Hepper) and mung bean (*V. radiata* (L.) Wilczek) were undertaken with respect to disease survey and epidemiology, purification and identification of the virus, protein constitution of healthy and diseased leaves of resistant and susceptible varieties, screening of varieties/germplasm lines for disease resistance and control measures.

A survey of different districts of Haryana during summer and kharif seasons of 1975, 1976 and 1979 revealed that the disease incidence was less in summer season as compared to kharif season. The disease continuously increased year after year throughout the state. It was more on urd bean crop than mung bean in all the districts during kharif season. In summer season the urd bean crop was not found cultivated in Haryana during years under survey.

The districts located in north of Haryana showed more disease in comparison to districts located in south. Among the commonly grown cultivars in Haryana, mung bean cv. Pusa Baisakhi and urd bean cv. H-70-3 had the least incidence followed by mung bean cvs. J-45 and Varsha and urd bean cvs. Mash 1-1 and T-9.

The loss in yield due to this disease ranged from 2.12 to 93.18 % in mung bean cv. Varsha and 2.82 to 95.17% in urd bean cv. T-9. Earlier, the infection greater was the loss in yield.

Studies on the effect of temperature and humidity in relation to disease incidence and development showed that the leaf crinkle symptoms were more conspicuous when the maximum temperature was around 35°C, minimum temperature was around 25°C, morning relative humidity more than 70 % and evening relative humidity more than 50 %. Symptoms masked when the temperature ranged from 38° to 45°C with morning and evening relative humidity of about 60 % and 40 %, respectively. The disease symptoms did not appear even if the maximum temperature came down abound 35°C if the host plants were already exposed between 41° to 45°C continuously for a week or to more than 47°C for a day with morning and evening relative humidity of 45 % and 20 %, respectively.

The efficiency of mechanical transmission was greatly increased when the inoculum was prepared in 0.1M phosphate buffer pH 7.0 and carborandum powder was used as an abrasive. Younger plants (1 - 2 weeks after sowing) of urd bean cv. Krishna were more sensitive/susceptible to mechanical inoculation than the older ones. _A.craccivora_

and A.gossypii transmitted the virus with a short acquisition feeding of 5 min. in a non-persistent manner. The virus was seed transmitted. Seed transmission was found to be 21.13 % and the percentage of seed transmission decreased with increasing plant age at the time of infection.

Host range was not strictly restricted to Leguminous plants. The virus also infected 5 weed plants namely C.arvensis, D.stramonium, D.metel, D.metaloides and D.incremis and some other plants namely G.globosa and R.communis var. Aruna belonging to the families other than Leguminosae. Among leguminous plants, 8 plant species were susceptible. Fifty seven plant species belonging to 17 families were not infected.

The virus had dilution end point (DEP) between 1:1,000 to 1:10,000, thermal inactivation point (TIP) between 64° to $66^{\circ}C$ and longevity in vitro (LIV) of 3 days at room temperature ($35^{\circ} \pm 2^{\circ}C$) and 9 days at refrigerator temperature.

Attempts were made to purify the virus employing different methods. Precipitation of the leaf sap at different pH and with ammonium sulphate, fractionation on Sephadex G-25 and Sephadex G-50 coloumns failed to purify

the virus. The virus was purified by chloroform:butanol (1:1) treatment and precipitation with 8 % PEG (polyethylene glycol, mol.wt. 6000) + 1.2 % NaCl followed by differential centrifugation of the leaf sap prepared in 0.1 M phosphate buffer pH 7.0.

The leaf crinkle virus described here had spherical particles with an average diameter of about 50 nm and containing 15.24 % nucleic acid. The information based on present studies helped in preparing the following cryptogram:

/ : */15.24 : S/S : S/Ap.

After going through the literature on plant viruses it was found that none of the plant virus reported so far, have the similarities in properties of the virus described in this dissertation. Therefore, the leaf crinkle virus of urd bean and mung bean purified for the first time as described in this dissertation, may be considered as a new virus. The comparison of properties of the closely related plant viruses already reported in literature indicated that this virus has greater similarities with pea enation mosaic virus and may be grouped along with it.

Studies on protein constitution by polyacrylamide gel electrophoresis technique revealed the presence of 4 proteins of slow mobility common in 7 days old seedlings

of both mung bean and urd bean. These 4 proteins were perhaps the characteristics of the genus *Vigna*. Presence of 4 or 5 more fast mobile proteins in urd bean and their absence or if present then in very low concentration in mung bean, indicated the difference in genetic make up and therefore, susceptibility/resistance to leaf crinkle virus at species level. Differences in protein pattern in resistant and susceptible varieties of both mung bean and urd bean before and after virus inoculation, in 7 days old seedlings and in leaves of 27 days old plants, led to the conclusion that there was reduction in protein number with progressing age and the presence or absence of fast mobile proteins were perhaps related to susceptibility/resistance of the varieties. Greater the number of fast mobile proteins or greater the change of slow mobile to fast mobile proteins, higher was the susceptibility of the varieties of mung bean and urd bean to leaf crinkle virus.

Out of 390 varieties/germplasm lines of mung bean and 138 varieties/germplasm lines of urd bean screened for susceptibility/resistance to leaf crinkle virus, none was found highly resistant after artificial inoculation (sap inoculation). Mung bean varieties L-24-2 and 15227 and urd bean varieties DLU 90 and DLU 487 proved resistant and not highly resistant to this virus after artificial inoculation in the screen house.

