Mycopath (2007) **5**(2)**:** 89-94

Screening of mungbean germplasm for resistance/tolerance against yellow mosaic disease

Sana Habib[1], *Nadeem Shad[1], Arshad Javaid[1] and Umer Iqbal[2]

[1]*Institute of Mycology and Plant Pathology, University of the Punjab, Quaid-e-Azam campus, Lahore, 54590 Pakistan.*
[2]*Pulses Program, National Agricultural Research Centre, Park Road, Islamabad, Pakistan*
**E-mail: shadbaloch@gmail.com*

Abstract

Absence of resistance/tolerance against diseases and insect pests in mungbean [*Vigna radiata* (L.) Wilczek] varieties, is one of the main reasons for their low yield in Pakistan. During the summer (Kharif) season, yellow mosaic epidemic damages the crop in most of the mungbean growing areas of Pakistan. For the purpose of identifying resistance/tolerance in mungbean germplasm, a disease screening nursery, comprising of 108 test entries, was developed. Screening was done under natural environmental conditions in 2007 at University of the Punjab, Lahore, Pakistan against yellow mosaic disease (YMD). All the test entries showed a highly susceptible response. Despite being highly susceptible, some test entries produced good yield and showed tolerance to YMD. Tolerance against YMD is a considerable factor to be included in breeding program to develop high yielding varieties of *V. radiata*.

Keywords: Mungbean, MYMV, susceptibility, tolerance, Yellow Mosaic Disease.

Introduction

Conventionally, pulses have been an important constituent of Pakistani diet. Affluent in protein and essential amino acids, they are consumed in various ways in different regions of the country. Mungbean [*Vigna radiata* (L.) Wilczek] is an imperative summer food legume in humid and sub-humid countries of the world. In Pakistan, the crop was cultivated on an area of 2, 53,000 hectares, total production of 130,000 tons of grain with yield of 577 kg/ha during 2006. (Anonymous, 2007). The crop is exceedingly prone to YMD caused by Mungbean Yellow Mosaic Begomovirus (MYMV). This disease is vital, serious, critical, open spread and inflicts heavy yield losses annually. It was first reported in India in 1955 and obviously transmitted by white fly (*Bemisia tabaci* Gennadius). It is not spread by mechanical inoculation or by seed. (Shad *et. al.*, 2005), but Thailand strain of MYMV is reported to be mechanically transmitted. (Honda *et. al.*, 1983)

MYMV infects mungbean, soybean, mothbean, cowpea and urdbean (Mash) and some other leguminous hosts (Dhingra and Chenululu, 1985, Qazi *et. al.* 2007). Yellow mosaic is reported to be the most destructive viral disease not only in Pakistan, but also in India, Bangladesh, Srilanka and contiguous areas of South East Asia (Bakar, 1981; Malik 1991, Biswass *et. al.*, 2008. John *et. al.*, 2008.). MYMV resembling other whitefly-transmitted Geminiviruses has appeared as the most important, serious and often overwhelming disease throughout Pakistan. The virus causes uneven yellow and green specks or patches on the leaves which finally turn entire yellow. Affected plants generate fewer flowers and pods, which also develop mottling and remain small and contain fewer, smaller and shrunken seeds.

MYMV belongs to genus *Begomovirus* of the family Geminiviridae (Bos, 1999). The virus has geminate particle morphology (20 x 30 nm) and the coat protein encapsulates spherical, single stranded DNA genome of approximately 2.8 Kb (Hull, 2004). In Pakistan, the virus has been partly described and identified on the basis of Polymerase Chain Reaction (PCR) and Epitope outline and DNA succession. (Hussain *et. al.* 2004; Hamid and Robinson 2004). In *Bemisia tabaci*, which transmits MYMV persistently, the adult females are 3 times more proficient transmitters than males. The white fly nymphs obtain the virus from diseased leaves (Honda and Ikegami, 1986).Whitefly-transmitted plant viruses are found in the humid and sub-humid countries. In legumes, MYMV is the major virus and found in all the mungbean growing countries of the globe.

