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# Influence of plant age on infection and symptomatological studies on urd bean leaf crinkle virus in urd bean (*vigna mungo*)

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## ABSTRACT

Studies were conducted to identify the susceptible stage and symptomatology of urd bean leaf crinkle virus (ULCV) on urd bean. The incubation period was short and symtoms developed on second trifoliate leaf stage onwards when the plants were inculated at younger stage as compared to older plants. In the infected plants the increase in leaf size was evident from third trifoliate leaf stage onwards. Reduction in rachis length of terminal leaflet of infected trifoliate and thickening of stem and petiole were evident in infeceted plant. The size of stipules increased prior to the symptom development in lamina in all the infected plants. These symptoms are of great use in eliminating infected plants in the early stages for effective management of the disease.

## INTRODUCTION

Urd bean (*Cigna mungo* L. Hepper) is an important pulse, very rich in protein (23.9%) and cardohydrate (60.4 per cent) and is used in the daily diet of Indians. Urd bean plant becomes a victim of a large number of diseases caused by both fungi and viruses. Among the virus diseases. Urd bean leaf crinkle virus (ULCV) is considered to be the most serious one causing considerable damage to the crop depending on season and variety cultivated. Studies on influence of plant age on infection and symptomatology of urd bean crinkle virus are important factors to access the losses due to disease.

## MATERIALS AND METHODS

**Source of inoculum :**

In the field, plant showing typical symptoms of urd bean leaf crinkle virus were marked, The seeds from such plants were harvested and sown in insect proof glass house. The resulting infected plants were used as source of virus inoculum and the culture of the virus was subsequently maintained in the T9 variety through sap inoculation in the glass house. The young leaves showing typical symptoms of leaf crinkle virus infection served as a source of inoculum for the following experiments.

**Influence of plant age on infection and symptom development :**

Urd bean plants age 7, 10, 15, 22, 29, 37, 43 and 49 days were raised in the insect proof glass house of find out the influence of plant age on infection of urd bean leaf crinkle virus (ULCV). The youngest trifoliate leaves of plants were inoculated by sap. The primary leaf was inoculated in 7 and 10 days old plants. The first, second, third, fourth, fifith and sixth trifoliate leaves were inoculated in 15, 22, 29, 37, 43 and 49 days old plants respectibely. Thirty plants were inoculated in each age.

**Effect of Urd bean leaf crinkle virus on leaf sizem, petiole, stipules and stem :**

In order to find out the effect of infection on growth of susceptible and resistant varieties, T9 (susceptible) and Karaikal (resistant) plants were inoculated on cotyledon leaves by sap. They were kept in the glass house. Sixty days after inoculation, eight trifoliate leaves were numbered from the base of the plant. The area of trifoliate leaves of healthy and infected plants was measured by non-destructive method (Balakrishnan *et. al.*, 1987). For this purpose, the maximum lenght (l) and breadth (b) of the leaflets of each trifoliate leaf were measured. The leaf area (Y) was worked out by using the regression equation $Y = 4.33 + 1.653 (x)$, where x is the product of l and b. For each cultivar and leaf position measurenments were made from ten plants.

Stem thickness was measured in first to eight intermodes. The thickness of petiole was measured in the middle of the petiole of trifoliate leaf by using screw guage. Lenght of rachis of terminal leaflet was measured.

To study the effect of Urd bean leaf crinkle virus (ULCV) on stipules, 50 plants of T9 cultivar were inoculated on 10 days old seedlings and they were kept in the glass house. The plants were under continuous observation for 30 days after inoculation. The plants which were showing stipule enlargement were tagged and subse-

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quent symptom appearance on lamina was observed. The stipules were detatched from the base of the trifoliate leaf of infected and healthy plants. Their lenght and breadth were measured for 20 plants.

## RESULTS AND DISCUSSION

**Influence of plant age on infection and symptom development :**

This experiment was taken up to find out, if age of the plant at the time of inoculation influenced the per cent transmission and incubation period. The experiment (Table 1) revealed that incoulation at primary leaf stages (7 and 10 DAS) gave 85 and 72 per cent infection respectively. In these stages of inoculation, the plants developed symptoms in the second and third trifoliate leaves. This was significfantly superior to inoculations of first, second and third trifoliate leaves which produced 59, 49 and 36 per cent infected plants. Inoculation of plants at fourth and subsequent trifoliate leaves failed to transmit the virus.

