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Effect of stage of mechanical inoculation on leaf crinkle disease development in urdbean (*Vigna mungo* L.) under controlled conditions

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Abstract
Vigna mungo (L.) Hepper, (chromosome no. 2n=24) commonly known as urdbean or blackgram, is a leguminous crop and belongs to family *Fabaceae.* Urdbean contribute about 11% of total pulse production in India. It is severely affected by various biotic and abiotic factors. Among biotic factors, urdbean leaf crinkle disease is major production constraint. The disease is characterized by crinkled and leathery leaves and floral malformation of plants. In case of severe infection, it caused yield loss up to 62 to 100%. Plant stage at time of infection is important factor, which determine severity of disease and yield loss. In present study, mechanical inoculation was done at different growth stage of urdbean plant. Lower disease incidence with reduced severity was observed for inoculation at later stage. Interestingly, 100% disease incidence with very high severity was observed when inoculation was done in germinated seed however; disease incidence was reduced to 28% in plants inoculated at first trifoliate stage at 50 days after inoculation. The outcome of this study would help in better understanding the host-pathogen interaction.

Keywords: Crinkle, stage, incidence, urdbean

Introduction
Vigna mungo (L.) Hepper, commonly known as urdbean or blackgram is leguminous crop and belongs to family *Fabaceae.* Its progenitor is *V. mungo* var. *silvestris* which is native to India, where it has been in cultivation from ancient times (Lukoki *et al.*, 1980) [18]. The urdbean plants are annual herbaceous, erect to spreading or trailing types in habit with 30-100cm in height. Leaves are trifoliate, alternate and sparsely hairy on both the surfaces. Urdbean is cultivated in different seasons in India like in northern plains, it is cultivated during spring as a catch crop and in southern and south-eastern states; it is cultivated in rice fallows during *Rabi* season. It required relatively heavier, well drained, moisture retentive, deep loam soils. It is grown as a rainfed crop in the warm plains as well as in the foot-hills and up to an altitude of 2,000m. Urdbean contributes about 11% of total pulse production in India. Despite advancement in agricultural production technology, there is stagnation in urdbean production in country due to various biotic and abiotic factors. Among the diseases, urdbean leaf crinkle disease (ULCD) is the most important constraint depending on variety cultivated and season (Reddy *et al.*, 2005; Sharma *et al.*, 2015) [22, 23]. Disease is characterized by stunting, rugosity and crinkling on affected leaves and production of few pods (Williams *et al.* 1968; Nene 1968) [25, 19]. Bindra (1971) [4] reported that urdbean infected plants showed crinkling, puckering, curling, malformation of leaves and flowers malformation. Khatri (1971) [14] described, reduced leaf size, leaf crinkling, witches brooms and sterility of plants. In case of early infection, complete sterility and malformation of inflorescence with proliferation of flowers was observed (Nene, 1972; Chowdhury and Chowdhury, 1977) [20, 8]. Temperature range of 30-35 ˚C is reported to be most conducive for disease development (Dubey *et al.*, 2019) [10]. The disease is reported to be transmitted through seed (Kolte and Nene 1973, Brar and Rataul 1987; Ahmad *et al.* 1997; Kanimozhi *et al.*, 2009) [15, 6, 1, 13], sap (Nene, 1972; Biwas *et al.*, 2012) [20, 5] and wide range of insect vectors (Khatri *et al.*, 1971; Bindra, 1971; Sharma, 2015) [14, 4, 23], but not transmitted through soil (Nene, 1972; Beniwal *et al.*, 1983) [20, 3]. However, conclusive experimental proof for its transmission is yet not available. Almost all varieties showed symptoms of ULCD, however, under field conditions, severity may vary from field to field. It was observed that primary inoculum comes from seed and secondary spread may take place through vector under open field conditions (data unpublished). So, plant stage at

secondary infection may play crucial role in the severity of disease. To study the effect of plant stage during infection on disease development, an experiment was conducted in glasshouse conditions at Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi-12.