The chemical management of the vector is not cost-effective since numerous sprays of insecticides are required to control whitefly. Recurrent sprayings also lead to health danger and ecological effluence. On the contrary, use of virus resistant varieties, if available, is the best approach

to alleviate occurrence of YMD in areas where the infection is a major constraint to production. The reasonable, robust and perfect method of controlling viral diseases is regarded as the use of resistant crop varieties. A good quality of research efforts have been directed towards screening mungbean germplasm against MYMV for the identification of resistant sources under diverse environmental conditions and a number of resistant lines have been reported by some workers (Murtaza *et. al.* 1983; Ghafoor *et. al.* 1992; Bashir, 2002; Shad *et. al.* 2006. Pandiyon *et. al.* 2007).It was, therefore, planned to screen accessible mungbean germplasm against YMD under natural environmental conditions in Lahore, where high population of viruliferous white fly is always present.

Materials and Methods

Disease screening nursery was established at Institute of Mycology and Plant Pathology, University of the Punjab, Lahore; during summer season of 2007. One hundred and eight mungbean germplasm accessions were evaluated against YMD under natural environmental conditions with high population of whitefly. The source of these accessions is shown in Table- 1. Mungbean accessions were stored at 4ºC prior to sowing.

Nursery sowing was done during the Ist week of July 2007. Pots of 12 inches length and 6 inches diameter were used. The pots were filled with Sandy Loam soil. Lay out in pot experiment comprised replicates in block design, accommodating 108 germplasm lines in three replications. Each germplasm comprised of four pots in each of the replicates. Germplasm lines were divided in to four sets; each having 27 lines. One row of a most susceptible spreader line (Burma Mash) was planted after every two test entries. Two rows of spreader were planted all around the experiment in order to attract white fly and enhance infection of MYMV. Recommended cultural practices were followed to maintain the experiment except that insecticide sprays were not given to encourage the white fly population for spread of the disease.

The crop was regularly monitored for the presence of whitefly and development of YMD. Whitefly started landing on the plants soon after germination and the disease made its first appearance 2nd week after planting. Infection and disease severity of MYMV progressed in the next 6 weeks. The disease was scored on 0-5 arbitrary scale, as suggested by Bashir *et. al.* (2005) which is described in Table-2.

Disease severity index (DSI) for MYMV was determined in all the mungbean lines at weekly interval. It was based on: 0 (no symptoms on any leaf), 1 (few visible spots/specks on the leaves), 2 (specks increase and occupy 20% leaf area), 3 (specks coalesce and cover more than 50% area), 4 (all leaves become yellow) and 5 (yellow plant with mottled pods).

Spread of MYMV in the pot experiment was recorded at weekly intervals till maximum infection was achieved. Percent increase in the infection was the difference between fortnight observations. The number of genotypes infected per week was calculated. Ripened pods in the individual row were gradually picked at appropriate times, sun dried for 10 days, and grains were separated and weighed to record the yield.

Results and Discussions

One hundred and eight lines of mungbean of diverse origin/source were sown under natural environmental conditions on Ist July 2007 in randomized block design. Germination was completed within a week and whitefly started landing soon after the plants emerged and continued till maturity of the crop. The fist appearance of yellow mosaic was recorded in several genotypes two weeks after planting. Mild to severe yellow specks were first observed on young leaves. Within next 2 weeks, these specks increased, coalesced and turned into yellow and green patches. The severity of disease increased with the passage of time. Thirty five days after sowing, all the infected plants turned completely yellow and pods developed mottling and contained few shriveled seeds. The spread of YMD in all mungbean lines was recorded at weekly interval for six weeks from 20th August to 26th August 2007. Based on symptoms expression, infected plants were counted and percent disease augmentation was calculated on each accession.