The incubation period of the virus in inoculated plants was gradually increased with the age of plants at the times of inoculation. It was 13 days when plants were inoculated at the age of seven days, but increased to 28 days when plants were inoculated at 29 DAS. This indicaters doubling of the incubation period when the third trifoliate leaf was inoculated, compared to inoculation of the primary leaf. There was no significant difference in incubation periods when inoculations were made on primary leaf (10 DAS) and first trifoliate (15 DAS) stages.

When primary leaves were inoculated, symptoms developed earlier that the inoculations made at 10, 15 and 22 days after sowing. The per cent transmission was gradually reduced with increase in plant age. There was o infection when plant were inoculated prior to flowering stage. Similar observation was recorded by Manjuvani (1987) in urd bean infected by filiform leaf virus. Soybean plants exhibited increased resistance to infection and systemic spread of tobacco ring spot virus after flowering (Owusu *et. al.*, 1968).

**Effect of Urd bean leaf crinkle virus on leaf size, petiole, stipules and stem :**

**Symptomatology :**

The symptoms were studied in detail in naturally infested and artificially inoculated plants. The sympotm under artificially inoculated plants resembled those on naturally infected plants in the field. In the field, the first recognizable symptoms usually appeared three to four weeks after sowing. Generally, the third trifoliate leaf developed the symptoms first by an increase in the size and turning light green in colour. After a week, the typical leaf crinkling became conspicuous. As the plant grew further, the extent of crinkling on the order leaves went on decreasing and crinkling was more pronounced on the younger leaves. Considerable malformation of the inflorescence was observed in early infected plants.The infected plants started lodgning when they attained fouth leaf stage. The plants became bushy in appearance and remained green in the field even after the healthy plants attained senescence.

**Leaf Size :**

The effect of Urd bean leaf crinkle virus (ULCV) on leaf size was studied in susceptible and resistant cultivars (Table 2 and 3, Fig. 1). In susceptible cultivar, significant enlargement of leaflets was seen conspicuously from third trifoliate onwards. The terminal leaflet of infected plant was found to be significantly larger than the left and right leaflets of the trifoliate leaf. But the left and right leaflets were of the same size. The interaction between plant and leaf position was significant only from third trifoliate leaf onwards. However, there was no significant increase in the leaf size in first and second trifoliates of infected plant. The maximum increase of 40.0, 28.9 and 25.3 per cent over healthy was recorded in 4$^{th}$ trifoliate leaf from base of the plant in the left, terminal and right leaflets, respectively. It was evident from Table 3 that there was no significant enlargement of leaflets in inoculated resistant cultivar over healthy.

Since this enlargement of leaf was intiated from third leaf onwards, it may be considered as a shock reaction produced by virus infection. The virus infection appears to have triggered the synthesis of growth regulators and hormones in early stage of infection. The role of plant growth regulators/ hormones in cell multiplication and cell enlargement of tissue organelles of many plants has been studied by several workers.

The elongation of cells, stimulated by auxins, occurs in different pcrtions of dicotyledonous plants (Barkely and Evans, 1970; Kazama and Katsumi, 1973). Dayanandan *et. al.*, (1976) have found that auxin, but not GA or kinetlin promoted cell elongation in parenchyma, collenchymas and vascular elements of the sheeth pulvinus of Avena Leaves. Skoog *et. al.*, (1967) reported that plant hormones like cytokinins are responsible for cell division in cooperation with an auxin. This indicates that hormones are incolved in hyperplasia.

Cytokinins also enhance cell expansion in young been leaves (Leopold and Kawase, 1964) and in other leaf types (Letham, 1969) and the increased expansions occur with little enhancement of cell division (Tsui *et. al.*, 1980, Letham, 1969), This indicates that cytokinins play a role in hypertrophy of cells.

**Stem and petiole thickness :**

The present experiment (Fig. 2) revealed that there was siginificant increase in the thicknes of infected stem in all internodes. There was gradual increase in per cent thickness of stem from first to fifth inter node

