

OCCURRENCE AND DISTRIBUTION OF VIRAL DISEASES OF MUNGBEAN AND MASHBEAN IN PUNJAB, PAKISTAN

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Abstract

In order to assess the occurrence, distribution and importance of viral diseases of mungbean and mashbean crops in Punjab, Pakistan, an extensive survey was conducted during summer season of 2004. The symptomatic plant samples were collected from farmer's fields and experimental plots at research stations and tested by Enzyme-linked Immunosorbent Assay (ELISA) to detect and identify viruses infecting these two crops. Based on field observations, the incidence of mungbean yellow mosaic virus (MYMV) ranged from 4 to 40% in mungbean depending upon crop variety and location. In case of mashbean, the MYMV incidence was from 5 to 100%. MYMV was observed as a major disease of both these crops distributed in all the districts surveyed with significant importance. Urdbean leaf crinkle virus (ULCV) was the second important viral disease with incidence of 5 to 28%, but was more serious in mashbean than mungbean.

Out of 540 symptomatic plant samples collected from commercial plots of mungbean and mashbean and tested by ELISA, only 213 (39%) samples reacted positively with one or more antisera. Based on ELISA results, only five viruses such as MYMV, ULCV, cucumber mosaic virus (CMV), bean yellow mosaic virus (BYMV) and alfalfa mosaic virus (AMV) were detected and identified. AMV was prevalent only at research stations, but not at farmer's fields. The incidence of CMV, BYMV and AMV was recorded as 6 to 20%. The natural infection of CMV, BYMV and AMV on mungbean seems to be first record in Pakistan. Widespread infection of viral diseases in commercial crops and experimental stations is of great concern, especially where individual virus incidence is high and two or more viruses are present. Incorporation of viral disease resistance in the present cultivars is the only practical way to control viral diseases.

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is grown at about 200 thousand hectares in Pakistan (Anon., 2001) under barani as well as irrigated conditions. About 80-85% of the total mungbean area is located in Mianwali, Bhakkar and Layyah districts of Punjab. Whereas, mashbean (*Vigna mungo* (L.) Hepper) also called as urdbean or blackgram is grown under rainfed conditions mainly in Sialkot, Narowal and Chakwal districts. The viral diseases of mungbean and mashbean are common. Some viruses may cause significant economic losses (Bashir *et al.*, 1991; Aftab *et al.*, 1993; Malik, 1991), whereas, the others are of minor importance. Under field conditions, the symptoms due to viral diseases may be confusing due to multiple infections by different viruses or nutritional deficiency in the same plant. The viruses of mungbean and urdbean need further critical study to identify their properties, distinguishing features, and distribution. Therefore, isolation and maintenance of virus cultures is important to study their properties.

During August, 2004, an extensive survey of mungbean and urdbean (mash) crops was conducted in districts of Minawali, Bhakkar, Layyah, Chakwal, Sialkot, Narowal and Shakargarh with the objective to assess the overall crop condition and to collect

information on the occurrence, incidence and distribution of viral diseases. During survey, the mungbean and mashbean plants showing virus-like symptoms (symptomatic) were collected and brought to National Agricultural Research Centre (NARC), Islamabad to detect and identify infecting viruses.

Materials and Methods

Survey methodology: During the last two weeks of August, 2004 when mungbean and urdbean crops were at flowering stage in Punjab, an extensive survey was conducted to assess the viral disease incidence, distribution and economic importance. During survey, the farmer's fields were visited along the road sides keeping a distance of 3 to 6 Km among sites depending upon the availability of mungbean and urdbean plots. In order to know the overall crop situation and problems, the farmers were also interviewed. A total of 87 mungbean and urdbean farmer's fields were selected and visited to collect symptomatic plants samples (plants showing virus-like symptoms). Around 5 to 10 plants from each field (depending upon availability of infected plants) showing virus-like symptoms were collected. A total of 540 samples showing virus-like symptoms were collected. In addition to farmer's fields, 43 experimental plots of mungbean and urdbean at research stations such as Barani Agricultural Research Institute (BARI), Chakwal, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Ayub Agricultural Research Institute (AARI), Faisalabad, University of Agriculture (UAF), Faisalabad, Pulses Research Sub-station (PRSS), Sahowali (Sialkot), Adaptive Research Farm (ARF-KN), Kot Nainan (Narowal) and Adaptive Research Farm (ARF-KR), Karor (Layyah), were also visited and 225 symptomatic plant samples from experimental plots were collected for laboratory tests.

