Plant Pathol. J. 25(4) : 422-428 (2009)

Notes

The Plant Pathology Journal

Field Evaluation of Mungbean Recombinant Inbred Lines against Mungbean Yellow Mosaic Disease Using New Disease Scale in Thailand

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(Received on August 28, 2009; Accepted on September 22, 2009)

Studies were conducted to identify the sources of resistance in mungbean recombinant inbred lines (RILs) in Thailand against mungbean yellow mosaic disease (MYMD). 146 mungbean RILs in F_8 series were evaluated in a field including resistant parent NM-10-12-1 and susceptible parent KPS 2 during summer 2008 under high inoculum pressure. The RILs were subsequently scored for disease symptom severity ratings (DSSR) using a new scale. Observations regarding DSSR and % disease index (%DI) showed that the tested RILs responded differently to the disease. A large number of RILs (132) were found highly susceptible, 12 were susceptible, 3 were tolerant and one was resistant. Overall screening results showed that three RILs, viz. line no. 30, 100 and 101 had minimum DSSR and % disease index thus they are good source of resistance to MYMD in spite of high disease pressure and can therefore be used directly as varieties to manage the disease in Thailand.

Keywords : Begomovirus, mungbean, natural infection, RILs, yellow mosaic, whitefly

Mungbean (*Vigna radiata* (L.) Wilczek), a rich source of dietary protein, is an important short duration grain legume crop in humid and sub humid countries of the world (Akhtar & Haq, 2003). Economic yield of mungbean is low due to various biotic and abiotic constraints and diseases are the major impediments to production (Malik and Bashir, 1992). When grown in the field, mungbean is exceedingly prone to various viral diseases, which were caused by mungbean yellow mosaic virus (MYMV), urdbean leaf crinkle virus (ULCV), cucumber mosaic virus (CMV), bean yellow mosaic virus (BYMV) and alfalfa mosaic virus (AMV), some of which can cause significant economic losses (Aftab et al., 1993; Bashir et al., 1991; Bashir et al., 2006; Malik, 1991). Mungbean yellow mosaic diseases (MYMD) is the major threat to mungbean production in India, Sri Lanka, Pakistan, Bangladesh, Papu New Guinea, Philippines and Thailand (Chenulu and Verma, 1988; Honda et al., 1983; Jones, 2003; Malik and Bashir, 1992) and inflict on heavy yields losses annually.

MYMD is caused by mungbean yellow mosaic begomovirus belonging to family Geminiviridae. Like other begomoviruses its viral particles are isometric and geminate having 18 to 30 nm in size with two single stranded DNA molecules (DNA A & DNA B) of 2726 and 2775 nucleotides, respectively (Bos, 1999; Hull, 2004; Morinaga et al., 1990 & 1993). This virus is transmitted by whitefly (*Bemisia tabaci* Genn) and through grafting but not through seed, sap and soil (Bashir 2003; Nariani, 1960; Nair and Nene 1973; Nene, 1972). Initially the disease appears as small yellow spots along the veins on young leaves and then spread over the leaves. Under severe infection, the entire leaf can show yellowing or chlorosis on the whole plant followed by necrosis, shortening of internode, severe stunting of plants with no yield or few flowers & deformed pods producing small, immature and shriveled seeds (Aftab et al., 1993; Akhtar and Haq, 2003; Bashir et al., 1991; Bashir et al., 2006; Malik, 1991).

The use of resistant genotypes is the best way to reduce the losses inflicted by MYMD but for a successful screening programme, a reliable assessment method is also required. Previously, resistance against MYMD in mungbean has been reported by different workers in different scales, as described by Ahmad (1975), Bashir (2005), Bashir et al. (2006), Khattak et al. (2008). However, these assessment methods are thought to be impractical, because all present methods are based to express the varietal response on percentage of diseased plants. Keeping this in view, the present study was performed to screen mungbean RILs from Thailand using a new disease scale.

A total of 146 RILs as presented in Table 2, they were grown together with the resistant parent (NM 10-12-1; introduced from Pakistan) and susceptible parent (KPS 2;

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Table 1. Disease scale for rating of mungbean yellow mosaic disease (MYMD)

Symptoms	Rating/Disease severity*	% Disease Index*	Disease reaction
Complete absence of symptoms	0	0	Immune*/Field immune
Few small yellow specks or spots on few leaves seen after careful observations.	1	0.01-10	Highly resistant
Bright yellow specks or spots common on leaves, easily observed and some coalesced.	2	10.01-25	Resistant
Mostly coalesced bright yellow specks or spots common on leaves, but no or minor reduction in yield.	3	25.01-40	Tolerant
Plants showing coalesced bright yellow specks or spots on all leaves, with no or minor stunting and set fewer normal pods.	4	40.01-60	Susceptible
Yellowing or chlorosis of all leaves on whole plant followed by necrosis, shortening of internode, severe stunting of plants with no yield or few flowers & deformed pods produced with small, immature and shriveled seeds.	5	>60.01	Highly susceptible

*The percentage disease index was calculated as (sum of all disease ratings/total # of plants)×20

the most popular mungbean cultivar grown in the lower northern part of Thailand). All RILs were developed and supplied by the Department of Agronomy, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand for evaluation against MYMD.

