



## Prevalence and transmission of leaf crinkle disease infecting urdbean in Tamil Nadu

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### Abstract

Urdbean leaf crinkle disease (ULCD) caused by an ungrouped virus is a destructive and serious disease of urdbean (*Vigna mungo* (L.) Hepper). Urdbean is commonly infected by leaf crinkle disease and is distinguished by the symptoms of extreme crinkling, curling, puckering, rugosity of leaves, stunting of plants and malformation of floral organs. An intensive survey was conducted during the year of 2018-2019 in major blackgram growing districts of Tamil Nadu viz., Pudukkottai, Tiruchirappalli, Villupuram, Tenkasi and Coimbatore. The highest disease incidence was recorded in Pudukkottai (24.95%) and lowest incidence was recorded in Coimbatore (17.89%). The transmission of ULCD by seed sprout abrasion and sap inoculation methods was 70.59% and 63.30% respectively. The transmission of ULCD by infected seeds was ranging from 71.89 to 83.62%.

**Keywords:** *Urdbean leaf crinkle disease (ULCD); Survey; Mechanical ; Seed transmission*

### Introduction

Urdbean (*Vigna mungo* (L.) Hepper) is an important leguminous crop of the Indian subcontinent and it is the rich source of protein in the human diet. It is one of the short-duration pulse crops cultivated throughout the year under various agro-climatic conditions (Jayasekhar and Ebenezer, 2016). In India, the major urdbean growing states are Madhya Pradesh, Rajasthan, Uttar Pradesh and Tamil Nadu. The highest production was recorded by the state of Madhya Pradesh (11.91 lakh tonnes) followed by Rajasthan (3.76



lakh tonnes) and Uttar Pradesh (3.25 lakh tonnes). In India, Tamil Nadu occupies 4<sup>th</sup> position under cultivation of 4.41 lakh hectares and 5<sup>th</sup> position under the production of 2.74 lakh tonnes (DES, 2019). The crop is highly prone to attack by many virus diseases. Among them, urdbean leaf crinkle disease (ULCD) is considered to be a serious one which resulted in drastic yield loss ranging from 35-81% depending upon the season and variety (Reddy *et al.*, 2005).

The disease was first reported from Delhi by Nariani (1960) and Williams *et al.* (1968). It was characterized by the symptoms *viz.*, extreme crinkling, puckering, curling of leaves, malformation of flowers and stunted growth (Iftikhar *et al.*, 2020). There is no detailed information related to etiology of ULCD but there are reports on the various transmission of ULCD. Seed transmission of ULCD was first reported by Kolte and Nene (1972). The percent transmission of ULCD in urdbean and mungbean was 34.50% and 26.25% respectively (Kanimozhiet *et al.*, 2009). The disease was also reported to be transmitted by various insect vectors (Narayanaswamy and Jaganathan, 1973; Beniwal and Bharathan, 1984) and sap (Biswas *et al.*, 2012) but not transmitted through soil (Beniwalet *et al.*, 1983). The economic importance of the disease was investigated by surveying for the incidence of ULCD in major urdbean growing districts of Tamil Nadu and also the transmission characters of ULCD was studied to understand the etiology which share the similar characters with already identified and established plant viruses of other crops.

## Materials and Methods

### *Survey for the incidence of ULCD*

Major urdbean growing villages of Pudukottai, Tenkasi, Villupuram, Coimbatore, Tiruchirappalli districts of Tamil Nadu were surveyed for the incidence of urdbean leaf crinkle disease (ULCD) during the year 2018-2019. The disease incidence of ULCD was recorded following the procedures of Bhavani and Manoj Kumar (2017). In each district, two villages (two fields/ village) were randomly selected and 25sq.m areas of each field were fixed to record the disease incidence at 45 days after sowing (DAS). The percent disease incidence (PDI) was calculated by using the standard formula.

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

### *Transmission of ULCD*

#### *Collection of infected and healthy seeds*

The transmission studies were conducted in the Glasshouse of Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore during the year of 2018-2019. The healthy urdbean cv. VBN 8 was used for mechanical transmission study. The ULCD infected plants cv. CO 5 and VBN 8 seeds were tagged individually in the experimental farms of Tamil Nadu Agricultural University, Coimbatore



during *Kharif*- 2019, *Rabi* 2019-20 and summer- 2020. The seeds were harvested from tagged plants at the time of maturity and used for seed transmission study.