The samples were placed in labelled polythene bags, placed on ice in cooler boxes and brought to National Agricultural Research Centre (NARC), Islamabad for testing against different antisera of legume viruses by Direct Antigen Coating Enzyme-linked Immunosorbent Assay (DAC-ELISA). Some samples showing bright yellowing and suspected to be infected with mungbean yellow mosaic virus (MYMV) were sent to National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad to confirm the identity of MYMV by Polymerase Chain Reaction (PCR) (Innes *et al.*, 1990). Disease incidence under field conditions was recorded by determining the average percentage of symptoms-affected plants at different location in each field in per square meter area (Latham & Jones, 2001).

Laboratory tests: A total of 540 symptomatic samples (Table 1) of mungbean and urdbean collected from farmer's fields in six districts (Chakwal, Bhakkar, Mianwali, Layyah, Sialkot and Narowal) were tested by Direct Antigen Enzyme-linked Immunosorbent Assay (DAC-ELISA) as described by Hobbs *et al.*, (1987). Two hundred and twenty five samples (Table 2) were collected from seven research stations and tested by DAC-ELISA for detection and identification of viruses infecting mungbean and urdbean (mash). All these samples were tested by DAC-ELISA using nine polyclonal antisera to legume viruses such as blackeye cowpea mosaic virus (BICMV), cowpea aphid-borne mosaic virus (CABMV), cucumber mosaic virus (CMV), bean yellow mosaic virus (BYMV), cowpea severe mosaic virus (CSMV), southern bean mosaic virus (SBMV), bean common mosaic virus (BCMV), urdbean leaf crinkle virus (ULCV) and

alfalfa mosaic virus (AMV). In case of MYMV, Triple Antibody Sandwich ELISA (TAS-ELISA) was applied using polyclonal antibody to African Cassava Mosaic Virus (ACMV) according to the procedure as described by Harrisin *et al.*, (1997). When ELISA was used, leaves were always tested in groups of 10 to determine virus incidence. When incidence was high, samples were re-pooled at appropriate lower levels for retesting or retested singly. Some of the samples which were positive for specific viruses with single infection in ELISA have been desiccated and preserved at -20°C for future reference.

Results

a. District-wise crop condition

Mianwali: In district Mianwali, the mungbean commercial plots in Kundian, Piplan, Hasanwala and Hafizwala areas were visited. Mungbean is the major kharif crop of this area. In general, the overall crop condition was good. Almost every field was observed with some plants showing symptoms of mungbean yellow mosaic virus (MYMV) with incidence of 3 to 40% depending upon crop variety and improved seed sown. The fields, where seed of improved varieties was not used (the seed source was local market) were observed with high incidence (more than 80%) of MYMV. The incidence of ULCV was recorded as 5-28%. The overall viral disease incidence ranged from 2-52% depending upon the crop variety and management practices followed. Mixed infection of both these viruses was also observed. The symptoms of other viruses were not clear at field level.

Bhakkar: In Bhakkar district, reverine area (kaccha) was visited in detail. Other areas visited include; Kalurkot, Darya Khan, Noutak, Sarai Muhajar and Khansar. Mungbean is major kharif crop in district Bhakkar. In general, the crop condition in the entire district was good. The MYMV incidence was from 5 to 25%. In some plots, a few plants were observed with severe upward leaf curling. These plots were near cotton fields. Cotton leaf curl virus (CLCuV) was detected from these plants when tested by Polymerase Chain Reaction (PCR). The incidence of ULCV was from 2 to 8%. Urdbean crop was very rare in this area. A few plots of urdbean crop were observed with 100% incidence of MYMV. According to the farmer's statement, imported seed of urdbean which was highly susceptible to MYMV was planted in this field.

Layyah: The areas visited in Layyah district include; Samtia, Karore, Duratta, Lalazar, Chowk Azam and Fatehpur. Mungbean is the major kharif crop of this district. Only two viruses MYMV and ULCV were observed in traces with low incidence (2 to 5%). Due to insecticidal sprays, whitefly population was also low. In general, the crop condition was good in this area due to good management practices adopted. No urdbean crop was observed in this area.

Chakwal: The area from Mandhra to Chakwal, and Chakwal to Talagang was surveyed. In this area urdbean crop was more common than mungbean. In urdbean, MYMV was a common disease with 6 to 40% disease incidence. In some fields high incidence of MYMV (70%) was observed. The seed source in these field was local market (susceptible cultivars). Similarly, ULCV was also observed with incidence of 5 to 11%. However, the incidence of ULCV was very high (35%) in experimental plots at Barani Agricultural Research Institute (BARI), Chakwal in some cultivars.