Each test entry was planted in triplicates in a row of 4 meter (24 to 28 plants per replicate) in length with 40 cm row to row distance in July 2008 at NIAB, Faisalabad, Pakistan. One row of susceptible parent KPS 2 was planted after every two test entries. Conventional agronomic practices (fertigation, irrigation, weeding, hoeing etc.) were followed to keep the crop in good condition. However, no plant protection measures were applied against the whitefly to ensured high inoculum pressure throughout the experiment. Experimental unit was observed weekly and data for disease symptom severity ratings (DSSR) were recorded according to the rating system described in Table 1 to calculate percent disease index (%DI) and the level of resistance/ susceptibility of the genotypes.

A low level of whiteflies started to appear immediately after the emergence of seedlings and it continued its build-up during the whole growth period of the crop. However, the 1st MYMD symptom started to appear on the susceptible RILs after about 15 days after seedlings emergence. Disease symptoms were started as scattered few small yellow specks on few young leaves. After 4-5 days most of the specks were coalesced, alternating between yellow and green patches with irregular margins developed in the first fully formed trifoliate leaf next to the apex. Complete yellowing or chlorosis was observed within 8-10 days followed by necrosis.

Reactions of the tested RILs ranged from resistance to highly susceptible, although the MYMD incidence and severity varied extensively depend to the RILs used. None of the tested plant/RILs was free from disease symptoms. Minimum %DI of 23.64% was recorded in NM-10-12-1 (resistant parent) and maximum as 100% in Line No-114 (Table 2). Out of 146 RILs evaluated, 132 were highly susceptible with high percentages DI ranging from 60.67 to 100% and symptom severity rating between 3-5. Among the remaining, 12 RILs were susceptible, three viz. Line no. 30, 100 and 101 were tolerant with 31.89, 40.0 and 39.26 %DI, respectively giving DSSR from 1-3, while NM-10-12-1 was resistant with 23.64 %DI and 1-2 DSSR.

Identification of reliable sources of resistance against serious and destructive MYMD is an important aspect of plant breeding. Accurate measurement of plant disease is crucial in all studies relating disease severity to disease losses and subsequent management tactics (Horsfall & Cowling, 1978; Akhtar & Khan, 2002). In all the present MYMD assessment systems the percentage of diseased plants (Ahmad et al., 1975; Bashir, 2005; Bashir et al., 2006; Khattak et al., 2008) are taken for the expression of varietal response (resistance/susceptible), however it is not an appropriate method for the quantitative assessment of varietal resistance/susceptible. Existing mungbean cultivars show a great variation in level of resistance/susceptibility, which have not been properly discussed in these assessment systems. It means a disease assessment method is needed which can properly describe different severity levels for proper rating of an individual plant or cultivar under high inoculum pressure. Based on our personal experiences using visual observations against 200 genotypes during summer 2007 and 2008, we have developed a new rating scale for the assessment of MYMD, which we believe can be useful for the accurate measurement of advance mungbean breeding genotypes against MYMD.

Use of resistant variety is an important aspect of integrated disease management programme as well as for successful breeding programme. A reliable screening method is a prerequisite (Akhtar & Haq, 2003). As the disease is vectored by whitefly, field screening is the most commonly

Table 2. Response of mungbean RILs against mungbean yellow mosaic virus in field under high inoculum pressure

