

## EVALUATION OF URDBEAN GERMPLASM FOR RESISTANCE AGAINST URDBEAN LEAF CRINKLE VIRUS

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### Abstract

Urdbean leaf crinkle virus (ULCV), an unclassified virus, seed-borne with narrow host range and probably aphid-transmitted, is relatively a destructive and serious disease of urdbean than other viruses infecting legumes in Pakistan. Urdbean germplasm consisting of 87 genotypes was screened against the virus under field conditions during spring and summer seasons 2005-06. More than 50% of the genotypes were infected during initial period of 3-4 weeks followed by slow down of infection. Eight genotypes viz., Mash-1, 6049-7-1, 9049-1, AARIM-174, AARIM-191, AARIM-220, 4cm-719, and 90cm-056 showed variable response to ULCV in both the seasons probably due to their genetic instability. On the basis of 0-5 disease rating scale and disease severity index, genotypes varied significantly in their reaction against ULCV. Nine genotypes viz., 2cm-703, 90cm-015, 93cm-006, 94cm-019, 99cm-001, IAM 382-1, IAM382-9, IAM382-15 and IAM133 remained free of infection and were classified as "Highly Resistant". Rest of the genotypes were infected, among which 19 were classified as "Resistant", 29 as "Moderately Resistant", 11-19 as "Moderately susceptible 3-6 as "Susceptible and 4-6 were highly susceptible. Some genotypes in spite of high disease pressure were good yielder, indicating a high degree of tolerance to ULCV with desirable quality for breeding.

### Introduction

Mash or urdbean, also called blackgram (*Vigna mungo* (L.) Hepper) is an important pulse crop grown all over the world including Pakistan. It occupies an area of 48.7 thousand hectares, yielding an annual production of 25.1 thousand tonnes of grains with an average grain yield of 515kg/ha (Anon., 2005). The crop is highly susceptible to leaf crinkle disease caused by urdbean leaf crinkle virus (ULCV) than greengram and other pulses (Kadian, 1980, Rishi, 1990). Thus the disease is economically important, destructive, widespread and inflicts heavy losses annually. It was observed that the incidence of ULCV was relatively higher at the Research Stations than at the farmer's fields and this variation may be attributed to low level of seed transmission in farmer's seed stocks and high number of accessions evaluated by the breeders and pathologists at the stations. The disease is characterized by the appearance of extreme crinkling, curling, puckering and rugosity of leaves, stunting of plants and malformation of floral organs. Pollen fertility and pod formation is severely reduced on infected plants (Nene, 1972). The virus has been reported to decrease grain yield from 35 to 81% depending upon genotype and time of infection (Bashir *et al.*, 1991). ULCV is transmitted through sap inoculation, seeds and insects (Nene, 1972; Kadian, 1980). According to Ahmad *et al.*, (1997) ULCV is transmitted through seed at the rate of 2.7 to 46%. Leaf feeding beetle (*Henosepilachna dodecastigma* (Wied), whitefly (*Bemisia tabaci* Glov.) and two aphid species (*Aphis craccivora* and *A. gossypii*) have been reported to be putative vectors of ULCV (Beniwal & Bharathan 1980, Narayansamy & Jaganthan 1973, Dhingra, 1975).

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**Table 1. Disease rating scale (0-5) for ULCV.**

Disease reaction	Disease severity index (DSI)	Reaction
All plants free of symptoms	1	HR
1-10% plants infected showing mild crinkling at the top, pods normal	2	R
11- 20% plants infected showing crinkling and curling of top leaves, pods normal	3	MR
21-30% plants infected with crinkling, puckering, malformation, shortening of pods	4	MS
31-40% plants infected showing all the typical disease symptoms	5	S
More than 40% plants infected showing all the plants with severe symptoms, few pods containing few seeds	6	HS

Use of disease resistant crop varieties is regarded as an economical and durable method for controlling plant diseases, especially those caused by viruses. A good deal of research work has been directed towards screening urdbean germplasm against ULCV, to identify resistant sources under diverse environmental conditions and a number of lines resistant to virus were selected (Iqbal *et al.*, 1991; Bashir *et al.*, 2005). They all suggested continuing screening of available varieties and new germplasm, which constitutes the basis of this work. Therefore, 87 urdbean genotypes of diverse origins were evaluated in this study against ULCV in both spring and summer epiphytotic conditions.