Sialkot: Sialkot and Narowal are the major mashbean growing areas of Punjab. Only few fields of mungbean were observed with 10 to 25% MYMV incidence. The incidence of MYMV in urdbean was (5-75%) in this area. The main reason is that most of the farmers plant local urdbean cultivars susceptible to MYMV. The farmers are not aware of the improved variety seed. In addition to MYMV, plants with symptoms of ULCV were also observed, but the disease incidence ranged from 3 to 19%. The overall crop condition was not good due to high incidence of MYMV and ULCV at farmer's fields. The symptoms of other viruses in commercial crop were not clear.

Narowal: Tehsils Shakargarh, Zafarwal and Narowal are the main areas where mashbean crop is grown. Only a few fields of mungbean were observed in this area. MYMV was the major disease with high incidence (70%) in urdbean crop. Local susceptible urdbean variety is commonly cultivated in this area. Whitefly population was also high. The incidence of ULCV at farmer's fields was low (3-7%). However, at research stations such as Pulses Research Sub-station, Sahowali (Sialkot) and Adaptive Research Farm, Kot Nainan (Shakargarh), the incidence was high (15-30%) depending upon crop genotypes. The introduction of improved varieties of mash is lacking in this area. Plant population was poor and the overall crop condition was not good. In some areas, the crop was also badly damaged due to high rainfall and standing water in commercial plots.

b. Symptomology and viral disease incidence

Variable virus-like symptoms were observed under field conditions depending upon virus and crop variety. The affected plants were showing yellow specks along the veinlets and spreading over the lamina, the pods became thin and curled upward. In case of susceptible cultivars the whole leaves became yellow. The next trifoliate leaf emerging from the growing apex was showing irregular yellow and green patches alternating each other. The leaf size was not much affected. The plants showing such symptoms were rated as MYMV-infected plants (Fig. 1). The other type of symptoms were leaf rugosity, crinkling and distortion. The leaf size was increased and more green than normal colour (Fig. 2). This was rated as ULCV. In a few fields, severe upward leaf curling was observed (Fig. 3). Such fields were observed near cotton fields and on testing, cotton leaf curl virus (CLCuV) was detected from these samples. But the incidence of CLCuV was very low (less than 2%) and was observed in a few plots only in Kaccha area of tehsil Bhakkar.

In experimental plots at University of Agriculture, Faisalabad (UAF) and Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad some mungbean plants were found with mottling of leaves, mild to severe yellowing, stunting and necrosis of terminal parts (Fig. 4). The incidence of this viral disease at UAF was high (11-32%). On testing, bean yellow mosaic virus (BYMV) mixed with MYMV was detected in these plants. However, a few plants were also found with single infection of BYMV. The incidence of BYMV at NIAB was from 5 to 13%. The disease incidence of MYMV and ULCV was higher at research stations than in commercial plots. The incidence of BYMV in the commercial field was very low (less than 1%).



Fig. 1. Typical and severe symptoms of mungbean yellow mosaic virus (MYMV) with leaf necrosis in urdbean.



Fig. 2. A mungbean plant showing severe upward leaf curling infected with cotton leaf curl virus (CLCuV).



Fig. 3. Typical symptoms of urdbean leaf crinkle virus (ULCV) in urdbean (mash) under field conditions.



Fig. 4. A mungbean plant showing symptoms of bean mosaic virus (BYMV) in experimental plots.

c. ELISA results and detection of viruses

Out of 540 samples of mungbean and mashbean when tested by indirect ELISA (DAC-ELISA) against nine polyclonal antisera to legume viruses, only 213 (39%) samples reacted positively with one or more antisera (Table 1). Majority of the samples 336 (63%) which were collected on the basis of virus-like symptoms did not react with any antiserum used in ELISA. Only four viruses; MYMV, ULCV, CMV and BYMV were detected from the samples collected from commercial plots. AMV was not detected in samples collected from farmer's fields. Three hundred and thirty six symptomatic samples (63%) did not react with any antiserum. Based on ELISA results maximum disease incidence (15 to 36%) was found in case of MYMV followed by ULCV (6 to 26%). Whereas, the incidence of CMV (6 to 12%) and BYMV (6 to 12%) was low as compared to MYMV and ULCV. We could not detect CABMV, BICMV, CSMV, BCMV and SBMV in any sample.

