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Studies on Seed Transmission of Urdbean Leaf Crinkle Virus (ULCV) in Blackgram

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Abstract

Blackgram is an important pulse crop in India. Among the viral diseases, it is more susceptible to yellow mosaic and leaf crinkle. ULCV (Urdbean Leaf Crinkle Virus) is more severe in blackgram than other pulses and causes yield loss ranging from 35 to 81 per cent. Seed transmission studies were carried out in blackgram infected by ULCV to characterize the pathogen. In grow-out test, it was revealed that ULCV was seed transmitted both under natural and controlled conditions.The per cent seed transmission was in the range of 59.20 to 95.45 under field conditions, while it was 56.00 to 72.91 in glass house plants. Further, the symptoms were recorded at trifoliate leaves under field conditions and at the two-leaf stage under glasshouse conditions. Sprout seed abrasion studies showed the maximum disease incidence in sap inoculated soaked seeds kept for germination for two days.

Keywords : Blackgram, ULCV, Seed transmission, Sprout seed abrasion

Pulses are a rich source of proteins among the cultivated crops in India. They are second most important crops in human diet after cereals. They fix atmospheric nitrogen and add organicmatter to the soil. They are used in crop rotation with cereals and increase the fertility of the soil. They have good fibre content with a low glycemic index and thus are beneficial todiabetic people. India is the largest producer and consumer of pulses. The important pulses grown in India are bengalgram, redgram, blackgram and greengram (Mudryj *et al*., 2014).

Blackgram [*Vigna mungo* (L.) Hepper], with chromosome number 2n=22, belongs to the family Fabaceae (Jayamani and Satya, 2013 and Thamodharan *et al*., 2016). Commonly known as Urdbean, it has a good protein content of 25 per cent. It is mainly a *kharif* crop in most of the states of India (Vishalakshi *et al*., 2017). The crop is susceptible to many fungal, bacterial and viral diseases. The important viral diseases include Mungbean Yellow Mosaic Virus (MYMV) and Urdbean Leaf CrinkleVirus(ULCV).

Among pulses, blackgram is more susceptible to ULCV than other pulses (Saha *et al*., 2017)). Urdbean leaf crinkle is the most devastating disease in blackgram resulting in 35-81 per cent of yield loss depending on the time of infection and genotype (Bhavani *et al*., 2018 and Sharma *et al*., 2015). It results in symptoms like mild to severe crinkle, puckering, stunting, rugosity, floral malformation resulting in significant damage and yield loss (Gautam *et al*., 2016). In India, it was first reported from Punjab by Chohan and Kalia (1967). It was named as Urdbean Leaf Crinkle by Kolte and Nene (1972). There are conflicting reports on the etiology and transmission studies of ULCV. Hence, seed transmission studies of ULCV were carried out in blackgram.

Seed transmission studies play an important role in crop viral diseases epidemiology. They serve as a primary source of inoculum of viruses, facilitating the introduction of viruses tonew areas and resulting in secondary transmission of the viruses. Seed transmitted viruses affect the germination of the crops, affecting the crop stand (Aishwarya *et al*., 2020). Seed transmission studies help in roguing of plants at the early stages of the crop, thus preventing the spread of the virus to other plants and other crops (Aishwarya, 2018 and Reddy, 2018). Hence, present seed transmission studies of ULCV were carried out in blackgram.

Material and Methods

Identification of ULCV Infected Blackgram Plants

Blackgram plants showing typical symptoms of crinkling, puckering, stunting and floral malformation were identified in the field. Fifteen plants showing ULCV were tagged.

Maintenance of ULCV Inoculum in the Glasshouse

Fresh leaves were harvested from symptomatic plants and sap inoculated to healthy blackgram plants grown in the glasshouse at the two-leaf stage. Plants that showed the ULCV symptoms upon sapinoculation were maintained.