Materials and Methods

Raising of plants and seed collection

The seeds of urdbean cultivar Barabanki local, a ULCD susceptible variety were collected from Division of Plant Pathology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi and grown in experimental field of Division of Plant Pathology (28.6377° N and 77.1571° E) in 20×25m^2 area to collect the seeds from symptomatic plants. Timely watering and fertilizer application as per standard agronomic package were done for proper growth of plants. The seeds were collected from symptomatic plants sown in insect proof glasshouse conditions. The symptomatic plants grown in glasshouse were used as source of inoculums for mechanical inoculation.

Mechanical inoculation and observation

To study the relation between plant stage during inoculation and symptom development, inoculation was done in germinated seed and plants with different growth stages. 75 germinated seeds were inoculated and maintained under insect proof glasshouse condition and observation was recorded at 20, 35 and 50 days after inoculation (DAI). Further, two sets of 75 seeds were sown in 4 inch plastic pots. In first set, inoculation was done at cotyledonary leaves and in second set; inoculation was done on true leaves (1st trifoliate) (Figure1). Sap transmission of ULCD in greenhouse conditions was done using method described by Biswas *et al.* (2012). The ULCD-infected symptomatic leaves of susceptible local cultivar, Barabanki local were collected from glasshouse and used as source of inoculum. The crude extract was obtained by grinding 1g diseased leaf sample in 5 ml of potassium phosphate buffer (0.05M, pH-7.0) supplemented with β-mercaptoethanol (0.1%) in sterilized mortar and pestle. Seeds from non-symptomatic plants were pre-soaked in water and kept on moist blotting paper in Petri plates for 48 h at room temperature for germination. The seed coat of germinated seeds were removed and mixed with crude extract in beaker and shaken gently, using 1% celite in the crude extract to create injury on the seed surface. Mock inoculation and healthy sap was also done to serve as control. The 3-4 inoculated seeds were sown in 4 inches plastic pots containing fresh potting media (uniform mixture of garden soil and FYM (1:1)). The seedlings were allowed to grow in insect proof glasshouse at 30 °C±2 conducive for crinkle symptom development. For inoculation in cotyledonary leaves and trifoliate leaves, carborundum powder was dusted on leaves and cotton swab dipped in crude sap was gently rubbed. After few minutes leaves were washed with running water. Observation for symptomatic plants was recorded at 20, 35 and 50 DAI. The disease incidence during both the experiments was calculated using the below mentioned formula:

Disease incidence = (Total number of diseased plants/Total number of plants) X 100

Result and Discussion

Sap inoculation of diseased crinkled leaf was done at three developmental stage *viz.*, germinated seed, cotyledonary leaf stage and trifoliate leaf stage. Disease incidence was recorded at 20 DAI, 35 DAI and 50 DAI. It was observed that disease incidence decreased when plants are inoculated at later stage. For seed inoculated plants, 100% disease incidence was recorded, while 50.6% and 28% disease incidence was recorded for cotyledonary leaf and trifoliate leafs, respectively at 50 DAI (Figure 1 and Figure 2). No symptom was observed in mock inoculated and un-inoculated plants. The number of symptomatic plants for different treatment at different time interval is given in table 1. Infectivity of causal agent and symptoms on host depend upon strains, environmental, host cultivar conditions, plant physiological stage and source of inoculum; (Gibson 1991, Le Romancer and Nedellec 1997, Draper *et al.*, 2002, Hamm *et al.*, 2010, Nie *et al.*, 2012, Basky and Alma´si 2005, Cervantes 2008; Shrestha, 2014) [11, 16, 9, 12, 21, 2, 7, 24].