A total of 225 samples of mungbean and urdbean were collected from 43 experimental plots at different research stations. Out of these only 105 (46%) samples reacted positively with one or more antisera to legume viruses (Table 2). Five viruses (AMV, BYMV, CMV, MYMV and ULCV) were detected from samples collected from research stations. AMV was detected only from mungbean and not from urdbean samples. Based on ELISA results, maximum incidence was found in case of MYMV (20

to 46%) followed by ULCV (20-33%). AMV, BYMV and CMV were detected with low incidence (2-6%). Majority of the samples (55%) did not react with any antisera tested. It was interesting that AMV was detected only from mungbean samples. These samples were collected from BARI-Chalwal, AARI-Faisalabad, and University of Agriculture, Faisalabad. This virus was not detected from any sample collected from farmer's fields. Based on ELISA results, the incidence of BYMV was 5 to 20%. In addition to single virus infection, mixed infection of one or two viruses was also detected from samples collected from farmer's fields or research stations. The mixed infection of MYMV and ULCV was very common. However, in experimental plots of Plant Breeding and Genetics, University of Agriculture, Faisalabad, the mixed infection of MYMV, ULCV and BYMV was detected. The identity of MYMV was confirmed either by TAS-ELISA or PCR conducted at NIBGE, Faisalabad, whereas, the identity of other viruses was confirmed by ELISA and mechanical transmission.

Discussion

Because of the damaging impact of viral diseases on yield and seed quality of infected crop legumes (Bos *et al.*, 1988), the extent of virus infection revealed by our surveys of commercial crops and experimental plots of mungbean and urdbean, is cause of great concern. This also warrants a reappraisal of the actual and potential importance currently assigned to the viral diseases of mungbean and urdbean. The widespread infection of MYMV and ULCV in mungbean and urdbean growing areas of Punjab, Pakistan provides a measure of economic importance they have already attained. Moreover, the occurrence of three new seed-borne viruses of mungbean (AMV, CMV, BYMV) reported in experimental plots at research stations of Punjab provides an indication of potential threat for commercial crops in the future. The infected experimental plots become source of infection in the vicinity of commercial plots both during growing season and through sowing of infected seed in subsequent years (Bos, 1992; Jones & McKirdy, 1990). Our survey data also illustrate the risks associated with legume crops and new crop genotypes in introducing viral diseases, increasing virus incidence and may cause economic losses in legume crops (Bos, 1992, 1996).

Mungbean is reported to be naturally infected by more than eight viruses under field conditions (Nene, 1973, Kaiser *et al.*, 1971). The viruses which may infect mungbean or urdbean crops are MYMV (Malik, 1991), blackgram mottle virus (BMoV) (Saleh *et al.*, 1986), ULCV (Bashir *et al.*, 1991), BCMV (Kaiser & Mossahebi, 1974), AMV (Kaiser, 1979), CMV (Kaiser *et al.*, 1971) and leaf curl virus (Nene, 1973). In Pakistan, MYMV has already been reported to be a major viral disease of mungbean and urdbean and causes heavy losses (Bashir & Zubair, 1985; Malik, 1991). In this survey, we found that MYMV was widely distributed with low to high incidence at farmer's fields in all the mungbean and urdbean growing areas of Punjab. Recently, due to introduction of MYMV-resistant cultivars of mungbean such as NM-92, NM-98 and Chakwal-97, the incidence of MYMV is low in major mungbean growing districts (Bhakkar, Layyah and Mianwali) of Punjab. In this survey, we also observed that the incidence of MYMV was low (5-10%) in areas where improved mungbean varieties (NM-92, NM-98, Chakwal-97) were grown. However, it was high (30 to 70%) only in fields where local land races were grown.

In case of urdbean, although four improved varieties (NARC-1, NARC-2, NARC-3, Chakwal-Mash-2002) resistant to MYMV have been developed, but due to non-availability of certified seed of these cultivars, the incidence of MYMV in urdbean crop is still high in mash growing areas of Sialkot, Narowal, Chakwal. MYMV was observed as the most economically important disease of both the crops, but it was more serious in urdbean than mungbean. Previously, similar observations have been reported (Bashir & Zubair, 1985).

Next to mungbean yellow mosaic (MYMV), leaf crinkle disease caused by ULCV was the second important disease commonly occurring on these two crops, but ULCV was more serious in urdbean than mungbean. It was observed that the incidence of ULCV was high at Research Stations than at farmer's fields. This may be due to the low level of seed transmission at farmer's field than at research stations as a number of germplasm accessions from different sources are being evaluated by the breeders or pathologists. Since ULCV is seed-borne, the initial source of infection under field condition can come from seed (Beniwal *et al.*, 1980). Infection at an early stage is known to cause sterility of plants, leading to heavy losses (Bashir *et al.*, 1991). Thus use of virus-free seeds would help in the management of ULCV.

