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SOURCES OF GENETIC RESISTANCE IN MUNGBEAN AND BLACKGRAM AGAINST URDBEAN LEAF CRINKLE VIRUS (ULCV)

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Abstract

In order to identify sources of genetic resistance in mungbean and blackgram (mash), 32 accessions (16 each of mungbean and blackgram) were evaluated under greenhouse conditions by sap inoculation method. The inoculated plants of each accession were also tested by enzyme-linked immunosorbent assay (ELISA) using polyclonal antiserum to ULCV to separate the resistant plants from susceptible ones. From mungbean, only five genotypes viz., VC-3960 (A-88), VC-3960 (A-89), 98-CMH-016, NM-2 and BRM-195 were found highly resistant to ULCV. These genotypes neither expressed disease symptoms nor virus was detected by ELISA. However, in case of 98MG-003 genotype, 10% plants were observed with mild symptoms of the leaf crinkle disease, and virus was also detected by ELISA at low titer. In case of blackgram (mash) only one genotype (VH-9440039-3) was found highly resistant and one ES-1 resistant to ULCV. The others were moderately susceptible to highly susceptible. In this study we report new genetic sources of resistance in mungbean and blackgram to ULCV which are available for breeding program to develop virus-resistant cultivars for commercial cultivation.

Introduction

Urdbean leaf crinkle disease caused by urdbean leaf crinkle virus (ULCV) is an important disease of mungbean (*Vigna mungo* (L.) Wilczek) and blackgram (*Vigna mungo* Hepper) in Pakistan (Bashir & Malik, 1988). Under field conditions ULCV is more serious in blackgram (mash) than mungbean (Bahir & Zubair, 1985). The symptoms of the disease appear in form 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 ULCV has been reported to decrease grain yield in blackgram from 35 to 81% depending upon host genotype and time of infection (Bashir *et al*., 1991). ULCV is transmitted through sap inoculation, grafting and seeds (Ahmad *et al.*, 1997, Kolte & Nene, 1972). Leaf feeding beetle (*Henosepilachna dodecastigma (*Wied) (Beniwal & Bharathan, 1980), whitefly (Narayansamy and Jaganthan, 1973), and two aphid species (Dhingra, 1975) have been reported as insect vectors of ULCV.

For the control of ULCV, although a number of approaches may be useful, but host plant resistance is the ideal and cheapest way to control this viral disease. In order to identify genetic sources of resistance in mungbean and blackgram against urdbean leaf crinkle virus (ULCV), this study was conducted under greenhouse conditions at National Agricultural Research Centre (NARC), Islamabad during 2002 using a seed-borne isolate of ULCV.

Material and Methods

Virus isolate: An isolate of ULCV was obtained from infected seeds of blackgram (mash), which have been preserved in refrigerator at 4^{o}C collected from previous year. The ULCV isolate was maintained in the greenhouse on mash plants (cv. Mash-3) by frequent sap inoculation throughout the season. The virus-infected leaves were used for sap inoculation to test the mungbean and blackgram lines/advanced genotypes under greenhouse conditions.