Collection and Sowing of Seeds from ULCV Symptomatic Plants

Seeds were harvested from 15 symptomatic tagged plants separately. Each of the harvested seeds of 10 plants was sown individually in the plant to row progeny in the field and five plants in a glasshouse under controlled conditions. The plants were later observed for the symptom expression and percent disease incidence was worked out

$$\text{Per cent disease incidence} = \frac{\text{No. of plants with symptoms}}{\text{Total no of germinated plants}} 100$$

Sprout Seed Abrasion Studies

ULCV sap was prepared using ULCVsymptomatic fresh leaves with phosphate buffer of 0.1 M. β mercapta ethanol (2 µl/ml of buffer) was added to sap to remove the host impurities. A small pinch of ceilite/ carborundum was added to sap to create abrasions. Healthy black gramseeds of different treatments (Table 1 and Fig. 1) were inoculated with ULCV sap for three hours (Fig. 2) to artificially detect the seed transmission.

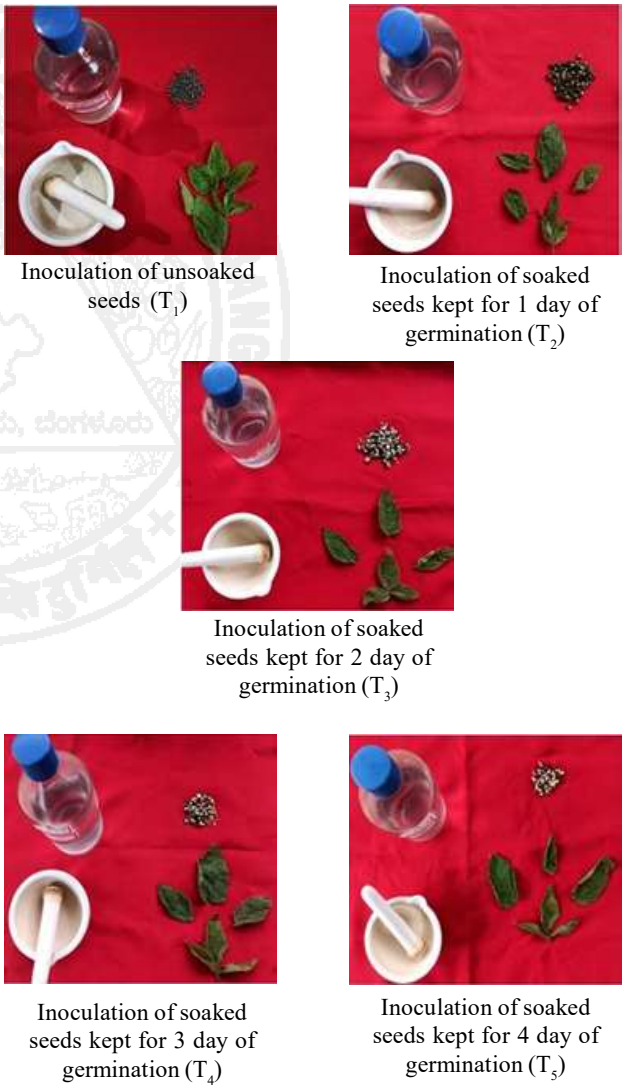
Results and Discussion

Upon sap inoculation at two-leaf stage, the plants started to show symptoms 25-33 days after sowing. Symptoms included crinkling, puckering, rugosity, twisting of leaves and floral malformation. Fig.3

Table 1

Different treatments used for sprout abrasion studies of ULCV in blackgram

T_1	Unsoaked seeds
T_2	Water-soaked seeds and kept for germination for one day
T_3	Water-soaked seeds and kept for germination for two days
T_4	Water-soaked seeds and kept for germination for three days
T_5	Water-soaked seeds and kept for germination for four days



Inoculation of unsoaked seeds (T_1)

Inoculation of soaked seeds kept for 1 day of germination (T_2)