DAC-ELISA procedure proved successful to test a large number of field collected samples without any non-specific reaction. However, in case of CSMV antiserum, in every test we had non-specific reaction and this antiserum did not work. The DAC form of ELISA is very useful for field surveys, because plant extracts can be used for coating plates without the necessity of adding either antisera or immunoglobulins. The usefulness of DAC-ELISA for testing a large number of samples during survey has been reported (Hobbs, *et al.*, 1987).

In ELISA, majority (63%) of the symptomatic samples collected from farmer's fields did not react with any antiserum, and similarly 55% samples collected from research stations did not give positive reaction to any antiserum. This may be either due to nutrient deficiency, physiological disorder or we could not detect the viruses due to non-availability of antisera to other legume viruses (Makkouk *et al.*, 2001).

Based on ELISA results, we were able to detect only five viruses such as MYMV, ULCV, AMV, CMV and BYMV. In Pakistan, MYMV and ULCV have already been reported on mungbean and urdbean (Ahmad, 1975; Bashir *et al.*, 1991), but the natural occurrence of AMV, BYMV and CMV on mungbean is being reported for the first time in Pakistan. AMV was detected only from mungbean samples and not from urdbean. At present, this virus is prevalent only at research stations and not at farmer's fields. Based on ELISA detection its incidence was 4-10%. The symptoms of AMV on mungbean were observed in form of stunting, leaf yellowing and leaflet deformation. Its symptoms resemble those of CMV, but is distinguishable from AMV by serology (Latham & Jones, 2001). As AMV was found only at research stations, this clearly indicates that the virus has been introduced in the country through infected seed either by breeders or plant pathologists. Its spread at farmer's fields can only be prevented to avoid planting of infected seeds or production of virus-free seeds at research stations. Although AMV infecting mungbean and cowpea has been reported in Iran (Kaiser, 1979), but natural occurrence of AMV on mungbean seems to be the first report in Pakistan.

Natural infection of cucumber mosaic virus (CMV) and BYMV have also been observed for the first time in Pakistan on mungbean. Natural infection of these two viruses in urdbean was not observed, but urdbean infection was obtained under

greenhouse tests by mechanical inoculation. Both viruses are present at farmer's fields as well as research stations. The disease symptoms of CMV in mungbean were observed in form of dark and light green mosaic pattern, puckering and blistering of trifoliate leaves and flower abortion. The incidence of CMV at field level was 5 to 15% and is distributed in all the major mungbean growing areas. Although CMV infecting mungbean has been reported in Iran (Kaiser *et al.*, 1971), but natural infection of mungbean by CMV seems to be the first report in Pakistan.

Bean yellow mosaic virus (BYMV) was detected from samples collected from farmer's fields as well as research stations with incidence of 5 to 20%. BYMV is distributed in districts Chakwal and Mianwali, whereas it was not detected in samples collected from other districts surveyed. Maximum (20%) incidence of BYMV was observed in experimental plots at University of Agriculture, Faisalabad. Mixed infection of BYMV with ULCV and MYMV was common. The symptoms of BYMV were observed in form of mottling of leaves, mild to severe yellowing, stunting and necrosis of terminal parts. In some plants, stunting, deformation of terminal parts, puckering and yellowing of leaves was also observed. We could not detect BYMV in urdbean samples. Although, BYMV has been reported in mungbean in Indonesia (Iwaki & Auzay, 1988), but natural occurrence of BYMV on mungbean is the first report in Pakistan.

Although BCMV has been reported in mungbean in Iran (Kaiser & Mossahebi, 1974), but we could not detect this virus in mungbean and urdbean samples. However, to know more about other viruses infecting legume crops in Pakistan, subsequent extensive surveys are required in future. AMV, BYMV and CMV are Potyviruses and seed transmitted (Latham & Jones, 2001). Under field conditions the spread is through aphid species by non-persistent manner. Presently, these viruses are of minor importance, but may become a potential threat in summer pulses if their spread is not checked through production of virus-free certified seed.

Control of viral diseases of legume crops rests with breeding new cultivars with virus resistance. The other approach is production of virus-free certified seed. Application of insecticides for vector control is feasible only in case of persistently transmitted viruses. Removal of weed hosts and cultural practices also limit virus spread (Makkouk *et al.*, 1993, Jones, 1993, Bwye *et al.*, 1999).

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