#### ***Mechanical transmissions by sprout seed abrasive method***

The seeds collected from healthy plants were pre-soaked for six hours and placed on moist blotter paper for 8 hrs for sprouting. The crude sap was prepared by grinding ULCD infected leaves (1:5 w/v) with 0.05M potassium phosphate buffer (pH 7.0) supplemented with 0.1% of 2-Mercaptoethanol in a sterilized ice-cold mortar and pestle. The sprouted seeds were soaked in the sap for one hour with intermittent shaking in the beaker containing crude sap mixed with carborundum powder (600 mesh) as abrasive.

The seeds soaked in the infected leaf sap were sown in 20 diameter plastic pots containing a mixture of farmyard manure, red soil and sand (1:2:1) with timely irrigation under insect proof conditions. The sprouted seed incubated with buffer was maintained as untreated control. In each sap-inoculated and uninoculated seeds, thirty number plants were used per replication (5 seeds/pot) in the experiments. The plants were observed periodically for symptom development and a number of plants showing symptoms were recorded at 45 DAS. The PDI was calculated by using a standard formula.

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of plants produced symptoms}}{\text{Number of inoculated seeds}} \times 100$$

#### ***Mechanical transmission by sap inoculation***

The ULCD infected symptomatic leaves were collected and ground ULCD infected leaves (1:5 w/v) with 0.05 M Phosphate buffer (pH 7.0) containing 0.15% Sodium sulfite and 1% 2-mercaptoethanol. The seeds were sown in potting mixture under insect-proof glasshouse conditions. At two leaf stage (7 DAS), the sap was inoculated on carborundum (600 mesh) dusted leaves and the leaves were washed with distilled water after 1 minute of inoculation in order to remove excessive sap. Thirty plants per replication were maintained to record the transmission of ULCD incidence. The sap inoculated plants were kept under insect proof condition and observed for symptoms expression. The disease incidence was recorded at 45 DAS.

#### ***Seed transmission***

The seeds collected from ULCD infected plants cv. CO 5 and VBN 8 were sown in the 20 diameter plastic pots filled with sterilized soil under insect-proof conditions. Hundred numbers of healthy and ULCD infected seeds were sown separately in twenty pots and five seeds per pot were sown. After sowing, the germination and symptoms development were periodically observed. The percent seed transmission was computed by the following formula

$$\text{Seed transmission (\%)} = \frac{\text{Number of plants showing symptoms produced symptoms}}{\text{Number of seeds germinated}} \times 100$$

#### ***Statistical analysis***

The percentage data were arcsine transformed for analysis. The Standard error of mean (SEm ±) and critical difference (C.D.) values were calculated by completely randomized design using the statistical tool developed by Gomez and Gomez (1984).



## Results and Discussion

The survey was conducted in major urdbean growing districts of Tamil Nadu and results revealed that percent disease incidence was ranged from 11.24 to 28.23% (Fig 1). The highest mean disease incidence was observed in Pudukkottai District (24.95%) followed by Villupuram (19.45%). The Coimbatore district was recorded with the lowest disease incidence of 17.89% under field conditions. The ULCD infected plants produced the typical symptoms of crinkling, stunting and malformation of flowers. A similar survey on ULCD incidence was carried out in which the disease incidence was recorded with a range of 28 to 85% in Uttar Pradesh (Srivastava, 2005). Priyanga et al. (2020) conducted a survey in six major urdbean growing districts of Tamil Nadu and reported that the highest incidence of 33.50% was recorded in Pudukkottai district where urdbean cultivar T9 was grown. Sprout seed abrasive and sap inoculation methods were followed to study the mechanical transmission of ULCD. The sprouted seed inoculated with sap showed the disease incidence up to 70.59% and no incidence was recorded in un-inoculated seeds (Fig 2). Similar experiment conducted for mechanical inoculation on cotyledon leaf was recorded the disease incidence of 63.30%. The transmission percentage of ULCD by mechanical sap inoculation on cotyledon leaves was studied by Dubey et al. (2020) and the incidence of ULCD by this method was 50.6% at 50 days after inoculation.