During the first week, 19 test lines became infected and the remaining 89 varieties were uninfected with disease incidence ranging from 8%-17%. In the second week, 74 test lines were infected with disease incidence ranging from 8%-42% and other 34 remaining test lines were symptomless. In the third week, none of the 108 test lines escaped from the infection and the disease incidence ranged from 8%-67%. The 2 test lines NM-38-203-34 and NCM-257-10-36 showed the minimum incidence of 8%, and 8 test lines exhibited 17% incidence. The remaining test lines showed the incidence ranging from 33%-67%, in the fourth week, 6 test lines M-2004, NCM-209-28, N-27, NCM-251-13-31, N-37, N-12 showed the incidence of 42%, 11 test lines showed 50%, and 75 lines showed 58%-75% and remaining 16 test lines showed 83%-92% incidence. In the fifth week, 2 test lines viz. N-28 and NCM-257-5-33

exhibited minimum disease incidence of 42% and 5 test lines showed 50%, 9 lines showed 67% incidence, 17 test lines showed 75% disease incidence and the remaining 75 test lines showed 83%-92% disease incidence. By the end of sixth week, 1 test line N-4 showed the minimum incidence of 75% and two test lines N-36 and N-26 showed 83% incidence, 18 test lines showed the incidence of 92% and the remaining 87 test lines had 100% incidence. These results clearly indicate that the initial period of mungbean crop is highly critical for the development and spread of yellow mosaic. Environmental conditions were highly conducive for spread of the disease due to high vector population and the build up of inoculum potential of virus from the very beginning. It also indicates that there are minimum chances that any disease escape mechanism could become operative except the environmental factors. Statistical analysis revealed that the yellow mosaic infection/week progressed significantly (Table 4).

Disease severity index generally followed the same trend as was visualized for MYMV infection presented in Table 2. In the first week with the initiation of infection, DSI was only ranging from 0-1 in 19 lines which progressed to 0-4 in 66 lines during second week and 3-5 in third week. During the fourth week, DSI was recorded between 3-5 and in fifth week 4-5, and by the end of sixth week, 5 DSI was recorded. Statistical analysis (Table 3) revealed that the changes in the D.S.I were significant for the first three weeks and reached maximum during the subsequent weeks.

As incidence of MYMV in various mungbean lines is concerned, disease incidence ranged from 8% to 100%. Therefore, almost all the lines were invariably infected by MYMV to the highest extent and appeared to be far below the boundaries of resistance i.e. test lines did not vary in their reaction to MYMV. Nineteen test lines were infected in the first week with the disease incidence of 8%. During the second week, 74 lines were infected with MYMV with incidence increasing up to 42%. During the third week, all the test lines infected with MYMV with incidence increasing up to 67%. During the fourth and fifth week, incidence increased up to 83%-92%.During sixth week, 83 test lines exhibited 100% incidence. By the end of sixth week, all the test lines exhibited highest disease severity index of 5.

It was difficult to classify the mungbean lines into various resistance categories because none appeared to be highly resistant, resistant, moderately resistant or moderately susceptible. Mungbean lines, based on the infection and severity index, could be placed in 1 class only, highly susceptible with the inclusion of 108 lines. The results clearly indicate that the source of resistance in mungbean against MYMV is absent or rare.

As regards grains yield; some tests lines were good yielder in spite of high disease incidence. However, the impact of infection of MYMV seems to be quite variable. 7 test lines yielded more than 8.3 grams per plant in spite of variable incidence of MYMV; 29 entries produced 6.6-8.3 g yield, 36 lines between 5-6.58 g, 21 lines between 3.33-4.96 g and 15 lines less than 3.33 g.
High disease incidences are encountered every year, which are attributed to the combination of factors such as high whitefly population, presence of imoculum potential of the virus, and favourable environmental/ecological conditions. Evolution and use of disease resistant varieties are considered as effective and durable solution of controlling YMD and therefore it remained a subject of extensive research with many scientists. In the first instance efforts were directed towards screening mungbean germplasm against YMD under natural environmental conditions (Verma *et. al.* 1983, Ayyub 1987, Ghafoor *et. al.* 1992, Singh *et. al.* 1996, Bashir 2003, Bashir *et. al.* 2006, Shad *et. al.* 2006). The study of literature revealed that only a limited number of cultivars expressed high or good resistance. Partial resistance was reported by Singh *et. al.* (1996), but Gill *et. al.* (1983) stated that resistance against YMD was rare and scarce. They found a good quality of resistance in urdbean and soybean which led them to successful hybridization and interspecific transfer of resistance.