Our study indicates the differential rate of ULCD incidence in urdbean plants mechanically inoculated at different growth stages. Urdbean seeds/plants inoculated at different stages viz. seed stage, cotyledonary leaf stage and trifoliate leaf stage were showing progressive significant reduction in disease incidence as well as disease severity. The increase in resistance of the plants along with progressive growth stages against pathogens in general, and plant viruses in particular is an already well-established fact. Lindblad and Sigvald (2004) [17] conducted field surveys and reported that winter wheat plants became resistant to Wheat dwarf virus infection at the stem elongation. Similarly, wheat plants growing under greenhouse condition were showing susceptible response until growth stage DC 30, however at growth stage DC 31 (first node detectable) and thereafter the resistance of the main shoot increased significantly.

Table 1: Number of inoculated and symptomatic plant at different days after inoculation for seed, cotyledonary leaves and first trifoliate leaves inoculation

Stage of Inoculation	Total number of plants/seeds inoculated	Diseased plants observed		
		20 DAI	35 DAI	50 DAI
Seed	75	36	75	75
Cotyledonary leaf	75	0	26	38
True leaf stage (First trifoliate)	75	0	14	21

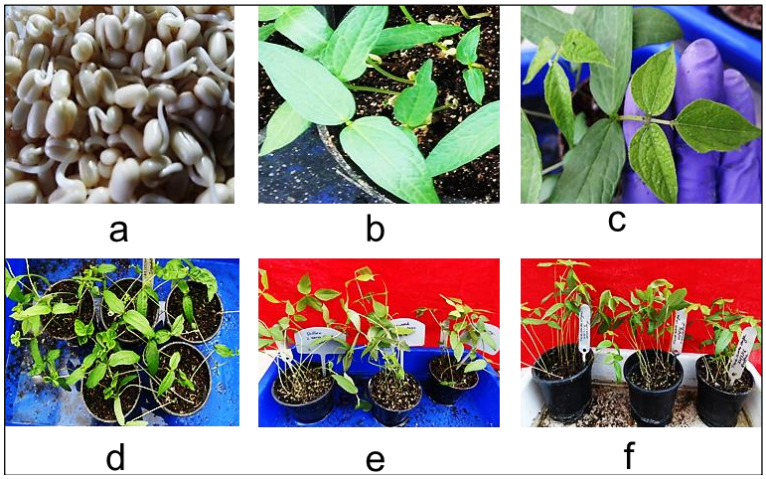

Fig 1: different stage of inoculation, seed **(a)**, cotyledonary leaves **(b)** and trifoliate leaves **(c)** and leaf crinkle symptom at 20 DAI on seed **(d),** cotyledonary leaves **(e)** and trifoliate leaves **(f)** inoculated plants.

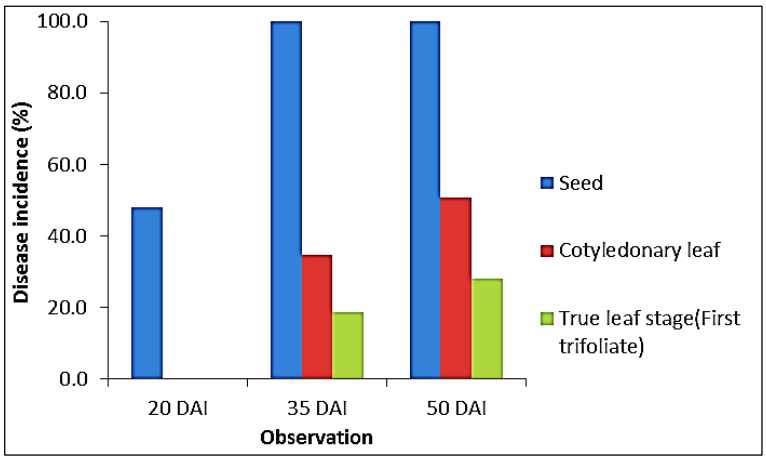

Fig 2: Graph showing disease incidence at different days after inoculation for seed, cotyledonary leaves and first trifoliate leaves inoculation.

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