

Studies on Seed Transmission of Urdbean Leaf Crinkle Virus on *Vigna mungo*

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Abstract

Seed transmission of Urdbean leaf crinkle virus was maximum in pods located at the base of the plant rather than pods produced subsequently. There was no relationship between extent of seed transmission and pod position on the plant while considering the individual plants showing infected leaves. The growing-on-test can be effectively ascertained for testing seed-borne infection. The virus was detected only in embryo and cotyledons. Seed lots showing 2.0 to 3.6 per cent seed borne infection recorded 45.2 to 86.5 per cent disease incidence in the post flowering stage in field conditions. The produce from this field yielded 3.6 to 8.6 per cent virus-infected seeds.

Keywords: Urdbean, *Vigna mungo*, Urdbean leaf crinkle virus, seed transmission

Introduction

Urdbean (*Vigna mungo* (L.) Hepper) is an important pulse, very rich in protein (23.9 per cent) and carbohydrate (60.4 per cent). Among the virus diseases, Urdbean leaf crinkle virus (ULCV) is the most serious one causing considerable damage to the crop (Reddy *et al.*, 2005). In Uttar Pradesh, seed transmission of ULCV in germplasm and varieties of urdbean ranged from 0 to 15% (Beniwal *et al.*, 1983). It was observed that the virus was carried in 17.6% of urdbean seeds from naturally infected plants (Dubey and Indu Sharma, 1985). Pod size, number of seeds per pod and test weight was reduced in infected seeds (Ashok-Mishra *et al.*, 1994). Kadian, 1994 reported that ULCV was seed-transmitted (21 per cent) and transmission rate increased when infected seeds were continuously reused. Seed transmission plays a substantial role in virus ecology and is vital for the survival and spread of the disease. Therefore, further studies on seed transmission of ULCV were conducted in the present investigation.

Materials and methods

The seed transmission studies in ULCV were carried out at Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore across alternate years in five phases up to 2002. The mean data are interpreted and discussed in

this paper.

Identification of first leaf position showing symptom

Three farmer's fields showing different percentages of ULCV infection were selected. The plants showing first symptoms in different leaf positions from the base of the plant *viz.*, 3rd leaf (28 DAS), 4th leaf (35 days), 5th leaf (42 DAS), 6th leaf (49 DAS) and 7th leaf (56 DAS) were tagged. Fifty plants were tagged for each age. A similar number of plants which remained healthy were selected at the time of harvest to serve as control. The seeds produced in different nodes were collected from the above plants. These seeds were sown in the glass house. In the resulting plants the position of leaf that showed first symptom of the disease was recorded.

Effect of first infected leaf position and pod location on seed germination and seed borne infection

From these marked plants the pods were harvested separately from first to eighth node from base. For each infected leaf position and each node 50 seeds were used for sowing in the glass house. The plants were regularly sprayed with systemic insecticides as an additional protection. Seeds from

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healthy plants were maintained as control. Percentage of seed germination and seed transmission were recorded.

Detection of seed-borne infection in the seed lots

To find out the percentage of seed-borne infection in the seed lots used for sowing in the three fields, seed samples were collected from each field. From each field 300 seeds were used for sowing in the glass house. The percentage of seed transmission was recorded. After harvesting the fields, seed samples were collected from the respective fields and 300 seeds were sown in the glass house and per cent seed transmission of ULCV was recorded.

Location of virus in seed parts

Dry seeds from these marked plants in field were collected. From each group of plants, 75 seeds were collected at random. The seeds were placed between two wet paper towels and incubated for 48 h in B.O.D. incubator at 25°C. Then the testa, cotyledons and embryo were dissected by using sterile forceps and scalpel under aseptic conditions. Each part was washed in 0.5% sodium hypochlorite solution for 30 seconds, rinsed several times in distilled water, and triturated in 0.1 M potassium phosphate buffer, pH 7.5 with a mortar and pestle. Each slurry was inoculated on 10-day old urdbean seedlings and the number of infected plants was recorded.

Results and discussion

First leaf to show symptom

When seeds collected from different nodes in infected plants were sown, the resulting plants developed symptom in leaf position ranging from 3.2 to 3.8 (Table 1). It was found that seeds collected from first and second nodes of infected plants expressed symptom earlier than seeds from other nodes. The nodes from 3 to 8 were on par. All the resulting plants developed symptoms before the appearance of the fourth leaf. When the leaf position showing first symptom in the seed transmitted plants was compared, it was found that symptom expression was quick when the seeds were collected from plants which had been infected in third leaf itself. The symptom expression in plants growing from seeds collected from plants infected at third and seventh leaves were 2.9 and 3.8, respectively. The leaf position to develop first symptoms ranged from 2.4 to 3.7 indicating early transmission.