### Materials and Methods

The study was conducted in the Research Area of Department of Plant Pathology, University of Agriculture, Faisalabad (UAF) during spring and summer seasons 2005 and 2006. The planting was done on 25<sup>th</sup> March and 25<sup>th</sup> July, 2005-06. Each test entry was sown in a row of 3 meter in length with 50 cm row-to-row distance and replicated 3 times. One row of a most susceptible check (imported mash) was planted after every two test entries in addition to two rows of susceptible check all round the experiment.

After germination, the plants were regularly examined and the progression of ULCV infection recorded at weekly intervals on visual symptoms. Plants showing clear symptoms such as crinkling, curling, puckering, rugosity and enlargement of the leaves were counted and percent infection was calculated. Disease incidence rating was based on 0-5 arbitrary scale (Table 1) as suggested by Bashir *et al.*, (2004). At maturity, ripened pods were gradually picked up at appropriate times, sun-dried for 10 days and threshed to record yield/plot.

### Results

Wavy appearance on the third trifoliolate leaves followed by crinkling, puckering and rugosity of leaves, shortening of petioles and crowding of leaves were observed on the susceptible check (imported mash) 20 to 25 days after planting. The check lines (spreader) manifested maximum disease and all plants were rated with DSI of "5" at the time of final observation.

It was observed that during spring 2005, 11 out of 87 genotypes i.e., 2cm-703, 90cm-015, 90cm-056, 93cm-006, 94cm-019, 99cm-001, SKG- Local, IAM382-1, IAM382-9, IAM382-15, IAM 133 remained asymptomatic up to maturity and therefore these accessions were considered as Highly Resistant (HR). The remaining 76 genotypes varied greatly in their reaction to ULCV. However 21 genotypes were graded as Resistant with DSI of 2 because their infection level ranged between 1-10%. Thirty five genotypes were classified as moderately resistant and followed by 11 moderately susceptible genotypes (21-30% infection) and three susceptible genotypes with infection level 31-40% and DSI 3 and 4, respectively. Highly susceptible group comprised of 6 genotypes i.e., Mash-97, 6036-22, 6022-22, Faisalabad- Local, 6065-3 and 62027 with maximum infection (>40%) with DSI of 5 (Table 2). Similarly, during summer 2005, 10 genotypes, 2cm-703, 90cm-015, 93cm-006, 94cm-019, 99cm-001, Local-SKG, IAM382-1, IAM382-9, IAM382-15 and IAM 133 did not show any symptoms and they were graded as Highly Resistant (HR) and their disease severity index rated as "0". Twenty and 38 lines were rated as resistant and moderately resistant, respectively. Out of remaining genotypes, 11 were moderately susceptible, 4 were susceptible and 4 were highly susceptible (Table 3).

It was observed that ULCV infection during 2006 increased with the advancement of age. Only 9 genotypes were found to be highly resistant and disease severity index rated as "0". Twenty genotypes were found to be resistant because they exhibited infection between 1-10% and rated as "1". However, 29 genotypes responded moderately resistant. The remaining genotypes were moderately susceptible to highly susceptible (Table 4). The disease reactions during summer 2006 indicted that 9 genotypes were graded as highly resistant and 19 and 34 lines were rated as resistant (1-10% infection) and moderately resistant (11-20% infection) respectively (Table 4), whereas, 16, 6 and 3 genotypes were moderately susceptible, susceptible and highly susceptible reaction to virus infection, respectively (Table 5).