Entry	Infection percentage	Infection type range	Percentage of plants showing disease severity ratings						Disease index (%age)	Disease reaction
			0	1	2	3	4	5		
KPS 2 (Parent)	100	3-5	0	0	0	12.0	72.0	16.0	80.80	HS
NM 10-12-1 (Parent)	100	1-2	0	81.82	18.18	0	0	0	23.64	R
Line # 1	100	3-5	0	0	0	13.8	75.8	10.4	79.31	HS
Line # 2	100	3-4	0	0	0	14.3	85.7	0	77.14	HS
Line # 3	100	3-4	0	0	0	10.0	90.0	0	78.00	HS
Line # 4	100	2-4	0	0	3.0	7.0	90.0	0	77.42	HS
Line # 5	100	3-4	0	0	0	15.6	84.4	0	76.88	HS
Line # 6	100	4-5	0	0	0	0	10.0	90.0	98.00	HS
Line # 7	100	3-4	0	0	0	37.9	62.1	0	86.90	HS
Line # 8	100	3-4	0	0	0	60.7	39.3	0	67.86	HS
Line # 9	100	3-4	0	0	0	62.5	37.5	0	67.50	HS
Line # 10	100	3-4	0	0	0	88.2	11.8	0	62.35	HS
Line # 11	100	3-4	0	0	0	80.0	20.0	0	64.00	HS
Line # 12	100	4-5	0	0	0	0	76.9	23.1	84.62	HS
Line # 13	100	4-5	0	0	0	0	38.1	61.9	92.38	HS
Line # 14	100	3-4	0	0	0	83.3	16.7	0	63.33	HS
Line # 15	100	4-5	0	0	0	0	88.2	11.8	82.35	HS
Line # 16	100	2-4	0	0	7.4	18.5	74.1	0	73.33	HS
Line # 17	100	4-5	0	0	0	0	51.7	48.3	89.66	HS
Line # 18	100	4-5	0	0	0	0	42.4	57.6	91.52	HS
Line # 19	100	3-5	0	0	0	18.8	59.4	21.8	80.63	HS
Line # 20	100	3-5	0	0	0	25.0	56.3	18.7	78.75	HS
Line # 21	100	2-4	0	0	12.5	75.0	12.5	0	60.00	S
Line # 22	100	4-5	0	0	0	0	85.2	14.8	82.96	HS
Line # 23	100	3-5	0	0	0	11.8	76.4	11.8	80.00	HS
Line # 24	100	3-5	0	0	0	17.3	72.4	10.3	78.62	HS
Line # 25	100	3-5	0	0	0	11.5	73.1	15.4	80.77	HS
Line # 26	100	2-4	0	0	10.7	78.6	10.7	0	60.00	S
Line # 27	100	2-4	0	0	7.7	80.8	11.5	0	60.77	HS
Line # 28	100	2-4	0	0	17.7	70.6	11.8	0	50.82	S
Line # 29	100	3-4	0	0	0	50.0	50.0	0	70.00	HS
Line # 30	100	1-3	0	21.6	62.2	16.2	0	0	31.89	T
Line # 31	100	4-5	0	0	0	0	61.3	38.7	87.74	HS
Line # 32	100	3-5	0	0	0	34.5	58.6	6.9	74.48	HS
Line # 33	100	3-4	0	0	0	26.9	73.1	0	74.62	HS
Line # 34	100	3-5	0	0	0	19.4	61.3	19.4	80.00	HS
Line # 35	100	2-4	0	0	21.9	59.4	18.7	0	59.38	S
Line # 36	100	2-4	0	0	43.3	50.0	6.7	0	52.67	S
Line # 37	100	4-5	0	0	0	0	74.1	25.9	85.19	HS
Line # 38	100	2-4	0	0	19.1	66.7	14.3	0	59.05	S
Line # 39	100	2-4	0	0	17.5	72.5	10.0	0	58.50	S
Line # 40	100	3-5	0	0	0	7.7	69.2	23.1	83.08	HS
Line # 41	100	3-5	0	0	0	3.8	71.4	25.0	84.29	HS
Line # 42	100	3-4	0	0	0	70.6	29.4	0	65.88	HS
Line # 43	100	4-5	0	0	0	0	76.7	23.3	84.67	HS
Line # 44	100	4-5	0	0	0	0	87.5	12.5	82.50	HS
Line # 45	100	3-4	0	0	0	40.0	60.0	0	72.00	HS
Line # 46	100	3-4	0	0	0	32.4	67.6	0	73.51	HS