Screening procedure: Sixteen mungbean and 16 of blackgram (mash) accessins/ advanced lines were evaluated under greenhouse conditions at Plant Genetic Resources Institute, National Agricultural Research Centre (NARC), Islamabad during summer season of 2002. Twenty four seeds of each mungbean and blackgram genotypes were planted in earthen pots (12 seed per pot) filled with sterilized soil, and later on 10 plants/pot were maintained in each pot of each test entry for mechanical inoculation. The planting was carried out in the first week of July, 2002. Two weeks after planting, when the primary leaves of the seedlings were fully expanded, they were mechanically inoculated with virus-infected sap. The leaves were dusted with carborundum powder (600 mesh) by an atomizer. The virus-infected leaves were harvested one day before inoculation and kept in refrigerator. Next day the ULCV-inoculum was prepared by grinding infected leaves in mortar with pestle in 0.05M Potashium Phosphate buffer (I g infected leave tuissue/20 ml inoculation buffer). The inoculum was applied on leaflets of the test seedlings of each genotype with fore-fingure of right hand by rubbing the leaflets of the seedlings with virus-infected sap. Ten plants of a susceptible mash variety (Mash-3) were also inoculated with the same inoculum to monitor the effectiveness of the inoculum. Ten plants were water-inoculated (no virus-infected sap) to keep as control. Just after inoculation, the plants were rinsed with tap water. Four weeks after inoculation, all the test lines were re-inoculated to ensure virus infection and to avoid any escape. The inoculated plants were kept for three months observations under insect-free greenhouse conditions to express crinkle disease symptoms. Observation on expression of virus symptoms were recorded after every 15 days by following 0-5 scale, 0: No visible symptoms and no virus detection by ELISA (Highly Resistant-HR); 1: Very mild symptoms and virus detection by ELISA with low titer, less than 15% infected plants (Resistant-R); 2: Moderately Resistant (MR), less than 25% infected plants; 3: Moderately Susceptible (MS), less than 50% infected plants; 4: Susceptible (S), less than 65% infected plants; 5: Highly susceptible (HS), very severe symptoms, 100% infected plants. The leaf samples collected from inoculated plants were tested by Direct Antigen Coating Enzyme-linked Immunosorbent Assay (DAC-ELISA) as described by (Hobbs *et al*., 1985) using polyclonal antiserum to ULCV to separate the virus infected from non-infected plants.

Results and Discussion

The ULCV transmission was successful by sap-inoculation method. The time between inoculation and appearance of the symptoms varied with mungean and blackgram genotypes and it took 12 to 20 days. Plants in the same pots showed symptoms on different dates. It was observed that mild leaf crinkling, downward leaf curling and

mosaic appeared three weeks after sap-inoculation on the second and third trifoliate leaves. Clear leaf crinkle symptoms were observed at fourth trifoliate stage. The infected plants remained stunted with malformed upper parts and bushy in appearance. The variation in symptoms expression among different genotypes and among plants of the same genotype might be due to variability in plant growth or genetic variation or some unknown reasons. Such observation in case of sap-inoculation of ULCV on blackgram have been reported by Nene (1972) and by Bashir & Hampton (1996) in case of cowpea evaluation against cowpea aphid-borne mosaic virus (CABMV).

The ULCV-disease reactions of each mungbean and blackgram genotype and DAC-ELISA results are given in Table 1. Five mungbean genotypes; VC-3960 (A-89), NCM-209, 98-CMH-016, NM-2 and BRM-195 were found free of disease symptoms. These five genotypes neither expressed disease symptoms nor virus was detected by ELISA and were rated as highly resistant (HR) to ULCV on 0-5 scoring scale. In case of 98 CMG-003 mungbean genotype, 10% plants showed mild disease symptoms and virus was also detected in low titer when tested by ELISA. All the other mungbean genotypes were rated as moderately susceptible (MS) to highly susceptible (HS). Virus was detected in low to high titer when tested by ELISA.

In case of blackgram, only one genotype viz., VH 9440039-3 was found as highly resistant (HR) to ULCV. This genotype was completely free of disease symptoms and no virus was detected by ELISA. One genotype; ES-1 was rated as resistant (R) as 25% plants of this genotype expressed disease symptoms, whereas the other genotypes proved to be moderately susceptible (MS) to highly susceptible (HS) with severe disease symptoms indicating susceptibility to ULCV.