Three genotypes viz. 9049-1, 4cm-719, 96cm-016 which were graded as resistant in spring-2005, turned out to be moderately resistant in summer-2005. Mash-1 and AARIM-191, 6049-7-1, were graded as moderately resistant in spring-2005 but responded as resistant, moderately susceptible, moderately susceptible, respectively, in summer-2005. Similarly during spring-2006, Mash-1, AARIM-191 which were graded as moderately resistant appeared to be resistant and moderately susceptible in summer- 2006. Genotypes 6049-7-1 which was graded as resistant and moderate susceptible in spring and summer-2005, respectively, behaved as moderately susceptible in spring and summer-2006. Varieties viz., 4cm-719 and 90cm-056 showing resistant response in spring 2005 became moderately resistant in other seasons but ranked as resistant in both seasons during 2005. These differences may be attributed to seed-borne nature of the virus which did not influence the level of resistance and susceptibility in other varieties. The response of urdbean genotypes under ULCV infection towards grain yield was quite variable. Some test lines were good yielder in spite of high disease severity. Ten genotypes yielded more than 200 gm/plot, 32 genotypes with 150-200 gm/plot, 23 lines between 100-149 gm/plot and 22 genotypes yielded less than 100 gm/plot.

Table 2. Reaction of urdbean germplasm to natural infection of ULCV during Spring-2005.

S. No.	Reaction grade	No. of genotypes	Disease severity	Genotypes
1.	Highly resistant (0% infection, all plants free of symptoms)	11	0	2cm-703, 90cm-015, 90cm-056, 93cm-006, 94cm-019, 99cm-001, SKG -Local, IAM382-1, IAM382-9, IAM382-15, IAM 133
2.	Resistant (1-10% plants infected )	21	1	3cm-704, 4cm-719, 4cm-721, 3cm-702, AARIM-220, NCH-7-2, NCH-9-3, 96cm-016, AARIM-56, 9049-1, Mash-Sialkot, IAM 5-60, Chakwal Mash-97, Chakwal Mash-2002, 1cm-710, 2cm-710, 2cm-724, 97cm-518, IAM-3, 3cm-705, , 4cm-718, NCH-1-2
3.	Moderately resistant (10-20% plants infected)	35	2	4cm-717, 90cm-048, 99cm-004, AARIM-5, AARIM-132, AARIM-176, NCH-9-7(NCH-7-5), NCH-10-1, NCH-9-2, 95024, Mash-95009, 6039-3, IAM 33-40, 4cm-715, 4cm-716, 4cm-720, 3cm-703, 3cm-706, AARIM-164, AARIM-174, 6036-7, 6039-1, 6049-7-1, NCH-9-5, M-95017, 6036-12, 3cm-708, 95cm-508, 98cm-525, NCH-3-3, NCH-3-4, NCH-9-5, Mash-1, 6036-14, 3cm-707
4.	Moderately susceptible(MS) (20-30% plants infected)	11	3	AARIM-4, AARIM-130, AARIM-136, AARIM-189, AARIM-191, 6065-5, 6036-21, 6049-11, 6026-5, ES-1, 95019,
5.	Susceptible (S) (30-40% plants infected)	3	4	Mash-3, M-17, Mash-88,
6.	Highly susceptible (HS) (>40% plants infected)	6	5	Mash-97, 6022-22, Faisalabad-Local, 6036-22, 6065-3, 62027

Table 3. Reaction of urdbean germplasm to natural infection of ULCV during Summer-2005.

S. No.	Reaction grade	No. of genotypes	Disease severity	Genotypes
1.	Highly resistant (0% infection, all plants free of symptoms)	10	0	2cm-703, 90cm-015, 93cm-006, 94cm-019, 99cm-001, SKG-Local, IAM382-1, IAM382-9, IAM382-15, IAM 133
2.	Resistant (1-10% plants infected)	20	1	Chakwal Mash-97, Chakwal Mash-2002, Mash-1, Mash- Sialkot, SKG- Local, 1cm-710, 2cm-710, 2cm-724, 3cm-702, 3cm-704, 3cm-705, 97cm-518, NCH-1-2, NCH-7-2, NCH-9-3, 4cm-718, AARIM-4, AARIM-56, AARIM-220, IAM 5-60
3.	Moderately resistant (10-20% plants infected)	38	2	3cm-703, 3cm-706, 3cm-707, 3cm-708, 4cm-717, 4cm-720, 4cm-721, 90cm-048, 90cm-056, 95cm-508, 96cm-016, AARIM-5, AARIM-32, AARIM-164, AARIM-174, AARIM-176, 6039-1, 62027, NCH-3-3, NCH-3-4, NCH-9-2, NCH-9-5, NCH-10-1, Mash-95017, Mash-95009, 6065-3, 6036-7, 6036-12, 6036-14, 95024, IAM 33-40, 4cm-716, 4cm-719, 99cm-004, 6026-5, 9049-1, 98cm-525, 4cm-715, NCH-9-7 (NCH-7-5), IAM-3, IAM 33-40
4.	Moderately susceptible (MS) (20-30% plants infected)	11	3	6036-21, 6049-7-1, 6065-5, 6049-11, 95019, AARIM-4, AARIM-130, AARIM-136, AARIM-189, AARIM-191, ES-1
5.	Susceptible (S) (30-40% plants infected)	4	4	6036-22, Mash-3, M-17, Mash-88
6.	Highly susceptible (HS) (>40% plants infected)	4	5	Mash-97, 6022-22, Faisalabad-Local, 6065-3