In case of seeds collected from plants showing symptoms in seventh leaf, it produced first symptom in leaf position 3.2 to 4.0 indicating delayed symptom production. The effect of seed transmission of viruses on seedlings and the degree to which young seedlings show symptoms varies

Table 1. Effect of infection in different leaf positions on seed transmission (%): Mean leaf position of resulting plants to show first symptom

Nodal position	Infected leaf position from base					Mean
	3	4	5	6	7	
1	3.3	2.0	3.2	3.0	3.3	3.2
2	2.5	3.0	3.2	3.6	3.3	3.2
3	3.7	3.2	4.1	3.3	3.2	3.5
4	3.1	3.3	3.5	3.5	4.0	3.5
5	3.2	3.0	3.5	4.1	4.4	3.6
6	2.3	4.1	4.1	4.3	4.0	3.8
7	2.4	3.5	3.3	4.1	4.0	3.5
8	2.4	4.0	3.1	4.1	4.0	3.5
9	2.9	3.4	3.5	3.7	3.8	

C D (P = 0.05)

Nodal position = 0.2

Leaf position = 0.1

Node x leaf = 0.3

considerably with different viruses and different host plants (Bennett, 1969).

Effect of pod location on seed germination and seed transmission

It is clear that the virus had no adverse effect on germination of seeds collected from plants showing first symptom in different leaf positions of infected plants (Table 2). It was found that in *Soybean mosaic virus* there was no correlation between the seed transmission and pod position on the plant (Bowers and Goodman, 1979). The location of pod on plant influenced seed transmission significantly. There was gradual decrease in the per cent transmission as the position of pod increased from the base. The maximum mean seed transmission was 19.7% from pods collected from plants showing first symptom in the third leaf. On the other hand, it was just 8.7 in pods collected from plants showing first symptom on seventh leaf. The decrease in per cent seed transmission was significant up to sixth node only. The highest mean seed transmission was 21.0% obtained from seeds of first node and lowest *viz.*, 5.98% from seeds of sixth node. The interaction was significant over healthy.

Seed-borne infection in the seed lots used for sowing in field

In order to find out whether the per cent transmission varied with the urdbean variety used by farmers, growing-on-test was conducted. The seed lot of Co 2 used for sowing in

Table 2. Extent of seed germination and seed transmission from seeds produced at different nodes in plants showing symptoms on different leaf positions

Nodal position from base	3rd leaf		4th leaf		5th leaf		6th leaf		7th leaf		Healthy		Mean	
	Germination (%)	Seed transmission (%)	Germination (%)	Seed transmission (%)	Germination (%)	Seed transmission (%)	Germination (%)	Seed transmission (%)	Germination (%)	Seed transmission (%)	Germination (%)	Seed transmission (%)	Germination (%)	Seed transmission (%)
1	96.0 (77.9)	41.6 (40.2)	90.6 (71.5)	37.9 (40.0)	91.4 (72.5)	9.3 (17.8)	90.3 (71.5)	19.1 (25.9)	94.5 (75.8)	18.1 (25.2)	92.0 (73.5)	0 (7.4)	92.5 (73.8)	21.0 (24.5)
2	89.6 (70.8)	11.5 (19.9)	92.3 (73.5)	21.0 (27.2)	92.4 (73.5)	15.6 (23.3)	93.4 (74.6)	8.7 (17.2)	93.4 (74.6)	17.4 (24.6)	88.0 (69.7)	0 (7.4)	91.3 (72.8)	12.4 (18.7)
3	91.6 (72.5)	20.8 (27.1)	90.4 (71.5)	21.0 (27.2)	89.4 (70.6)	12.1 (20.4)	91.3 (92.5)	5.6 (13.8)	93.6 (74.6)	9.5 (18.0)	92.0 (73.1)	0 (7.4)	91.0 (72.5)	11.5 (17.7)
4	87.6 (68.8)	18.1 (25.2)	90.9 (71.5)	20.0 (26.5)	87.5 (68.8)	9.5 (18.0)	94.4 (75.8)	2.9 (9.8)	92.8 (73.5)	9.2 (17.7)	90.0 (71.5)	0 (7.4)	90.0 (71.6)	9.9 (16.2)
5	96.4 (78.4)	17.8 (25.0)	87.5 (68.8)	9.5 (17.9)	91.6 (72.5)	12.1 (20.4)	93.4 (74.6)	4.2 (11.9)	90.0 (71.5)	3.7 (11.0)	90.0 (71.5)	0 (7.4)	91.3 (72.9)	7.8 (14.4)
6	95.5 (77.0)	19.6 (26.3)	88.8 (69.7)	6.2 (14.50)	90.4 (71.50)	2.6 (9.4)	93.2 (74.6)	5.4 (13.5)	87.2 (68.8)	2.1 (8.3)	92.0 (73.5)	0 (7.4)	91.0 (72.5)	5.9 (12.0)
7	91.6 (72.5)	20.0 (26.3)	87.5 (68.8)	14.2 (12.2)	90.3 (71.5)	11.5 (19.9)	91.1 (72.5)	2.4 (9.0)	90.0 (71.5)	7.4 (15.7)	94.0 (75.9)	0 (7.4)	90.5 (72.1)	9.3 (15.5)
8	91.6 (72.5)	10.0 (18.4)	88.2 (69.7)	13.3 (21.40)	87.5 (68.8)	9.5 (17.9)	91.9 (72.5)	1.7 (7.7)	91.1 (72.5)	2.4 (9.0)	92.0 (73.5)	0 (7.4)	90.0 (71.6)	6.2 (12.4)
Mean	92.2 (73.8)	19.7 (26.0)	89.0 (70.6)	17.9 (24.4)	89.6 (71.2)	10.3 (18.4)	92.0 (73.6)	6.3 (13.6)	91.3 (72.8)	8.7 (16.2)	91.3 (72.8)	0 (7.4)		