Table 1 : Influence of plant age during sap inoculation on incubation period and symptom development

| Position of Inoculated leaf | Age of plant (days) | Position of first leaf developing symptom | Incubation period (days) | Per cent transmission |
|---|---|---|---|---|
| Primary leaf | 7 | Second trifoliate | 13 (3.67) | 85 (67.37) |
| Primary leaf | 10 | Third trifoliate | 16 (4.06) | 72 (58.03) |
| First trifoliate | 15 | Fourth trifoliate | 17 (4.18) | 59 (50.17) |
| Second trifoliate | 22 | Sixth trifoliate | 22 (4.74) | 49 (44.40) |
| Third trifoliate | 29 | Eighth trifoliate | 28 (5.33) | 36 (36.84) |
| Fourth trifoliate | 37 | No symptoms | -- | 0 (7.4) |
| Fifth trifoliate | 43 | No symptoms | -- | 0 (7.4) |
| Sixth trifoliate | 49 | No symptoms | -- | 0 (7.4) |
| CD (P=0.05) | | | **0.15** | **3.46** |

(Figure in parenthesis are sine transformed values)

Table 2 : Leaf area ($cm^2$) of healthy and infected plants of susceptible variety T9

| Leaflets | Plants | Leaf position from the base | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Mean |
| Left | Healthy | 27.8 | 40.4 | 45.2 | 46.0 | 54.6 | 37.5 | 32.7 | 22.2 | 38.9 |
| | Infected | 28.4 | 41.0 | 58.0 | 64.4 | 66.0 | 52.5 | 41.2 | 31.2 | 47.8 |
| | Mean | 28.1 | 40.07 | 51.6 | 55.2 | 60.3 | 44.5 | 36.9 | 26.7 | 43.4 |
| Terminal | Healthy | 29.4 | 42.6 | 50.2 | 53.2 | 60.6 | 47.5 | 39.7 | 25.8 | 44.2 |
| | Infected | 30.2 | 43.4 | 62.7 | 68.6 | 72.5 | 60.4 | 52.4 | 38.2 | 53.5 |
| | Mean | 29.8 | 43.0 | 56.4 | 60.9 | 66.5 | 53.4 | 46.1 | 32.1 | 48.9 |
| Right | Healthy | 27.4 | 39.8 | 44.8 | 50.4 | 55.8 | 36.1 | 33.3 | 23.7 | 39.0 |
| | Infected | 27.0 | 40.9 | 59.0 | 63.2 | 64.9 | 54.6 | 40.2 | 29.4 | 47.4 |
| | Mean | 27.2 | 40.3 | 51.9 | 56.8 | 60.3 | 45.3 | 36.7 | 26.5 | 43.2 |
| | Healthy | 28.2 | 40.9 | 46.7 | 49.8 | 57.0 | 40.3 | 35.2 | 23.9 | 40.3 |
| | Infected | 28.5 | 41.7 | 59.9 | 65.4 | 67.8 | 56.1 | 44.6 | 32.9 | 49.6 |
| | Mean | 28.3 | 41.3 | 53.3 | 57.4 | 62.4 | 48.2 | 39.9 | 28.4 | |

**CD (P=0.05)**

| | | | |
|---|---|---|---|
| Leaf let | = | 0.33 | Leaflet x Position = 0.95 |
| Plant | = | 0.27 | Plant x position = 0.78 |
| Position | = | 0.55 | Leaflet x Plant = 1.35 |
| Leaflet x Plant | = | 0.47 | |

of infected plant. The thickness of the petiole of the first leaf of healthy and infected plant did not significantly differ. However, it increased significantly in diseased plant from the second trifoliate leaf. The thickness of petiole gradually increased from second to eight trifoliate leaf of infected plant. In the eight leaves, the petiole thickess increased from 1.3 mm in healthy plant to 3mm in diseased plant amounting to 230% over healthy. On the contrary, the healthy plant there was gradual decrease.

Fonnesbech (1972) reported that gibberellic acid enhances the leaf growth in developing orchid protocorms. The expansion of etiolated been leaves was stimulated byGA, which synergistically enhanced by IAA (Sharma

Table 3 : Leaf area ($cm^2$) of healthy and inoculated plants of resistant variety Karaikal