Table 4. Reaction of urdbean germplasm to natural infection of ULCV during Spring-2006.

S. No.	Reaction grade	No. of genotypes	Disease severity	Genotypes
1.	Highly resistant (0% infection, all plants free of symptoms)	9	0	2cm-703, 90cm-015, 93cm-006, 94cm-019, 99cm-001, IAM382-1, IAM382-9, IAM382-15, IAM 133
2.	Resistant (1-10% plants infected)	20	1	1cm-710, 2cm-724, 3cm-704, 3cm-705, 4cm718, 90cm-048, 90cm-056, 97cm-518, 6036-7, 9049-1, IAM 5-60, Chakwal Mash-97, Chakwal Mash-2002, NCH-1-2, NCH-9-2, NCH-9-3, SKG-Local, Mash-SialKot, IAM-3, IAM-5-60
3.	Moderately resistant (10-20% plants infected)	29	2	2cm-710, 3cm-702, 3cm-703, 3cm-706, 3cm-707, 3cm-708, 4cm-716, 4cm-717, 4cm-719, 4cm-721, 95cm-508, 98cm-525, 99cm-004, AARIM-191, AARIM-32, AARIM-164, Mash-1, Mash-95017, 95019, 95024, 6036-12, 6036-14, 6039-1, 6039-3, IAM 33-40, NCH-3-3, NCH-3-4, NCH-7-2, NCH-9-5,
4.	Moderately susceptible (MS) (20-30% plants infected)	19	3	4cm-715, 4cm-720, 96cm-016, AARIM-4, AARIM-130, AARIM-56, AARIM-136, AARIM-174, AARIM-189, AARIM-220, 6036-21, 6065-5, 6049-11, 6049-7-1, 95009, ES-1, NCH-9-7 (NCH-7-5), NCH-10-1
5.	Susceptible (S) (30-40% plants infected)	6	4	AARIM-5, 6026-5, Mash-3, Mash-88, M-17, Mash-97,
6.	Highly susceptible (HS) (>40% plants infected)	4	5	62027, 6022-22, 6036-22, Faisalabad- Local

Table 5. Reaction of urdbean germplasm to natural infection of ULCV during Summer-2006.