None of the test entries appeared to be highly resistant, resistant, moderately resistant or susceptible. All of 108 test entries were rated as "highly susceptible" (Table 3). These results agree at large with Dey and Singh (1973), Gill *et. al.* (1975), Pandya *et. al.* (1977), Patel and Srivastava (1990), Singh *et. al.* (1996) and Shad *et. al.* (2006) who evaluated a number of mungbean lines/cultivars against yellow mosaic and found good resistance only in few lines. Bashir (2003) screened 276 lines and only 10 were rated as resistant (95013, C1/95-3-140, C1/94-04-19, C1/95-03-20, C1/95-3-8, C1/94-3-140, C1/94-4-26, C1/94-4-5, C5/95-4-5, C5/95-5-19 and C1/95-3-17). Some of the reported resistant lines or closely related lines were included in this study but turned out to be highly susceptible. It was, however, reported by Bashir (2003) that infection of yellow mosaic was low because of sufficient rainfall and low whitefly population in the sprig season. Absence of resistant lines from the test germplasm population highlight the need for extensive work for exploring new sources of germplasm collection. Could that be possible that some varieties might show resistance, if planted in spring?

Table 1: Source of mungbean germplasm evaluated against YMD during summer of 2007.

SR. NO.	SOURCE	NO. OF LINES
1	National Agricultural Research Centre, (NARC), Islamabad.	42
2	Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad.	40
3	Ayub Agricultural Research Institute Faisalabad, (AARI).	20
4	University of Agriculture, Faisalabad (UAF).	6
	Total	**108**

Table 2: Disease Scoring Scale (0-5) for YMD

SEVERITY	% INFECTION	INFECTION CATEGORY	REACTION GROUP
0	All plants free of virus symptoms	Highly resistant	HR
1	1-10% infection	Resistant	RR
2	11-20% infection	Moderately resistant	MR
3	21-30% infection	Moderately susceptible	MS
4	30-50% infection	Susceptible	S
5	More than50 %	Highly susceptible	HS

Source: Bashir *et. al.* (2005)

Table 3: Distribution of Mungbean Lines in Various Infection Categories of MYMV.

Infection Category	Disease Severity Index	No. of Genotypes	Lines Involved
Highly Resistant	0	0	0
Resistant	1	0	0
Moderately Resistant	2	0	0
Moderately Susceptible	3	0	0
Susceptible	4	0	0
Highly Susceptible	5	108	AD-1 KHAN1, NM-92, NCM 20 9 3, C1/95-3-1454, NCM 252-7 5, M-8 MUNGO 7, 98C MG-018 8, SWAT-MUNG-1 11, NFMI2-12 12, 98CMG-003 13, V-BRM 268 15,V-BRM 288 14, VC-3960-88 17, AZRI, MUNG J-18, 2CMG 504 19, NM-98-20, M-1 21, AEM 6120 K-23, E/M-6 24, C2/94-4-43 25, DM-2 26, NM-3-27, NCM-209 28, 99CMG-05829, VC 3960 (A89)30, NCM-251-13-31, NCM-254-3 32, NCM-257-5 33, NM-38-203 34, NM-15-11 35, NCM-257-10 36, NCM-254-7 37, NM-49-7 38, NCM-251-4 39, NCM-252-1 40, NM-20-4 41, NCM-252-10-42, 98CMG-016, 2CMG-501, MUNG-F,VC-3960(A-88), LIP5/5/89, N-1, N-2, N-3, N-4, N-5, N-6, N-7, N-8, N-9, N-10, N-11,N-12, N-13, N-14, N-15, N-16, N-17, N-18, N-19, N-20, N-21, N-22, N-23, N-24,N-25, N-26, N-27, N-28, N-29, N-30, N-31, N-32, N-33, N-34, N-35, N-36, N-37, N-38, N-39, N-40, M 2001, AUM 18, 56-2, AUM 9, M 2004, AUM31, 96001 1, 96002 2, 96006 3, 96009 4, 97003 7, 97001 6, 98007 12, 98003 10, 970019 9, 98006 11, 97008 8,98011 14, 98010 13, 98004 20, 960012 5, NM-2006 17, M-1 18, 96004 19, M-615, NM-92 16