The incidence of ULCD by this mechanical sap inoculation method was 63.30% (Fig 3). Similar experiment was conducted by Ramya et al. (2020) on mechanical inoculation of ULCD by sprout seed abrasive method in urdbean cv. CO 5. A typical crinkling symptom was observed on third trifoliolate leaves followed by floral malformation and the transmission of 43%. The seed transmission study revealed that the ULCD symptoms appeared initially at third trifoliolate stage (Fig 4). The germination percentage was affected by the infection of ULCD. There was a reduction in germination percentage ranging 82 to 88% in the infected seeds than healthy seeds (96%). The percent seed transmission cv. VBN 8 was 83.62%, 80.65% and 81.32% during *Kharif-2019*, *Rabi- 2019-20* and *summer-2020* respectively. Whereas, percent seed transmission cv. CO 5 was 76.73%, 71.89% and 74.89% during *Kharif-2019*, *Rabi- 2019-20* and *summer-2020* respectively (Fig 5). Kanimozhiet al. (2009) recorded the seed transmission of 34.50% in urdbean. Latakeet al. (2020) reported that the rate of seed transmission of ULCD was 40 to 54%. Reddy et al. (2005) experimented on grow-out test for testing ULCD seed infection, which recorded seed transmission ranging 45.20% to 86.50%. The results of present study were confirmed with earlier workers (Narayanasamy and Jaganathan, 1975; Beniwalet al., 1984; Mishra et al., 1994, Rao and Reddy, 2005). Dubey et al. (2021) studied the seed transmission using seeds collected from mechanically inoculated plants under insect-free environmental conditions and reported 83.62 to 100% of seed transmission which clearly showed that the leaf crinkle disease is highly seed transmitted.

## Conclusions

The ULCD was prevailing in all the districts of Tamil Nadu under survey for the incidence of leaf crinkle in urdbean which was alarmingly increasing under natural conditions. The present studies confirmed that the ULCD is highly transmitted through infected seeds under natural condition and artificially through



mechanical sap inoculation. The infected seed was accountable for the widespread of the disease and severe yield losses. The UCLD infected seeds need to be eliminated from the seed lot to avoid the further spread of the disease. In this consideration, the causative agent of ULCD disease needs to be identified and the transmissions other than seed and sap which respect to ULCD are study to clarify the etiology agent.

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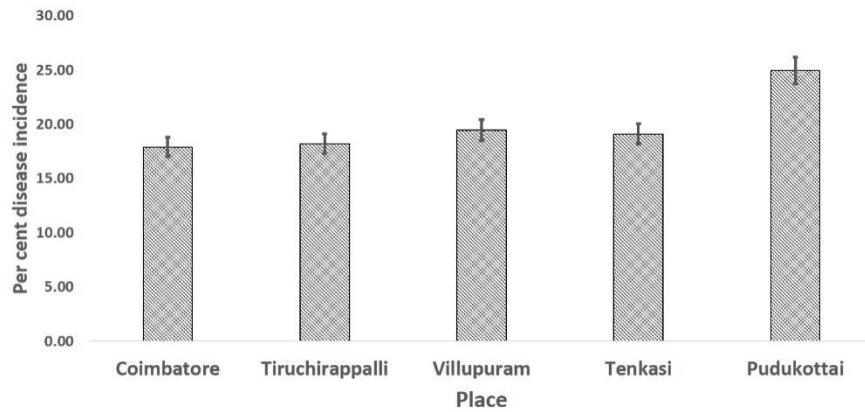


Figure 1. Incidence of ULCD during the year of 2018-2019 under natural conditions. Error bars represent the standard deviation of the mean from three independent villages.

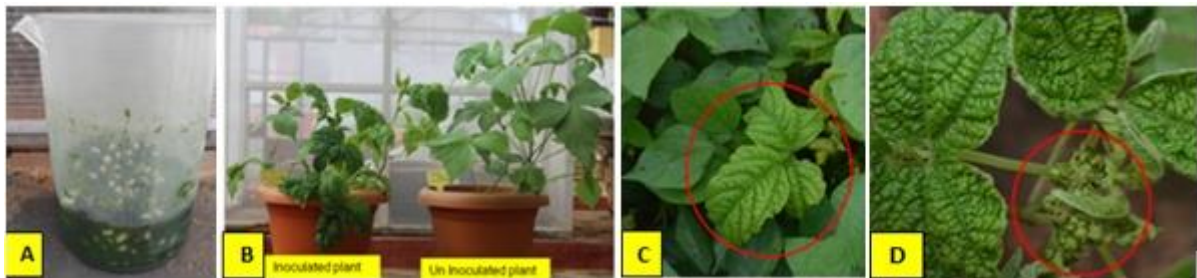


Figure 2. Mechanical transmission by seed sprout abrasive method A. Soaking of sprout seeds in infected sap; B. Inoculated and un-inoculated plants; C. Crinkling of leaves D. Floral malformation