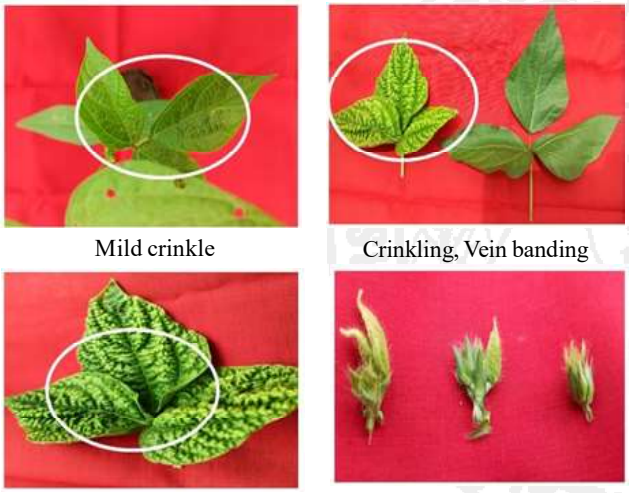
Inoculation of soaked seeds kept for 2 day of germination (T_3)

Inoculation of soaked seeds kept for 3 day of germination (T_4)

Inoculation of soaked seeds kept for 4 day of germination (T_5)

Fig.1 : Different treatments used in sprout seed abrasion studies

Fig 2 : Inoculation of blackgram seeds with ULCV sap for three hours



Mild crinkle | Crinkling, Vein banding

Rugosity and twisting of leaves | Floral malformation

Fig 3: Different types of symptoms recorded in the glasshouse upon sap inoculation

represents the ULCV symptoms recorded in the glasshouse.

Symptoms were observed in the field and glasshouse in the plants sown from the seeds of 15 symptomatic plants. In the field, mild crinkle symptoms were recorded on first trifoliate leaves, while the symptoms were recorded in cotyledonary leaves in the glasshouse (Fig. 4). The incubation period was 8-10 days for symptom expression in the glasshouse, while in the field it was for 15-20 days.This showed that the incubation period for symptom expression is more

Seed transmission in glass house | Seed transmission in field

Fig 4 : Mild crinkle symptoms in the plants raised from seeds of symptomatic plants in the glass house and field

under field conditions compared to controlled conditions.

The per cent disease incidence (PDI) differed among the plants raised (plants sown from the seeds of 15 symptomatic plants) in the field and glasshouse. PDI was in the range between 59.20 to 95.45 in the field while it was 56.00 to 72.91 in glass house plants (Table 2 and Table 3). It was further observed that there was a cent per cent disease incidence in field plants after five to ten days of symptom expression. Sprout seed abrasion studies showed that the symptoms started to express 26 days after sowing the sprouted inoculated seeds. The per cent seed transmission was 71.40 for unsoaked sap treated seeds (T_1) at 26 and 31 DAS (Days After Sowing), while it was 55.50 per cent at 25 DAS and 66.60 per cent at 30 DAS for one day germinated sap treated seeds (T_2). The two days germinated sap treated seeds (T_3) showed 83.30 per cent at 28 DAS and 33 DAS, whilethree days germinated sap treated seeds (T_4) showed 60 per cent transmission at 26 DAS and40 per cent at 31 DAS. The per cent seed transmission for four days of germinated sap treated seeds (T_5) was 50 per cent at 26 DAS and 70 per cent at 31 DAS (Fig. 5).

The maximum per cent seed transmission was recorded in the treatment T_3 (Seeds soaked and kept for germination for two days) (Table 4).This indicated that seed transmission efficiency was seen when seeds were soaked and kept for germination for two days compared to other treatments.

