International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(1): 2694-2697

Received: 01-11-2019
Accepted: 03-12-2019

Abhishek K Dubey
Division of Crop Research, ICAR-Research Complex for Eastern Region, Patna, Bihar, India

Sajad Un Nabi
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Saurabh K Dubey
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Manoj K Yadav
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Pankhuri Singhal
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Saritha RK
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

VK Baranwal
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Corresponding Author:
VK Baranwal
Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi, India

Development of arbitrary scale and host range studies of leaf crinkle disease in five different leguminous crops

Abhishek K Dubey, Sajad Un Nabi, Saurabh K Dubey, Manoj K Yadav, Pankhuri Singhal, Saritha RK and VK Baranwal

DOI: https://doi.org/10.22271/chemi.2020.v8.i1ao.8676

Abstract
Leaf crinkle disease (LCD) is a serious threat to the urdbean cultivation. The disease was first reported in 1968 from Delhi and surrounding areas and has become a serious threat to urdbean production in different states of India. Disease is prevalent in India and Pakistan and causes crop losses ranging from 62-100%. Based on field observation, apart from urdbean, ULCD has been reported from other pulse crops but experimental evidence for host range study in controlled condition is not available. In present study, an attempt was made to study the host range on five different leguminous crops (urdbean, mungbean, cowpea, groundnut and soybean) by mechanical inoculation under controlled conditions. The results confirmed that urdbean is highly susceptible to leaf crinkle disease with 98% disease incidence and higher severity than other leguminous crops tested in the present study. Plant disease assessment is important in predicting yield loss, judging host resistance and for studying fundamental biological host–pathogen interaction. In present study, an arbitrary disease severity rating scale (DSRS) was established based on symptom severity, consisting of five levels: 0:healthy, 1:initiation, 2:low, 3:moderate, 4:high. This study will help in screening of germplasm of urdbean and other pulse crops for resistance against LCD.

Keywords: Host range, scale, severity, crinkle

Introduction
Pulses are important food crops in the developing countries as these are the major and economical source of protein. Other nutrients like carbohydrates, Phosphorus, Riboflavin, Vitamin C and essential amino acids are also major constituents (Singh 2017) [31]. Pulses are grown in more than 170 countries across the world. Total pulse area under cultivation is 83.3 mha with production of 81.8 mt and productivity of 1001.8 kg/ha. The highest area is contributed by India (34%) which contributes 24% of total world production. In India, pulses are grown across the country under varied soil and climatic conditions. *Vigna mungo* (L.) Hepper, commonly called as blackgram is an important pulse crop. It is grown throughout India, but most important growing states are Maharashtra (0.34 mha, 0.164 mt, 483 kg/ha), Andhra Pradesh (0.389 mha, 0.329 mt, 846 kg/ha), Madhya Pradesh (0.847mha, 0.469 mt, 553 kg/ha), Uttar Pradesh (0.587 mha, 0.304 mt, 518 kg/ha), Tamil Nadu (0.358 mha,0.280 mt, 778 kg/ha), Rajasthan (0.261 mha, 0.135 mt, 516 kg/ha), Odisha (0.089 mha, 0.029 mt, 325 kg/ha) and Karnataka (0.092 mha, 0.038 mt, 416 kg/ha) (Annonymous, 2019) [3]. Urdbean contributed about 11% of total pulse production in India. Despite, advancement in agricultural production technology, due to various factors there is stagnation in urdbean production in the country. Crop losses due to emerging plant diseases particularly those of viral origin are of great concern (Anderson *et al*., 2004) [2]. Among diseases, urdbean leaf crinkle disease (ULCD) is the most important depending on variety cultivated and season (Reddy *et al.*, 2005, Sharma *et al*., 2015) [28, 29]. Leaf crinkle disease is characterized by the appearance of extreme crinkling, curling, puckering and rugosity of leaves, stunting and malformation of floral organs (Kolte and Nene 1972) [20]. The disease was first reported in 1960's from Delhi and surrounding areas (Nariani 1960; Williams *et al*., 1968) [22, 35] and has become a serious threat to urdbean production in different states of India, viz., Delhi (Amin *et al*., 1978) [1], Haryana (Singh and Allen 1979) [33], Himachal Pradesh (Gupta, 1974) [15], Punjab (Khatri *et al*., 1971)

