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

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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 (0.847 mha, 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) (Anonymous, 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, 39] and has become a serious threat to urdbean production in different states of India, viz., Delhi (Amin et al., 1978) [30], Haryana (Singh and Allen 1979) [33], Himachal Pradesh (Gupta, 1974) [15], Punjab (Khatri et al., 1971)
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             | -        |

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. Urdbean 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.
**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 leatheryness)
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)

![Symptoms observation at 30 DAI in Soybean (a), Mungbean (b), Groundnut (c) and Urdbean (d)](image)

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 [19]). 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.

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