S. No.	Reaction grade	No. of genotypes	Disease severity	Genotypes
1.	Highly resistant (0% infection, all plants free of symptoms)	9	0	2cm-703, 90cm-015, 93cm-006, 94cm-019, 99cm-001, IAM382-1, IAM382-9, IAM382-15, IAM 133
2.	Resistant (1-10% plants infected)	19	1	1cm710, 2cm-710, 2cm-724, 3cm-704, 3cm-705, 4cm-717, 90cm-048, 90cm-056, 97cm-518, AARIM-32, Chakwam Mash-97, Chakwal Mash-2002, Mash-Sialkot, SKG-Local, IAM-3, IAM 5-60, Mash-1, NCH-1-2, NCH-9-3,
3.	Moderately resistant (10-20% plants infected)	34	2	3cm-702, 3cm-703, 3cm-706, 3cm-707, 3cm-708, 4CM-718, 4cm-719, 4cm-720, 4cm-721, 95cm-508, 96cm-016, 98cm-525, 99cm-004, 6039-1, IAM 33-40, 6036-7, 6036-12, 6036-14, 6039-3, 9049-1, Mash-95017, 95019, 95024, AARIM-56, AARIM-164, AARIM-174, AARIM-176, NCH-3-3, NCH-3-4, NCH-7-2 NCH-9-2, NCH-9-5, NCH-10-1, NCH-9-7(NCH-7-5),
4.	Moderately susceptible (MS) (20-30% plants infected)	16	3	4cm-715, 4cm-716, 6026-5, 6036-12, 6036-21, 6049-7-1, 6049-11, 6065-5, Mash-95009, ES-1, AARIM-4, AARIM-130, AARIM-136, AARIM-189, AARIM-191, AARIM-220,
5.	Susceptible (S) (30-40% plants infected)	6	4	Mash-3, M-17, Mash-88, Mash-97, AARIM-5, AARIM-136
6.	Highly susceptible (HS) (>40% plants infected)	3	5	62027, 6022-22, Faisalabad-Local

## Discussion

Urdbean leaf crinkle virus (ULCV) is undoubtedly an important, serious and destructive disease in all the urdbean growing countries of the world including Pakistan (Kadian, 1980; Rishi, 1990; Bashir *et al.*, 1991). As high as 81 % yield losses due to ULCV are on record (Bashir *et al.*, 1991). The epidemiological factors responsible for disease development and spread have not been fully analyzed or appreciated, and heavy losses are continuously occurring which result from early infection, disease severity and poor pod and seed setting. As regards disease resistance and severity index manifestation in urdbean genotypes, variations have been reported in the germplasm screening efforts. Moreover, durability of resistance, definite identity of resistant source(s), or any niches in an ecosystem remain questionable, with the results that serious infections of ULCV continue to be occurring year after year.

In view of ubiquitous nature of this disease, 87 urdbean genotypes were evaluated against ULCV under field conditions for two consecutive years (2005,2006) during two seasons each Spring and Summer. The genotypes were classified into six reaction groups based upon % infected plants and disease severity index (0-5). These were: highly resistant, resistant, moderately resistant, moderately susceptible, Susceptible and highly susceptible. The mean percentage genotypes falling in the categories were: 11.5, 23.5, 36.5, 17.2, 5.2 and 5.7% in the spring 2005-2006 and 10.9, 22.4, 41.4, 15.5, 5.7 and 3.9%, respectively, in summer 2005-2006. The results suggest that urdbean genotypes varied greatly in their reaction to ULCV in both the years, but the differences in infection in each case were not significant, except that disease severity index was slightly higher during spring season. Eleven percent of the genotypes were found to be consistently and highly resistant to ULCV and almost similar number as highly susceptible, and of 78 % genotype population ranged between 15-23%. This distribution indicated that some genetic ratio or mechanism must be involved in conditioning resistance and susceptibility which need to be investigated and analyzed in detail. Highly resistant genotypes as 2cm-703, 90cm-015, 93cm-006, 94cm-019, 99cm-001, IAM382-1, IAM382-9, IAM382-15 and IAM 133 remained asymptomatic in the field, and majority of them showed no or mild reaction under mechanical inoculation. These genotypes need to be maintained for further studies for locating resistance sources under field conditions and artificial inoculation .for genetic manipulations and breeding purpose. One main problem in germplasm evaluation is that some genotypes found resistance at one location turn out to be susceptible at another place (Iqbal *et al.*, 1991, Bashir *et al.*, 2005), therefore environmental genotype interaction should also be studied for durable resistance. This work may be regarded at least only the first step and the candidate and additional genotypes be further evaluated with particular emphases on durability of resistance established by trialing them at multiple sites (Sahay *et al.*, 1999). Till such time, an ideal or desirable cultivar of urdbean, resistant to ULCV, becomes available; the effect of ULCV could be alleviated by growing apparently tolerant and high yielding varieties and combined with use of virus free and certified seed, weed and vector control and manipulation of agronomic practices (Kadian, 1983, Nawaz & Narayanasamy, 1983, Beniwal *et al.*, 1980).



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