[19], Tamil Nadu (Narayansamy, *et al*., 1973) [23], Uttar Pradesh (Nene, 1968) [24] and West Bengal (Chowdhury and Shah, 1985) [11]. Temperature range of 30-35˚C is reported to be most conducive for ULCD symptom development (Dubey *et al*., 2019) [14]. Although, etiological agent for ULCD has been attributed to a virus, but definite association of virus is yet to be established. Various workers reported the association of isometric virus particles with leaf crinkle disease (Bhaktavalsam *et al*., 1983; Dubey *et al*., 1983) [6, 13]. Sharma *et al*. (2014) [30] reported the presence of long filamentous virus particles measuring between 1600 to 2100 nm with a modal length of 1950 nm based on electron microscopic observations of >100 symptomatic samples. Baranwal *et al*. (2015) [4] reported three viruses viz., Cowpea mild mottle virus (CpMMV), Groundnut bud necrosis virus (GBNV) and Soybean yellow mottle mosaic virus (SoYMMV) in ULCD infected urdbean samples from field. Through deep sequencing of ULCD infected urdbean samples, large number of sequences obtained were matching with three earlier reported viruses viz., CpMMV, Mungbean yellow mosaic India virus (MYMIV) and Peanut bud necrosis virus (PBNV) (Iquebal, 2017) [18]. Based on field observation, apart from urdbean (*V. Mungo* L.), ULCD has been reported from other pulse crops viz., *Phaseolus vulgaris* L., *P. aconitifolius* Jacq., *V. radiata* (L.) Wilz. and *V. unguiculata* (L.) Walp. (Kolte and Nene, 1975 [21]; Nene, 1972 [25]; Beniwal *et al*., 1983 [5]; Singh, 1984 [32]) but host range of ULCD in various crops has not been experimentally studied yet. Scale for disease assessment is also not available for leaf crinkle disease. In present study, an attempt has been made to study the host range and development of arbitrary scale for disease assessment for the urdbean leaf crinkle disease.

Material and method

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 20×25 m^2 area to collect the seeds from the 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 seeds of cowpea (Pusa Komal), soybean (PS1347), groundnut (TAG 24), mungbean (Pusa Vishal) and urdbean (Barabanki Local). A total of 100 germinated seeds were inoculated and maintained under insect proof glasshouse condition and observation was recorded at 5days interval up to 45 days after inoculation (DAI). Sap transmission of ULCD in glasshouse conditions was done using method described by Biswas *et al.* (2012) [7]. 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 a control. The 5-10 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. Observation for symptomatic plants was recorded at 45 DAI. The disease incidence during both the experiments was calculated as;

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

Sampling for arbitrary scale development

It is difficult to achieve an exact measure of disease intensity of ULCD, therefore a visual estimation method, based on our own experience of nearly 2 years, a scale was developed. The leaf samples were collected randomly from urdbean fields at two experimental fields (ICAR-IARI, ICAR-NBPGR) and were grouped according to the disease severity. Total 500 leaf samples were evaluated for various grades of disease severity.

Result and discussion

Host range

Leguminous crops taken in our study showed different response towards leaf crinkle disease. The results revealed, that crinkling of leaves only in urdbean and mungbean, however no crinkling was observed in cowpea, soybean and groundnut. Disease incidence was higher in urdbean than that of mungbean. Urbdbean and mungbean were also varying in severity of disease (Figure 1). Severe crinkling was found in urdbean, while low crinkling severity was recorded in mungbean. The presence and absence of crinkling, disease incidence and severity of various leguminous crops are shown in Table 1.