C D (P=0.05)

	Germination	Seed transmission
Infected leaf	= 0.8	0.1
Nodal position	= 0.9	0.1
Leaf x Nodal position	= 2.2	0.3

Figures in parentheses are arcsine transformed values

field No.1 gave 2.6% seed transmission. When the farmer had sown this lot of infected seed, the resulting crop in the post flowering stage recorded 66.6% infection (Table 3). The seed lots of local variety used for sowing in field No.2 and 3 showed 2.0 and 3.6% seed transmission, respectively. When sown in the field, these two lots further produced 45.2 and 82.6% infected plants. Mahajan and Joi (1999) reported 2-30 per cent ULCV incidence at field level with 2-16 per cent seed transmission.

Table 3. Per cent seed-borne infection in the seed lots for sowing

Field No.	Variety	Seed transmission (%)	Post flowering infection in the field
1	Co2	2.6 (9.2)	66.6 (54.4)
2	Local	2.0 (8.2)	45.2 (41.7)
3	Local	3.6 (10.9)	82.6 (64.9)
CD (P=0.05)		0.61	1.8

Figures in parentheses are arcsine transformed values

The seed samples collected from the selected fields were sown in the glass house to find out the extent of seed-borne infection. The results presented in Table 4 revealed that higher percentage of field infection in field No.3 had given significantly higher seed-borne infection (8.6 per cent). The low field infection (66.6 and 42.4 per cent) has given correspondingly lower seed-borne infection (5.6 and 3.6 per cent). Manadhare *et al.*, (1999) reported that yield/plant was decreased by 47.3 per cent in ULCV infected plants, associated with a decrease in pod number/plant (32.0 per cent). Seeds from diseased plants had 73.3 per cent germination compared with 85.1 per cent in healthy plants, and also showed reduced vigour. The present study also indicated differences in rate of transmission between cultivars. Difference in extent of seed transmission in various varieties has been reported in urdbean cultivars infected by ULCV (Patel *et al.*, 1999, Prasad *et al.*, 1999) and cowpea cultivars infected by *Cowpea aphid borne*

Table 4. Percentage seed transmission of ULCV through seeds harvested from naturally infected crop

Field No.	Variety	Infected plants in the field	Transmission from harvested seed (%)
1	Co2	66.6 (54.4)	5.6 (13.7)
2	Local	45.2 (41.7)	3.6 (10.9)
3	Local	82.6 (64.9)	8.6 (17.1)
CD (P=0.05)		0.38	0.37

Figures in parentheses are arcsine transformed values

mosaic virus (Ladipo, 1977).

Transmission (%) of virus from different seed parts

The inoculum from cotyledons and embryos from infected seeds harvested from different infected leaf position plants transmitted the virus. But no transmission was observed from seed coat extracts and seed washings. There was significantly higher percentage of transmission from embryo extracts (27.0 per cent) than the cotyledon (22.2 per cent). Patel *et al.*, (1999) reported that ULCV was readily transmitted by seed (10-30 per cent) with reduction in test weight of 22.5 per cent. The presence of virus was observed in cotyledons and embryo. The infected whole seed gave 29.1% transmission which was on par with that of embryo (Table 5). The mean per cent transmission was 40.2 from the extract of seeds collected from plants, which showed first symptom in the third leaf as against 19.4 in seventh leaf. The interaction between infected leaf position and seeds parts was non-significant. Seed transmission of most plant viruses is dependent on infection of the embryo (Shepherd, 1972). Varma *et al.*, (1992) correlated with the amount of *Blackgram mottle virus* present in the embryonic axis and later in primary leaves. The presence of virus in the testa alone did not result in its transmission through seeds. Virus concentration in different tissues varied; the mean amount of virus in the 3 cultivars was 48-1234 ng per embryonic axis, 15-24 ng per cotyledon, and 12-20 ng per testa. The infection of primary leaves through the seed also resulted in systemic infection if the amount of virus in primary leaves exceeded 100 ng/100 mg of tissue. Close agreement was found between the percentage of seedlings with systemic infection and the percentage of seeds with embryonic axes containing less than 100 ng virus. The cultivars that resisted seed transmission contained relatively small amounts of the virus in the embryonic axes.

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