| Leaflets | Plants | Leaf position from the base | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **Mean** |
| Left | Healthy | 25.7 | 34.3 | 48.7 | 51.4 | 34.1 | 20.0 | 13.7 | 7.7 | 29.4 |
| | Infected | 25.2 | 35.1 | 48.3 | 50.7 | 34.6 | 20.1 | 12.4 | 7.7 | 29.3 |
| | Mean | 25.4 | 34.7 | 48.5 | 51.1 | 34.3 | 20.1 | 13.1 | 7.7 | 29.3 |
| Terminal | Healthy | 28.3 | 40.3 | 61.1 | 66.0 | 44.5 | 28.6 | 17.4 | 8.8 | 36.8 |
| | Infected | 27.8 | 40.6 | 60.8 | 65.6 | 44.3 | 27.5 | 18.7 | 8.7 | 36.7 |
| | Mean | 28.1 | 40.4 | 60.9 | 65.8 | 44.4 | 27.7 | 13.1 | 8.7 | 36.8 |
| Right | Healthy | 24.9 | 33.6 | 45.6 | 50.0 | 32.0 | 19.8 | 12.6 | 7.4 | 28.3 |
| | Infected | 24.6 | 34.3 | 48.8 | 49.5 | 33.1 | 20.7 | 13.0 | 7.6 | 28.4 |
| | Mean | 24.7 | 33.9 | 45.2 | 49.5 | 32.5 | 20.3 | 12.8 | 7.5 | 28.3 |
| | Healthy | 26.3 | 36.1 | 51.8 | 55.8 | 36.8 | 32.6 | 14.6 | 7.9 | 31.5 |
| | Infected | 25.8 | 36.6 | 51.3 | 55.1 | 37.3 | 32.5 | 14.7 | 8.0 | 31.5 |
| | Mean | 26.1 | 36.4 | 51.5 | 55.3 | 37.1 | 22.7 | 14.6 | 7.9 | |

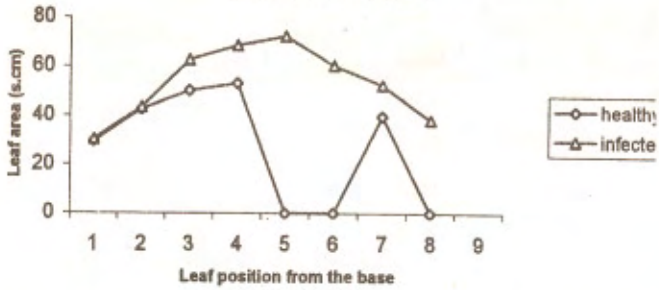
**CD (P=0.05)**

| | | | | | |
|---|---|---|---|---|---|
| Leaf let | = | 0.39 | Leaflet x Position | = | 1.12 |
| Plant | = | NS | Plant x position | = | NS |
| Position | = | 0.64 | Leaflet x Plant | = | NS |
| Leaflet x Plant | = | NS | | | |

Table 4 : Comprasion of stpules (cm) of healthy & infected plants

| Plants number | Plants number | | Plants number | |
|---|---|---|---|---|
| | Length | Breadth | Length | Breadth |
| 1 | 6.0 | 3.0 | 12.0 | 6.0 |
| 2 | 10.0 | 3.0 | 11.0 | 5.3 |
| 3 | 7.0 | 3.0 | 10.5 | 5.0 |
| 4 | 8.0 | 3.0 | 10.5 | 5.0 |
| 5 | 7.0 | 3.5 | 9.5 | 5.5 |
| 6 | 7.0 | 3.0 | 11.0 | 6.0 |
| 7 | 9.5 | 3.5 | 12.0 | 6.0 |
| 8 | 7.0 | 3.0 | 13.0 | 6.0 |
| 9 | 6.0 | 3.0 | 11.0 | 5.5 |
| 10 | 8.0 | 3.0 | 12.5 | 6.0 |
| 11 | 6.5 | 3.5 | 13.0 | 6.0 |
| 12 | 6.0 | 3.0 | 12.5 | 7.0 |
| 13 | 8.5 | 3.0 | 12.0 | 6.0 |
| 14 | 9.0 | 2.5 | 11.0 | 7.0 |
| 15 | 9.0 | 3.5 | 11.0 | 6.5 |
| 16 | 6.0 | 3.0 | 10.0 | 7.0 |
| 17 | 8.0 | 3.0 | 10.0 | 7.0 |
| 18 | 9.0 | 3.0 | 11.0 | 6.5 |
| 19 | 7.0 | 3.0 | 10.0 | 5.5 |
| 20 | 7.5 | 3.0 | 9.5 | 9.5 |
| Mean | 7.6 | 3.0 | 11.3 | 5.7 |