Figure3. Mechanical transmission by sap inoculation A. Cotyledon leaves dusted with carborundum; B. Sap inoculated plants; C. Un-inoculated plants

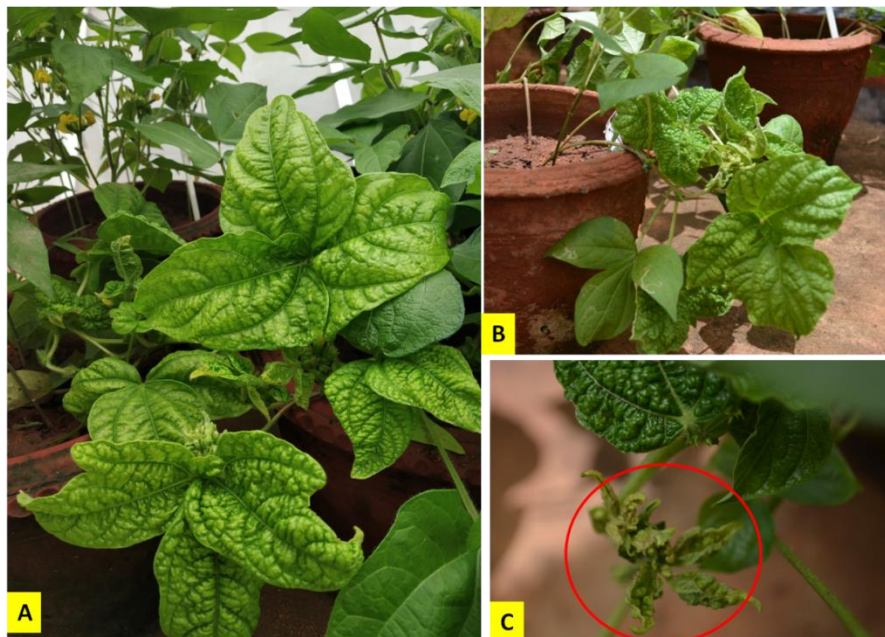






Figure4. Seed transmission of ULCD under glasshouse conditionsA. Symptoms produced from infected seeds; B. Crinkling of leaves; C. Floral malformation

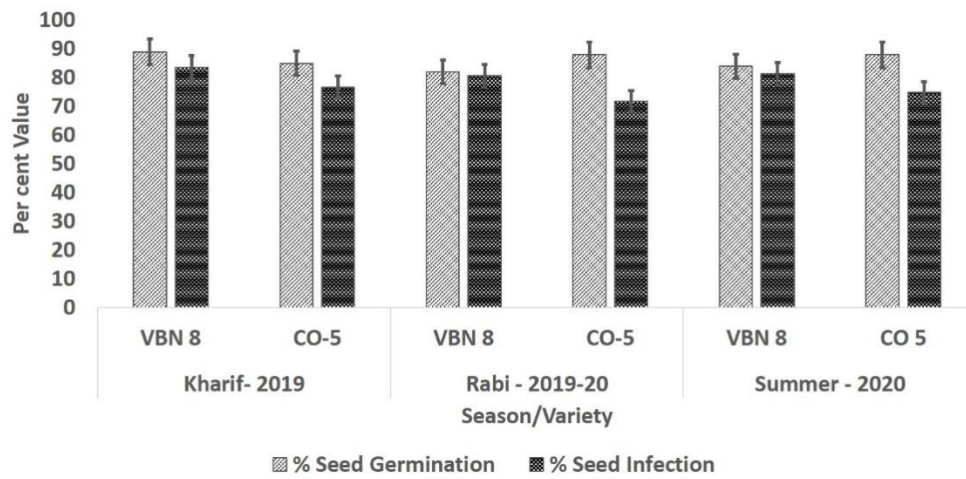


Figure5. Percentage seed transmission of ULCD from naturally infected plants. Error bars represent the standard deviation of the mean from three independent biological experiments.