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TABLE 2

Per cent seed transmission from seeds of symptomatic plants under field conditions

Plants raised from the seeds of symptomatic plants (Plant to row progeny)	The total number of plants germinated	Number of plants with symptoms	Number of plants without symptoms	Per cent seed transmission
Plant 1	69	48	21	69.56 %
Plant 2	198	137	61	69.10 %
Plant 3	41	33	8	80.40 %
Plant 4	22	21	1	95.45 %
Plant 5	34	24	10	70.50 %
Plant 6	66	42	24	63.60 %
Plant 7	45	32	13	71.10 %
Plant 8	40	27	13	67.50 %
Plant 9	51	34	17	66.60 %
Plant 10	54	32	22	59.20 %

Average percent seed transmission under field conditions : 71.30

TABLE 3

Percent seed transmission from seeds of symptomatic plants under controlled conditions

Plants raised from the seeds of symptomatic plants (Plant to row progeny)	The total number of plants germinated	Number of plants with symptoms	Number of plants without symptoms	Per cent seed transmission
Plant 11	50	28	22	56.00 %
Plant 12	49	32	17	65.30 %
Plant 13	66	49	17	74.20 %
Plant 14	44	34	10	77.20 %
Plant 15	48	35	13	72.91 %

Average percent seed transmission under field conditions : 69.12

TABLE 4

Effect of sprout seed abrasion on seed transmission of ULCV

Number of days soaked seeds left for germination	Number of plants with symptoms		Number of plants without symptoms		Total number of plants	Per cent seed transmission (%)	
	1st reading	2nd reading (5days after 1st reading)	1st reading	2nd reading (5days after 1st reading)		1st reading	2nd reading (5days after 1st reading)
Zeroday (Unsoaked seeds)	5	5	2	2	7	71.40	71.40
Firstday	5	6	4	3	9	55.50	66.60
Second day	5	5	1	1	6	83.30	83.30
Third day	3	3	2	3	5	60.00	40.00
Fourth day	5	7	5	3	10	50.00	70.00

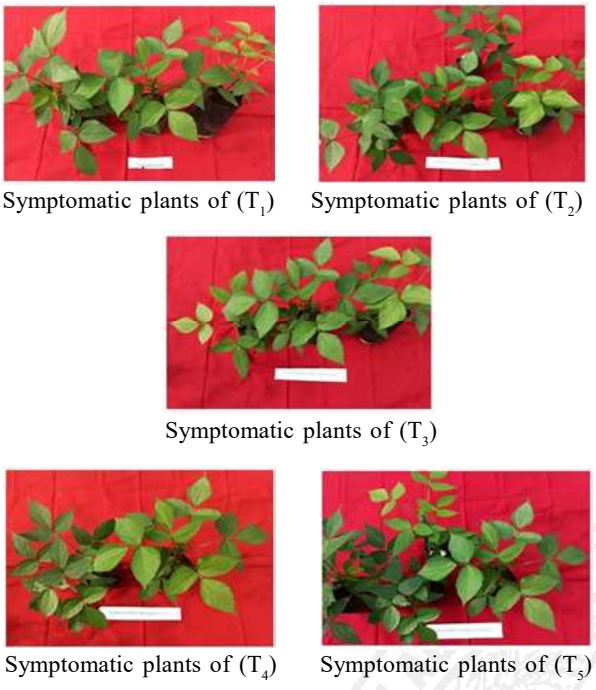

Fig 5 : Plants showing symptoms of ULCV of different treatments subjected to sprout seed abrasion studies

Seed transmission studies help biologically to characterise the pathogen, determine host-pathogen interaction and helps to devise better disease management strategies. The grow-outtest confirmed the seed transmission nature of ULCV both in the field (natural conditions) and in the glasshouse (controlled conditions). On an average, the per cent seed transmission of ULCV was 71.30 under field conditions and 69.12 under controlled conditions. It took more number of days for symptom expression under field conditions (symptoms seen at trifoliate leaves) than in glass house (symptoms seen at a two-leaf stage). Sprout seed abrasion studies revealed that sap inoculated sprouted seeds kept for two days of germination (T_3) showed maximum seed transmission compared to other treatments.

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