Although some blackgram genotypes such as AARI M-13, AARI- M-26, AARI M-27, AARI M-196 and AARI M-202 have been reported to possess moderately field resistance to ULCV when tested under natural infection conditions (Haq, 1991), but these genotypes have not been tested by sap-inoculation method. Bashir & Zubair (2002) reported 26 blackgram breeding lines/cultivars as highly resistant and 59 as resistant respectively when they screened 132 lines under field conditions. However, the resistance of these lines was not confirmed by sap inoculation method. Under field conditions there might be disease escape, but in this study we have reported genetic resistance in mungbean and blackgram when ULCV was challenged by sap-inoculation method. This seems to be the first report of resistance in mungbean and balckgram when tested by sap-inoculation. All previous reports in Pakistan regarding blackgram screening against ULCV are under natural infection conditions. Iqbal *et al*., (1991) evaluated 19 blackgram genotypes under field conditions and reported four genotypes viz., S-210, NM 5-60, S-250 and Mash Sialkot as resistant to ULCV. But these genotypes were not tested by sap inoculation and no ELISA was performed to know latent infection of ULCV.

In Pakistan, there is no report of mungbean screening against ULCV either by sap-inoculation method or under natural infection conditions. In this study we have reported five mungbean genotypes viz., VC-3960 (A-89), NCM-209, 98-CMH-016, NM-2 and BRM-195 as highly resistant (HR) to ULCV when tested by sap-inoculation. However, in India, Kadian (1982) reported nine mungbean genotypes viz., 15176, 15225, 15227, 15229, 15227, L-24-2, ML-5, T-44, and T-51 as resistant to ULCV when they evaluated 390 mungbean germplasm/varieties by sap-inoculation method. Nene & Kolte (1972) identified five mungbean cultivars viz., 24-3, Baisakhi, T-2, T-44 and T-51 as resistant to ULCV by following sap inoculation method. Since ULCV is seed-borne, the initial source of infection under field conditions comes from seed (Beniwal *et al*., 1980). The

Table 1. Reaction expressed by mungbean and blackgram genotypes/advanced lines after sap inoculation with urdbean leaf crinkle virus (ULCV) under greenhouse conditions.

No	Accessions No. (MUNG)	No. of Plants inoculated	No. of Plants infected	% infection	ELISA Results	Reaction Group	S. No	Accessions No. (MASH)	No. of Plants inoculated	No. of Plants infected	% infection	ELISA Results	Reaction Group
1	VC-3960 (A-88)	20	8	40	++	MS	1	98CM-522	20	20	100	+++	HS
2	VC-3960 (A-89)	20	0	0	-	HR	2	98CM-525	20	20	100	+++	HS
3	NM-1	20	11	55	++	HS	3	Mash-97	20	17	85	+++	HS
4	M-6	20	9	45	++	MS	4	Mash-3	20	20	100	+++	HS
5	98 CMG-003	20	2	10	+	R	5	VH-9440039-3	20	0	0	-	HR
6	98 CMG-018	20	10	50	++	HS	6	ES-1	20	5	25	+	R
7	C1/94-4-19	20	9	45	+	MS	7	98CM-524	20	20	100	+++	HS
8	LIPS/589	20	11	55	+	HS	8	98CM-523	20	20	100	+++	HS
9	Swat Mung	20	10	50	+	HS	9	VH-9440034-8	20	7	35	++	MS
10	BRM-188	20	13	65	+	HS	10	VH-940034-9	20	9	45	++	MS
11	NCM-209	20	0	0	-	HR	11	VH-9440039-1	20	20	100	+++	HS
12	98 CMG-016	20	0	0	-	HR	12	VH-9440039-2	20	18	90	+	HS
13	C2/94-2-42	20	8	40	++	MS	13	VH-9440034-6	20	19	95	+++	HS
14	MN-2	20	0	0	-	HR	14	VH-9440034-1	20	18	90	+++	HS
15	BRM-195	20	0	0	-	HR	15	VH-940034-2	20	20	100	+++	HS
16	BRM-202	20	7	35	++	MS	16	VH-9440034-7	20	20	100	+++	HS

ELISA Results:- No virus detection; + virus detected with low titer, ++: virus detected with medium titer, +++ ; virus detected with high titer VH: Vigna Hybrid

virus infection at an early stage of the plants is known to cause heavy losses (Bashir *et al*., 1991), therefore use of virus-free seed and development of virus-resistant mungbean and blackgram cultivars using the present source of resistance would help to control the disease and its further spread in new localities.

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