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Mechanical Transmission of Leaf Crinkle Disease in Urdbean (*Vigna mungo* (L.) Hepper) by Sprout Seed Abrasion Method

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A B S T R A C T

Urdbean or blackgram (*Vigna mungo* (L.) Hepper) is one of the important leguminous cropscultivated in India, Pakistan and South-East Asian countries. Among the biotic stresses, Urdbean leaf crinkle disease (ULCD), is economically significant and devastating in major urdbean growing countries. ULCD causes severe crinkling and rugosity of lamina, stunting of plants, malformation of buds inflicting heavy yield losses. Due to occurrence of ULCD as mixed infections along with other major virus diseases of pulses, maintenance of pure inoculum under glasshouse conditions is a prerequisite. In the present study, seeds were mechanically sap inoculated by sprout seed abrasion method. Influence of artificial inoculation on ULCD incidence has been studied in CO5 cultivar of blackgram. Symptoms started appearing 30-35 DPI and disease incidence was up to43.3%. This protocol will help in maintenance of pure inoculum of ULCD under glasshouse conditions and further investigations are under process to standardize efficient protocol to obtain higher transmission efficiency.

Introduction

Urdbean otherwise known as blackgram (*Vigna mungo* (L.) Hepper) is one of the important grain legumes grown in Asia and Africa. Several biotic and abiotic factors including viral diseases attribute to low productivity of this crop. From time to time, number of viruses causing substantial losses in urdbean has been reported. Among them a thrip transmitted G*roundnut bud necrosis virus* (GBNV) (Bhat, 2001; Sarita and Jain, 2007; Biswas *et al.,* 2009) and two whitefly transmitted Begomo virus as viz., *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic Indian virus* (MYMIV) appeared to be a serious concern and major factors for severe crop losses in Indian subcontinent (Biswas and Varma, 2001; Biswas *et al.,* 2008; Varma and Biswas, 2009).

Urdbean leaf crinkle disease (ULCD), still unknown of its etiology, became widely spread nowadays all over India and causes havoc losses of yield of urdbean (Kadian,

1982; Sharma *et al.*, 2014; Kanimozhi *et al.*, 2009). In India the incidence of this disease was first observed in 1966 in the states of Delhi and Uttar Pradesh (Williams *et al.*, 1968) and the causal organism was identified as Urdbean Leaf Crinkle Virus (ULCV) (Kolte and Nene, 1972).

Typical symptoms include enlargement of lamina, crinkling of lamina, stunting of plants and Malformation of floral buds. ULCD is highly seed transmitted and grain yield loss up to 35-81% has been reported (Bashir *et al.*, 1991).

Urdbean is reported to be infected by yellow mosaic disease (YMD), Bud necrosis disease (BND), Urdbean leaf crinkle disease (ULCD) at the same time in Delhi condition (Biswas *et al.*, 2009). However, the status of mixed infection and the etiology of ULCD in urdbean has not yet been reported so far. Therefore, effort is being made to study the etiology of ULCD, so as to determine the specific molecular diagnostic tools. In the present study, attempt was made to maintain the pure inoculum of urdbean leaf crinkle disease pathogen by sprout seed abrasion method.

Materials and Methods

The experiment was carried out in the glass house of Department of Plant Pathology of Tamil Nadu Agricultural University (TNAU), Coimbatore.

Source of inoculum

In order to maintain pure inoculum, ULCD Infected plants from field were tagged and seeds were collected and sown in glasshouse under insect proof cages. Leaves showing typical leaf crinkle symptoms were collected from glasshouse sown plants and used as inoculum.

Inoculation by sprout seed abrasion method

For mechanical sap inoculation of seed, urdbean cultivar CO5 was soaked in distilled water for 6 hrs. Pre-soaked seeds were placed on moist blotter paper for 8 hrs for the sprouts to emerge. Inoculum was prepared by grinding 1g of infected leaf sample in 5ml of potassium phosphate buffer (0.05M, pH 7.0)and supplemented with 0.1% of 2-Mercaptoethanol before grinding in sterilized ice-cold mortar and pestle. (0.05M Potassium phosphate buffer was prepared using 2.335g of Dipotassium hydrogen phosphate in 500ml of sterile distilled water as Sol A and 1.575g of Potassium dihydrogen orthophosphate in 500ml of sterile distilled water as Sol B. 38.9ml of Sol A and 61.1ml of Sol B are mixed to attain pH 7.0).

Sprouted seeds were incubated in the sapalong with carborundum as abrasive. Seeds were soaked for one hour with intermittent shaking and sown in pots (dia. 20") with nutrient supplement and timely irrigation. Incubating sprouted seeds with buffer was maintained as control. Inoculated seeds were sown in three replications (10 seeds per pot).

Observation on ULCD incidence

Symptom development was observed from 30-35 days post inoculation (DPI) on third trifoliate stage of the crop. Number of seedlings showing symptoms were recorded up to 50 DPI. Typical symptoms *viz.*, crinkling and enlargement of lamina, malformation of buds were observed. Percent disease incidence (PDI) was calculated as:

$$\textbf{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Results and Discussion

Sprout seed inoculated urdbean plants, maintained under insect proof cage were observed at regular intervals and observations were taken and noted as Disease incidence (Table 1). Per cent disease incidence was recorded and mean of three replications was calculated. Germination of inoculated seeds was 100%. Symptoms started as mild crinkling of lamina from 30-35 days post inoculation and progressed to enlargement and rugosity of lamina. Most of the plants started expressing symptoms from third trifoliate stage of crop and few plants on fourth trifoliate. Malformation of floral buds was observed in most of the plants. Data was recorded up to 50 DPI and mean of replications were calculated. No symptom was observed on control plants.