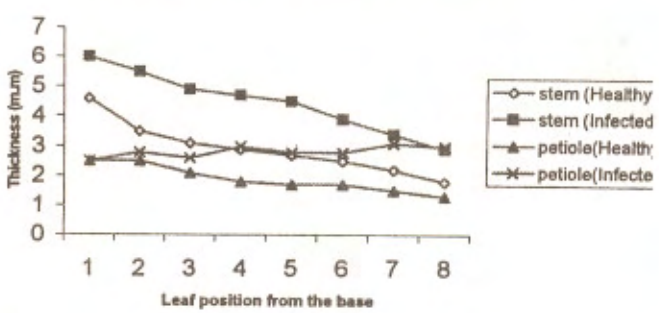
**Fig 1. Leaf area of healthy and infected plants of suceptible variety T9**




1979). Shoots treated with GA frequently exhibited large increase in cell number but no increase in cell size (Loy, 1977). The most striking effect of GA on cell expansions is its enhancement of internode and cell elongation in *Avena* stem segments (Kaufman *et. al.*, 1969 Kaufman and Jones, 1974). Further work to trace the levels of these hormones from the time of inoculation in leaves will throw light on the sequence of physiological changes taking place in the inoculated plant.

Bhaktavatsalam *et al.*, (1983a) reported that increase in IAA up to 59.0 , 59.5 and 100.0% in young , medium and old Urd bean leaf crinkle virus (ULCV) infected plants, respectively. They suggested that the increase

**Fig 2. Effect of ULCV on thickness of stem and petiole**




in IAA in diseased leaves might be involved in enlargement of leaves. Although virus infections are gnerally known to reduce auxin content in plants (Matthews, 1981), enhanced auxin levels were also noted in tomato and potato infected by spotted wilt virus and potato virus-X (Jones 1956).

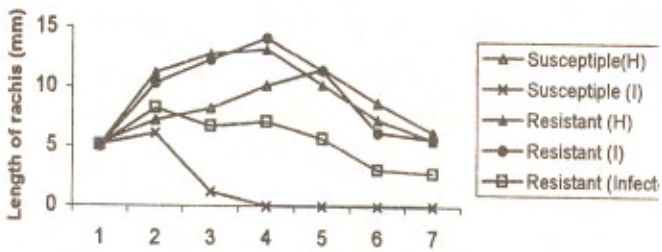
**Rachies length :**

The results in (Fig. 3) indicated that there was significant reduction in the rachis length in susceptible cultivar. The rachis development decreased significantly from second trifoliate leaf and ceased completely from fourth trifoliate onwards in infected susceptible cultivar. Therefore, the leaflets appeared to be sessile. However, there was no significant reduction of rachis length in inoculated plant of resistant cultivar.

**Stipule enlargement :**

Another important symptom prouduced by the virus was enlargment of the stipules even before the appearance of symptom on the lamina. In order to find out whether the stipule enlargement can be used as a reliable criterion to identify infected plants before appearence of symptom on lamina, 20 plants in which such enlargement occurred were marked after sap inoculation on cotyledon leaves. It was found that all the plants developed symptoms 5-7 days after stipule enlargement, The two stipules became thicker, broader and dark green than in healthy plants. Similarly, stipule en-

**Fig 3. Length of rachis (mm) in healthy and infected urd bean plants**




largement was found in plants grown in from infected seeds and also in case of naturally infected plants infectede field. The length and breadth of stipules of healthy and infected plants were measured (Table 4). The stipule length of healthy plants measured 7.6 mm whereas in infected plants, it was 11.3 mm amounting to an increase of 48.6 per cent. Similarly, the stipules width of diseased plants also increased by 90 per cent. This effect can be made use of in indentifying the infected plants five days before development of leaf crinkle. It is possible to remove infected plants by indetifying enlarged stipules.

**CONCLUSION :**

Studies on infulence of plant age on infection of Urd bean leaf crinkle virus (ULVC) revealed that inocualtion at primary leaf stage (1 and 10 DAS) gave 85 and 72 per cent infection. The incubation period of the virus in inoculated plants was gradually increased with the age of plants at the time of inoculation and the per cent transmission was gradually reduced with increase in plant age. There was no infection when plants were inoculated prior to flowering stage. This information can be effectively utilized for the management of the disease by reducing the spread of virus, through application of suitable insecticides in the early stages of the crop. Significant enlargement of leaflets was observed in Urd bean leaf crinkle virus (ULCV) infected plants from third trifoliate on wards. From these experiments, significant increase

in the thickness of infected stem in all internodes and stipule enlargement were also noticed. But significant reduction in the rachies length was observed in susceptible cultivars. These symptoms will be in great use in eliminating infected plants in the early stages for effective management of the disease.

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