Table 1: Response of mechanical inoculation with ULCD crude sap in five different leguminous crops at 45 DAI

Crop	Leaf crinkling	Incidence (%)	Severity
Urdbean	present	98	severe
Mungbean	present	23	low
Cowpea	absent	0	-
Soybean	absent	0	-
Groundnut	absent	0	-

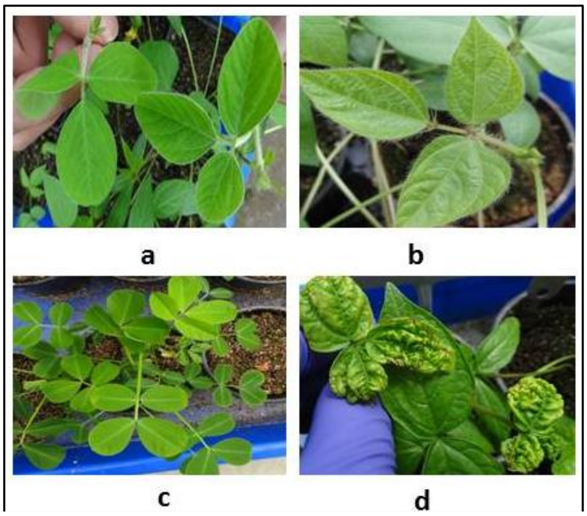
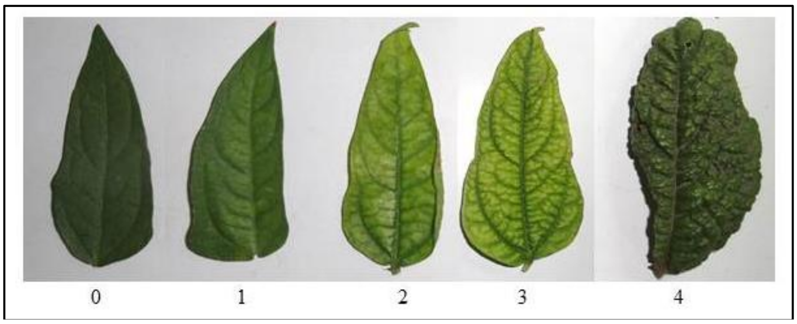


Fig 1: Symptoms observation at 30 DAI in Soybean (a), Mungbean (b), Groundnut (c) and Urdbean (d)

Development of arbitrary scale

The symptoms of the ULCD observed were leaf crinklings, puckering, leatheryness, expanded leaves. Based on our previous experience, an arbitrary disease severity rating scale (DSRS) has been established, consisting of five levels: 0=healthy,1=initiation, 2=low, 3=Moderate, 4=High. In order to achieve a better and more efficient measure of disease intensity, we tried to quantify this visual scale using mathematical criteria as below. The pictorial presentation of various levels of disease severity is shown in figure below

0 = Healthy (no visible symptoms)
1 = Initial (healthy appearance with slight rough texture of leaves).
2 = Low (light curling at margin of leaves, appearance of mild crinkling and initiation of leatheriness)
3 = Moderate (prominent crinkling with thick and leathery texture of leaves).
4 = High (severe crinkling and twisting of leaves with or without complete bud deformation)



The pictorial presentation of various levels of disease severity is shown in figure below

To know the prevalence and extent of damage caused by a disease and develop effective management strategies, disease measurement is necessary. Detection of plant disease and its severity assessment are very important for predicting yield loss, judging host resistance, monitoring and forecasting epidemics and for studying fundamental host–pathogen interaction (Bock and Nutter Jr, 2011) [8]. Cobb (1892) [12] was the first to develop a diagrams based assessment of the severity of wheat rust. Other several early attempts have been made to quantify disease severity based on diagrammatic keys and various rating scales (Horsfall and Barrat, 1945 [17]; Chester, 1950 [10]: Nutter and Esker, 2001 [26]; Bock, *et al*., 2010 [9]). In present study, an arbitrary disease severity rating scale (DSRS) has been established, consisting of five levels: 0 = healthy, 1 = initiation, 2 = low, 3 = moderate, 4 = high. Symptom based scales has been developed for other viral disease like yellow mosaic virus in mungbean and urdbean (Singh, 1992) [34].