Table 4: Weekly environmental data (average) for the experimental period, PU (July-August-September, 2007)

Week period	**Av. Temp. °C**	**R.H%**	**Rainfall**	**Operations**
1st Jul.-8TH Jul.	31.38	76.92	0.58	
8th Jul.-15nd Jul.	30.22	66.43	1.81	Sowing and Germination, whitefly landing
16rd Jul.-22nd Jul.	30.64	78.43	3.46	Whitefly feeding and emergence of YMD
23rd Jul.-29th Aug.	31.32	67.33	3.31	YMD prevalence rising
30thJul.- 5th Aug.	31.76	68.71	0.83	//
6thAug.-12th Aug.	30.76	64.93	1.81	YMD incidence and severity increasing
13th Aug.-19th Aug.	37.24	74.64	1.49	//
20th Aug.-26th Aug.	31.17	64.01	8.94	YMD frequency and severity maximum
27th Aug.- 2nd Sep.	31.46	75.78	1.97	Highest and regular infection.

References

Anonymous. 2007. Agricultural statistics of Pakistan. Ministry of Food, Agriculture and Live stock, Economies Wing. Govt. Pakistan. Islamabad.

Ayub A, 1987. Studies on the screening of mungbean germplasm and growth responses of mungbean plant to mungbean yellow mosaic virus infection. M. Sc. (Hons) Thesis. Dept of Plant Pathology, Univ. of Agri. Faisalabad. pp. 67-68.

Bakar AK, 1981. Pest and disease problems of mung bean in west Malaysia. *Malaysian J. Agric.*, **53**: 29-33.

Bashir M, Zubair M, 2005. Studies on viral diseases of major pulse crops and identification of resistant sourses. Technical Annual Report (April, 2004 to June. 2005) of APL Project. Crop sciences Institute, National Agricultural Research Centre, Islamabad. pp: 169.

Bashir M, Zubair M, 2002. Identification of resistance in urdbean (*Vinga mango*) against two different viral diseases. *Pak. J. Bot*., **34**(1): 49-51

Bashir M, 2005. Studies on viral disease of major pulse crops and identification of resistant sources. Technical Annual Report (April, 2004 to June, 2005) of ALP Project. Crop Sciences Institute, National Agricultural Research Center, Islamabad. Pp:169

Bashir M, 2003. Studies on viral diseases of major pulse crops and identification of resistant sources. Tech. Annual Report Project. ALP Project; NARC. Islamabad. Pp 76.

Biswass KK, Malathi VG, Varma A, 2008. Diagnosis of symptoless yellow mosaic begomovirus infection in pigeon pea by using cloned mungbean yellow mosaic India virus a probe. *J. Plant Biochem. Biotech*., **17**(1) 9-14.

Bos L, 1999. Plant Viruses: Unique and Intriguing Pathogens: A Text Book of Plant Virology, Backhuys Publishers, the Netherlands pp: 305-306

Dey SK, Singh DR, 1973. Resistance test of mung (*Phaseolus aureus* L.) against yellow mosaic in Uttar Pradesh. *Indian J. Farm Sci*., **1**(1):113-117

Dhingra KL, Chenulu VV, 1985. Effect of yellow mosaic on yield and nodulation of soybean. *Indian J. Phytopathol*., **38**(2): 248-251.

Ghafoor A, Zubair M, Malik BA, Iqbal SM, 1992. Evaluation of selected germplasm of mungbean.*Pak. J. Bot*., **24**: 112-118.