There was appreciable reduction in seed transmission of the virus when seeds were treated with wet

heat (70°C for 20 min. or 65°C for 30 min.) or with solar heat (3 h soaking in water then 4 h exposure to solar heat in the months of May/June) without affecting seed viability. Seed treatment with dry heat or with chemicals (antiviral chemicals, insecticides and fungicides) either did not check the seed transmission of the virus appreciably or the seed viability was affected. Seed treatment did not check the spread of virus through vector.

Out of 7 insecticides tested in this study either as soil application or foliar application in the field, Dimecron (0.035 % a.i. in water) was found most effective with maximum net benefit when sprayed on plants 10 days and 25 days after sowing.

Amongst the combinations of seed treatment and foliar sprays, seed treatment either with wet heat (70°C for 20 min. or 65°C for 30 min.) or with solar heat (3 h soaking in water than 4 h exposure to solar heat) followed by two sprays of Dimecron (0.035 % a.i.) at 10 and 25 days after sowing were found most effective in checking the transmission of the virus through seed and controlling vectored spread of the disease.

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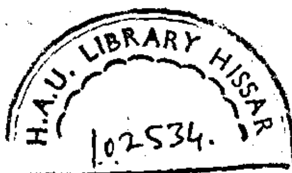
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STUDIES ON LEAF CRINKLE DISEASE OF URD BEAN (VIGNA MUNGO (L.) Hepper)/MUNG BEAN(VIGNA RADIATA (L.) Wilczek) AND ITS CONTROL

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(An Abstract of the dissertation presented in partial fulfilment of the requirements for the degree of Ph.D., Haryana Agricultural University, Hissar, India).

A survey of different districts of Haryana during summer and kharif seasons of 1975, 1976 and 1979 revealed that the leaf crinkle disease of urd bean and mung bean was less in summer season than kharif season. The disease continuously increased year after year through out Haryana. The disease was more on urd bean crop than mung bean in kharif season. It was more in northern region than southern region of Haryana. Mung bean cultivar Pusa Baisakhi and urd bean cultivars H-70-3 had least disease incidence followed by J-45 and Varsha cultivars of mung bean and Mash 1-1 and T-9 cultivars of urd bean.

The loss in yield due to this disease was found from 2.12 to 93.18% in mung bean cv. Varsha and from 2.82 to 95.17% in urd bean cv. T-9. Earlier the infection greater was the loss in yield.

The maximum disease incidence and development was observed when the maximum temperature was around 35°C, minimum temperature around 25°C, morning andevening relative humidity more than 70% and 50 % respectively. Higher temperature and low humidity had adverse effect on the virus in the host.

The virus causing the disease was transmissible by mechanical means,insects(*Aphis craccivora* and *A.gossypii*) and through seed. The efficiency of mechanical transmission was greatly enhanced by using carborandum powder as abrasive. Young plants (7-14 days old) of urd bean cv. Krishna were more sensitive/susceptible to the virus than the older ones. *Aphis craccivora* and *A.gossypii* transmitted the virus in a non-persistent manner. Transmission of the virus through seed was 21.13%. Percentage of seed transmission decreased with increasing plant age at the time of infection.

Host range of the virus was not confined to leguminous plants. Five weed and 2 cultivated plant species belonging to the families other than Leguminosae and 8 cultivated plant species belonging to Leguminosae were susceptible. Fifty seven plant species belonging to 17 families were not infected.

The virus had the dilution end point(DEP) between 1:1,000 to 1:10,000, thermal inactivation point(TIP) between 64° to $66^{o}C$ and longevity in vivo (LIV) of three days at room temperature ($35^{o} \pm 2^{o}C$) and 9 days at refrigerator temperature.

The virus was purified for the Ist time using Chloroform:butanol (1:1) treatment and precipitation with 8% PEG (polyethylene glycol, mol.wt. 6,000) plus 1.2 % NaCl followed by differential centrifugation of the leaf sap prepared in 0.1M phosphate buffer pH 7.0

The virus described here had spherical particles with an average diameter of about 60 nm and contained 15.24 % nucleic acid. This may be considered as a new virus having cryptogram (based on the present studies) as: */* : */15.24: S/S : S/Ap. This virus has greater similarities with pea enation mosaic virus and may be grouped along with it.

Studies on protein constitution of resistant and susceptible varieties of mung bean and urd bean using polyacrylamide gel electrophoresis technique, indicated difference in protein pattern in resistant and susceptible varieties at different age of the plant and before and after virus inoculation. It was concluded that the susceptibility/resistance of the mung bean and urd bean varieties depended upon the synthesis of fast mobile proteins or on how much slow mobile proteins changed into fast mobile proteins during virus multiplication.

None of the variety/germplasm line of mung bean and urd bean was found highly resistant after artificial inoculation(sap inoculation) but L-24-2 and 15227 varieties of mung bean and DLU 90 and DLU 487 varieties of urd bean were found resistant.

Seed treatment with wet,dry and solar heat,antiviral chemicals, systemic insecticides and systemic fungicides alone or soil or foliar application of insecticides alone did not satisfactorily control the disease. When the combinations of seed treatment with wet or solar heat and foliar application of Dimecron (0.035 % a.i. in water) were used, the disease was effectively controlled.

This dissertation contains pages 1 to 117, plates 9 having 23 photographs, Tables 29, Figs. 9 and References 114.

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