Host range, indicates total of host species infected by a pathogen. It is important to understand the epidemiology and pathogenicity of the pathogen. Large host range is very important factor in transmission dynamics of pathogen and also the survival which is considered to be a major factor in pathogen evolution. Host range has been used by the plant virologists as a criterion for attempting to identify and classify viruses even since the beginning of the twentieth century. Plant viruses are generally considered as host generalists and vector specialists (Power & Flecker, 2003) [27]. It is also important in defining viruses and virus strains like other biological criteria such as disease symptoms, cross-protection and methods of transmission etc. Likewise, simply by recording the differences on an extended host range, symptom differences between two virus strains could be emphasized. Study of host range of viruses becomes even more important, when any virus of economic importance which is highly variable in nature such as Alfalfa mosaic virus (Hajimorad and Francki, 1988) [16]. In present study, urdbean was found most susceptible than other leguminous crops against leaf crinkle disease. It indicates that, urdbean cultivation is highly prone to ULCD. However, as the symptomatic sample for mechanical inoculation was collected from urdbean plant, experiment using samples from other symptomatic leguminous crop should also be carried out to study the host range. The outcome of this study would help in better understanding of the disease, its severity level and could be used in better disease management.

References

1. Amin KS, Baladev B, Williams FJ. Transmission of leaf crinkle virus of urdbean by vacuum infusion of extract into water soaked seeds. Curr. Res. 1978; 6:98-99.
2. Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnological drivers. Trends Ecol. Evol. 2004; 19:535-544.
3. Annonymous. Press Imformation Bureau, Minister of Agriculture & Farmers Welfare, Government of India, 2019.
4. Baranwal VK, Jain P, Saritha RK, Jain RK, Gautam NK. Detection and partial characterization of Cowpea mild mottle virus in mungbean and urdbean by deep sequencing and RT-PCR. Crop Protect. 2015; 75:77–79.
5. Beniwal SPS, Bharathan N, Chaubey SN. Two cucurbitous hosts of urdbean leaf crinkle virus. Indian Phytopathology. 1983; 36(3):577-579.
6. Bhaktavatsalam G, Nene YL, Beniwal SPS. Influence of certain physic-chemical factors on the infectivity and stability of urdbeanleaf crinkle diseases. Indian Phytopath. 1983; 36:489-493.
7. Biswas KK, Biswas K, Malathi VG, Chattopadhyay C. Evaluation of urdbean cultivars for identification of resistance to leaf crinkle disease by mechanical sap inoculation. Indian Phytopath. 2012; 65(4):416-417.
8. Bock CH, Nutter FW. Detection and measurement of plant disease symptoms using visible-wavelength photography and image analysis. Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 2011, 6(027).
9. Bock CH, Poole GH, Parker PE, Gottwald TR. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Critical Reviews in Plant Sciences. 2010; 29:59-107.
10. Chester KS. Plant disease losses: their appraisal and interpretation. Plant Disease Reporter Supplement. 1950; 190-198(S193):190-362.
11. Chowdhury AK, Shah NK. Inhibition of urdbean leaf crinkle disease by different plant extracts. Indian Phytopathology. 1985; 38:566-568.
12. Cobb NA. Contribution to an economic knowledge of the Australian rusts (Uredinae). Agricultural Gazette (NSW). 1892; 3:60