Gill KS, Sandhu TS, Brar JS, 1975. Evaluation of mungbean (*Vigna radiata* (L.) Wilczek) germplasm. Crop improvement 2. 94-104. (*Field crop Abstr*. 33: 34-64, 1980).

Gill AS, Verma MM, Dhaliwal HS, Sandhu TS, 1983. Inter specific transfer of resistance to mungbean yellow mosaic virus from *Vigna mungo* to *Vigna radiata*. *Current Sci*., **52**: 31-33

Hamid S, Robinson DJ, 2004. Begomoviruses from mungbeans in Pakistan: epitope profiles. DNA A sequences and phytogenic relationships. *Archives virol***., 149**:809-819.

Honda Y, Ikegami M, 1986. Mungbean Yellow Mosaic Virus. *CMI/AAB* Description of Plant Viruses, No. 323.

Honda Y, Iwaki M, Saito Y, Thongmeearkom P, Kittisak K, Deema N, 1983. Mechanical transimission, purification and some properties of whitefly-borne mungbean yellow mosaic virus in Thailand. *Plant Dis*., **67**: 801-804.

Hull R, 2004. Mathew's Plant Virology, Fourth Edition. Elsevier Publishers, India. pp.180-182.

Hussain M, Qazi J, Mansoor S, Irum S, Bashir M, Zafar Y, 2004. First report of mungbean yellow mosaic virus on mungbean in Pakistan. *Plant Pathol*., **53**(4): 518.

John P, Sivalingam PN, Haq QMI, Kumar N, 2008. Cowpea golden mosaic disease in Gujrat is caused by a mungbean yellow mosaic India virus isolate with a DNA B variant. *Archives Virol*., **153**(7): 1359-1365.

Malik JA, 1991. Breeding for resistance to MYMV and its vector in Pakistan.Pp:41-53. Mungbean YMDs. Proceedings of an international workshop. Bankok,Thailand, 23 July, 1991. AVRDC. (Eds.): S.K Green and D. Kim.

Murtaza, Bhatti MA, Qayyum, 1983. Susceptibility of mung bean lines to white fly and yellow mosaic virus. *Pak. Entomol*., **5**: 51-56.

Pandya BP, Singh DP, Sharma BL, 1977. Screening of mungbean (*Vigna radiata* (L.) Wilczek) germplasm for field resistance to yellow mosaic virus. *Trop. Gram Leg. Bull*., **7**:13-14.

Pandiyon M, Subbalakshmi B, Alice D, Marimethn R, 2007. Screening of mungbean (*Vigna radiata* (L.) Wilczek) germplasm for mungbean yellow mosaic virus. *Plant Archives*., **7**(1): 375-376.

Patel MR, Srivastava KP, 1990. Field screening of some high yielding genotypes of mungbean (*Vigna radiata* (L.)Wilczek) to white fly (*Bemisia tabaci* Genn.) and yellow mosaic virus. *J. Entomol*., **52**: 547-553.

Qazi J, Ilyas M, Mansoor S, Briddon RW, 2007. Legume yellow mosaic viruses: genetically isolated begomoviruses. *Mole. Plant Pathol*., **8**(4): 343-348.

Shad N, Mughal SM, Bashir M, 2005. Transmission of mungbean yellow mosaic Begomovirus (MYMV). *Pak. J. Phytopathol.*, **17**(2): 141-143.

Shad N, Mughal SM, Farooq K, Bashir M, 2006. Evaluation of Mungbeen Germplasm for resistance against Mungbean Yellow Mosaic Begomovirus. *Pak. J. Bot*., **38**(2): 449-457.

Singh K, Singh S, Gumber RK, 1996. Resistance to mungbean yellow mosaic virus in mungbean. *Ind. J. Pulses Res*., **9**: 90-97

Verma HN, Rastogi P, Parasad V, Srivavastava A, 1983. Possible control of natural virus infection of *Vigna radiata* and *Vigna mungo* by plant extracts. *Indian J. Plant Pathol*., **3**: 21-24.