As new viruses with mixed infection in pulse crops are emerging, maintenance of virus inoculum free of vector borne contamination is a requisite for understanding etiology of virus, host specificity and symptom expression. In case of urdbean leaf crinkle disease, as etiology is still unknown maintenance of its pure inoculum assists in removing mixed infections as well as in studying host pathogen interactions. Sprout seed abrasion method is effective as leaf crinkle infection is taken by host plants at the initial stage before germination and symptom expression is early (Fig. 1 and 2).

Table.1 Disease incidence of leaf crinkle disease in urdbean under greenhouse condition

Parameters	CO5	Healthy control (CO5)
No. of seeds germinated/No. of inoculated seed sown*	30/30 (100%)	30/30 (100%)
No. of Plants showing symptoms*	14	0
Days taken to express symptoms	30-35	0
Per cent Incidence	43%	0

*Mean of three replications

Fig.1 Sprout seed abrasion methodology

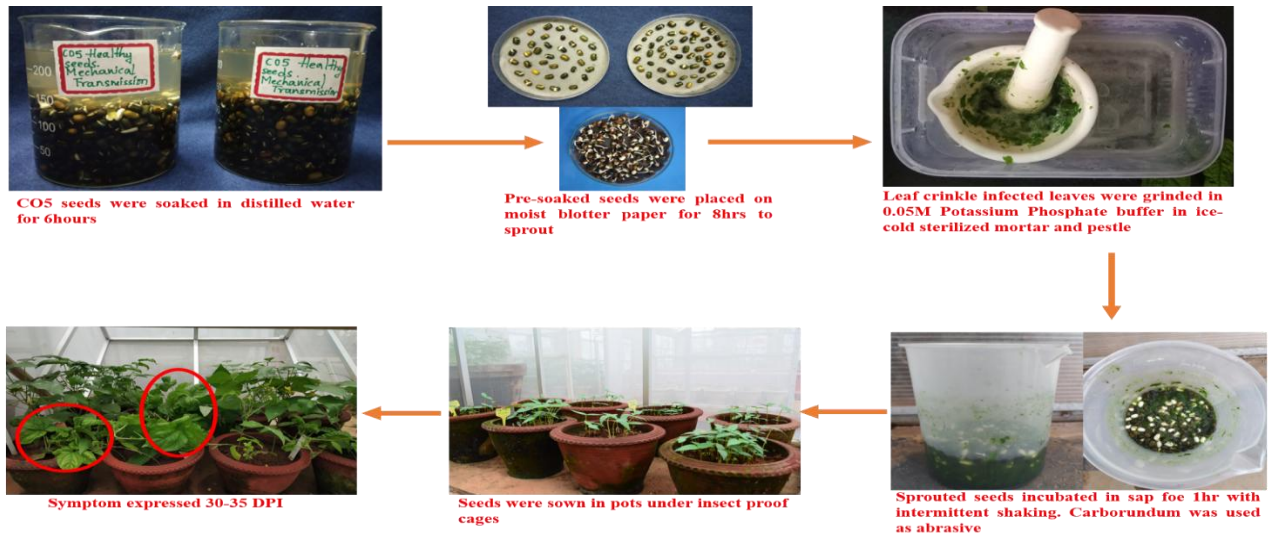
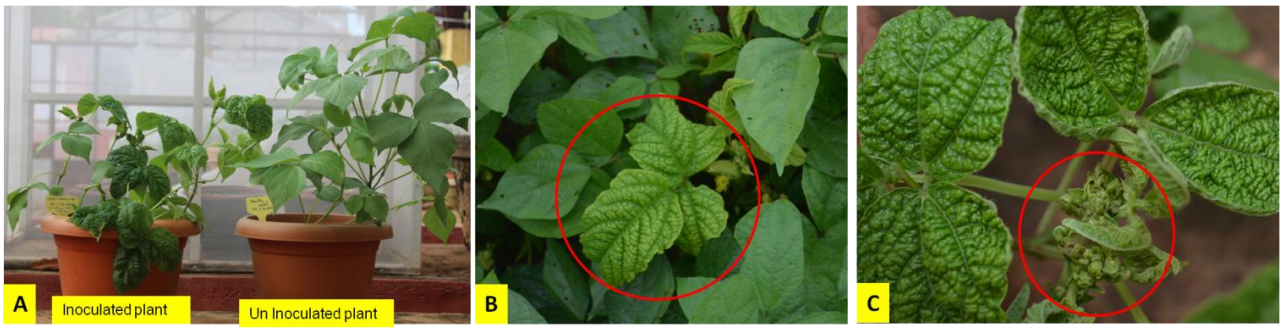


Fig.2 Symptom development by sprout seed abrasion method; A: Inoculated and Uninoculated plants; B: Crinkling of Lamina; C:Malformation of floral parts



In the present study seeds were mechanically sap inoculated by sprout seed abrasion method (Biswas *et al.*, 2012). Influence of artificial inoculation on ULCD incidence has been studied on CO5 cultivars. The present investigation is helpful to suggest that the ULCD is transmitted by mechanical sap inoculation through sprouted seed. It is increasingly becoming clear that outcome of plant-virus interaction depends on stage of inoculum, abrasive and additives used. Therefore, greater attention is needed in optimizing the sprout seed abrasion protocol by standardizing the abrasive, additives and time of soaking seeds in the inoculum. Further studies will be taken to ascertain the influence of sprout seed abrasion method on different genotypes of urdbean.

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