13. Dubey GS, Sharma I, Prakash N. Some properties of urdbean crinkle virus. Indian Phytopathology. 1983; 36:762-764.
14. Dubey AK, Sinha P, Baranwal VK, Mishra S, Saritha RK. Temperature influence on leaf crinkle disease expression in urdbean (*Vigna mungo* (L.) Hepper) and potential distribution of the disease in India. Crop Protection. 2019; 120:84-90.
15. Gupta VK. Leaf crinkle, a virus disease of *Phaseolus mungo* L. in Himachal Pradesh. In. J. Exp. Biol. 1974; 12:477-478.
16. Hajimorad MR, Francki RIB. Alfalfa mosaic virus isolates from lucerne in South Australia: biological variability and antigenic similarity. Annals of Applied Biology. 1988; 113(1):45-54.
17. Horsfall JG, Barrat RW. An improved grading system for measuring plant disease. Phytopathology. 1945; 35:655. (Abstract).
18. Iquebal MA. Deep sequencing of RNAs from *Vigna mungo* plants showing crinkle symptoms reveals the multiple virus infection (abstract). Virol Mycol. In: Proceedings of 9th International Virology Congress and Expo, March 13-14, 2017, London, UK. 2017; 6:1.
19. Khatri L, Bhatia DS, Chohan JS. Brief account of the work done on disease of kharif pulse crops at the Dept. of Botany and Pl. Path., PAU, Ludhiana during 1970–71. Fifth All India Pulse Improvement Programme Workshop, Hisar, 1971.
20. Kolte SJ, Nene YL. Studies on the symptoms and mode of transmission of leaf crinkle virus of urdbean (*Phaseolus mungo*). Indian Phytopathol. 1972; 25:401-404.
21. Kolte SJ, Nene YL. Host range and properties of urd bean leaf crinkle virus. Indian Phytopathol. 1975; 28:430-431.
22. Naraini TK. Yellow mosaic of mung (*Phaseolus aureus* L.) Indian Phytopath. 1960; 13:24-29.
23. Narayanasamy P, Jaganathan T. Vector transmission of blackgram leaf crinkle virus. Madras Agric. J. 1973; 60:651-652.
24. Nene YL. A survey of viral disease of pulse crops in Uttar Pradesh First Annual Report, FG-in-358, U.P. Agri. University, 1968, 1-25p.
25. Nene YL. A survey of viral disease of pulse crops in Uttar Pradesh. G.B. Univ. Agric. And Tech. Res. Bull. No 4, 1972, 191p.
26. Nutter Jr FW, Esker PD. Disease assessment keys. In: Maloy OC, Murray TD, editors. Encyclopedia of Plant Pathology. John Wiley and Sons, New York, NY, 2001, 323-6p.
27. Power AG, Flecker AS. Virus specificity in disease systems: are species redundant. The importance of species: perspectives on expendability and triage. Princeton University Press, Princeton, 2003, 330-346.
28. Reddy, Tonapi Ch VA, Varanasiappan S, Navi SS, Jayarajan R. Influence of plant age on infection and symptomatological studies on urdbean leaf crinkle virus in urdbean (*Vigna mungo*). Int. J. Agri. Sci. 2005; 1:1-6.
29. Sharma PN, Sharma A, Singh M. Effect of leaf crinkle disease on yield and quality of urdbean (*Vigna mungo* L. Hepper) in Himachal Pradesh. Himachal Journal of Agricultural Research. 2015; 41(1):80-82.
30. Sharma PN, Sharma A, Sharma OP, Sharma SK, Garg ID. Association of an Unusual Filamentous Virus with Leaf Crinkle Disease of Urdbean in Himachal Pradesh. J Mycol Plant Pathol. 2014; 44(3):257-26.
31. Singh N. Pulses: An overview. J Food Sci Technol. 2017; 54(4):853-857
32. Singh RN. Further studies on leaf crinkle disease of cowpea. Indian Phytopath. 1984; 2:199-200
33. Singh SR, Allen DJ. Cowpea pests and diseases. Manual series No. 2 International Institute of Tropical Agriculture, Ibadan, 1979.
34. Singh G, Sharma YR, Kaur L. Method of rating mungbean yellow mosaic virus in mungbean and urdbean. Plant Disease Research. 1992; 7:1-6.
35. Williams FJ, Grewal JS, Amin KS. Serious disease of pulse crops on India. Pl. Dis. Reptr. 1968; 52:300-304.