



RESEARCH ARTICLE

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, the 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 the 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 a 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 4th position under cultivation of 4.41 lakh hectares and 5th 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 that resulted in drastic yield loss ranging from 35-81% depending upon the season and variety (Reddy et al., 2005).

The disease was first reported in 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 the 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 (Kanimozhi 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 (Beniwal et al., 1983). The economic importance of the disease was investigated by surveying the incidence of ULCD in major urdbean growing districts of Tamil Nadu and also the transmission characteristics of ULCD were studied to understand the etiology which share similar characteristics with already identified and established plant viruses of other crops.

MATERIAL AND METHODS

Survey for the incidence of ULCD

Major urdbean growing villages of Pudukkottai, 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 25 sq.m areas of each field were

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fixed to record the disease incidence at 45 days after sowing (DAS). The percent disease incidence (PDI) was calculated by using the standard formula.

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 2018-2019. The healthy urdbean cv. VBN8 was used for the mechanical transmission study. The ULCD infected plants cv. CO5 and VBN8 seeds were tagged individually in the experimental farms of Tamil Nadu Agricultural University, Coimbatore during Kharif- 2019, Rabi 2019-20, and summer- of 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 (600mesh) 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 seed, 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.

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 (7DAS), the sap was inoculated on carborundum (600mesh) 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 conditions and observed for symptom expression. The disease incidence was recorded at 45 DAS.

Seed transmission

The seeds collected from ULCD infected plants cv. CO5 and VBN8 were sown in the 20 diameter plastic pots filled with sterilized soil under insect-proof conditions. A 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

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 the mean (SEm ±) and critical difference (C.D.) values was calculated by a 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 the per cent disease incidence ranged from 11.24 to 28.23% (Figure 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 the 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 upto 70.59% and no incidence was recorded in un-inoculated seeds (Figure 2). A similar

experiment conducted for mechanical inoculation on cotyledon leaf 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% (Figure 3). A 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 trifoliate leaves followed by floral malformation and the transmission of 43%. The seed transmission study revealed that the ULCD symptoms appeared initially at the third trifoliate stage (Figure 4). The germination percentage was affected by the infection of ULCD. There was a reduction in germination percentage ranging from 82 to 88% in the infected seeds to 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 (Figure 5). Kanimozhi *et al.* (2009) recorded the seed transmission of 34.50% in urdbean. Latake *et 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 the present study were confirmed by earlier workers (Narayanasamy and Jaganathan, 1975; Beniwal *et 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.

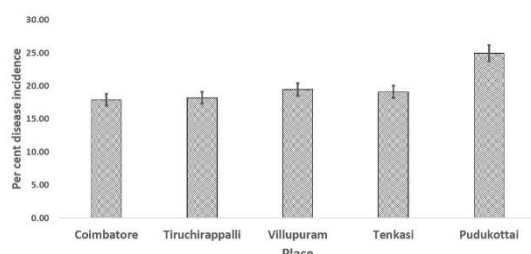


Figure 1. Incidence of ULCD during the year of 2018-2019 under natural conditions. Error bars represent the standard deviation of the mean from three independent villages

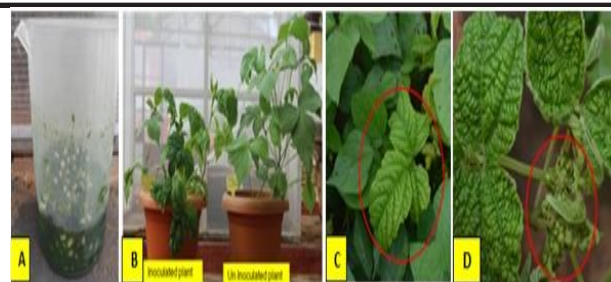


Figure 2. Mechanical transmission by seed sprout abrasive method A. Soaking of sprout seeds in infected sap; B. Inoculated and un-inoculated plants; C. Crinkling of leaves D. Floral malformation



Figure 3. Mechanical transmission by sap inoculation A. Cotyledon leaves dusted with carborundum; B. Sap inoculated plants; C. Un-inoculated plants



Figure 4. Seed transmission of ULCD under glasshouse conditions: A. Symptoms produced from infected seeds; B. Crinkling of leaves; C. Floral malformation

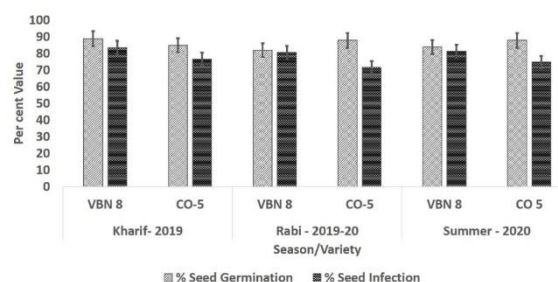


Figure 5. Percentage seed transmission of ULCD from naturally infected plants. Error bars represent the standard deviation of the mean from three independent biological experiments

CONCLUSION

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 conditions and artificially through mechanical sap inoculation. The infected seed was accountable for the widespread disease and severe yield losses. The ULCD 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 studied to clarify the etiology agent.

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There was no conflict of interest in the publication of this content

Data availability

Not applicable

Author contributions

T.K.S.L., G.K., M.R. have conceptualized the research; T.P., T.R., S.R. have performed the experiment related to this manuscript; T.K.S.L., K.P and V.B. have written the manuscript. All authors have proofread and reviewed the manuscript.

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