Table 2. Continued

Entry	Infection percentage	Infection type range	Percentage of plants showing disease severity ratings						Disease index (%age)	Disease reaction
			0	1	2	3	4	5		
Line # 47	100	3-5	0	0	0	26.7	63.3	10.0	76.67	HS
Line # 48	100	2-4	0	0	10.8	76.4	11.8	0	60.00	S
Line # 49	100	4-5	0	0	0	0	45.5	54.6	90.91	HS
Line # 50	100	4-5	0	0	0	0	46.9	53.1	90.63	HS
Line # 51	100	4-5	0	0	0	0	47.9	52.1	90.44	HS
Line # 52	100	4-5	0	0	0	0	48.3	51.7	90.35	HS
Line # 53	100	4-5	0	0	0	0	44.1	55.9	91.18	HS
Line # 54	100	3-5	0	0	0	4.8	47.6	47.6	88.57	HS
Line # 55	100	3-5	0	0	0	80.0	14.3	5.7	65.14	HS
Line # 56	100	3-4	0	0	0	79.3	20.7	0	64.14	HS
Line # 57	100	3-5	0	0	0	75.9	20.6	3.5	65.52	HS
Line # 58	100	3-5	0	0	0	66.7	29.6	3.7	67.41	HS
Line # 59	100	3-5	0	0	0	5.9	82.4	11.8	81.18	HS
Line # 60	100	3-5	0	0	0	5.4	83.8	10.8	81.08	HS
Line # 61	100	3-5	0	0	0	5.5	81.1	13.5	81.62	HS
Line # 62	100	3-5	0	0	0	55.0	40.0	50	70.00	HS
Line # 63	100	3-5	0	0	0	15.2	75.7	9.1	78.79	HS
Line # 64	100	2-4	0	0	7.7	23.1	69.2	0	72.31	HS
Line # 65	100	3-5	0	0	0	32.0	60.0	8.0	75.20	HS
Line # 66	100	2-4	0	0	6.9	68.9	24.2	0	63.45	HS
Line # 67	100	3-5	0	0	0	14.3	71.4	14.3	80.00	HS
Line # 68	100	2-4	0	0	12.9	64.5	22.6	0	61.94	HS
Line # 69	100	3-5	0	0	0	7.9	78.9	13.2	81.05	HS
Line # 70	100	3-5	0	0	0	2.3	83.7	14.0	82.33	HS
Line # 71	100	2-4	0	0	15.4	64.1	20.5	0	61.03	HS
Line # 72	100	2-4	0	0	19.2	59.3	11.5	0	58.46	S
Line # 73	100	3-5	0	0	0	4.8	71.4	23.8	83.81	HS
Line # 74	100	3-5	0	0	0	18.5	74.1	7.4	77.78	HS
Line # 75	100	3-5	0	0	0	23.1	73.1	3.8	76.15	HS
Line # 76	100	2-4	0	0	6.2	46.9	46.9	0	68.13	HS
Line # 77	100	4-5	0	0	0	0	62.5	37.5	87.50	HS
Line # 78	100	3-5	0	0	0	4.0	80.0	16.0	82.40	HS
Line # 79	100	4-5	0	0	0	0	88.9	11.1	82.22	HS
Line # 80	100	3-4	0	0	0	75.0	25.0	0	65.00	HS
Line # 81	100	2-4	0	0	8.3	79.2	12.5	0	60.83	HS
Line # 82	100	2-4	0	0	19.2	69.2	11.6	0	58.46	S
Line # 83	100	3-4	0	0	0	73.1	26.9	0	65.39	HS
Line # 84	100	3-4	0	0	0	76.9	23.1	0	64.62	HS
Line # 85	100	3-4	0	0	0	66.7	33.3	0	66.67	HS
Line # 86	100	3-4	0	0	0	77.3	22.7	0	64.55	HS
Line # 87	100	3-4	0	0	0	52.4	47.6	0	69.52	HS
Line # 88	100	3-5	0	0	0	12.9	67.7	19.4	81.29	HS
Line # 89	100	2-4	0	0	4.8	81.0	14.2	0	61.91	HS
Line # 90	100	3-4	0	0	0	71.0	29.0	0	65.81	HS
Line # 91	100	3-5	0	0	0	13.5	81.1	5.4	78.38	HS
Line # 92	100	3-5	0	0	0	13.3	73.4	13.3	80.00	HS
Line # 93	100	2-4	0	0	3.6	71.4	25.0	0	64.29	HS
Line # 94	100	3-5	0	0	0	7.4	74.1	18.5	82.22	HS

Table 2. Continued

Entry	Infection percentage	Infection type range	Percentage of plants showing disease severity ratings						Disease index (%age)	Disease reaction
			0	1	2	3	4	5		
Line # 95	100	3-5	0	0	0	4.0	84.0	12.0	81.60	HS
Line # 96	100	3-4	0	0	0	75.0	25.0	0	65.00	HS
Line # 97	100	3-4	0	0	0	23.1	76.9	0	75.39	HS
Line # 98	100	4-5	0	0	0	0	76.9	231	84.62	HS
Line # 99	100	4-5	0	0	0	0	84.6	15.4	83.08	HS
Line # 100	100	1-3	0	17.2	65.6	17.2	0	0	40.00	T
Line # 101	100	1-3	0	18.5	66.7	14.8	0	0	39.26	T
Line # 102	100	2-3	0	0	50.0	50.0	0	0	50.00	S
Line # 103	100	2-4	0	0	31.4	54.3	14.3	0	56.57	S
Line # 104	100	4-5	0	0	0	0	75.0	25.0	85.00	HS
Line # 105	100	4-5	0	0	0	0	84.9	15.2	83.03	HS
Line # 106	100	4-5	0	0	0	0	76.2	23.8	87.62	HS
Line # 107	100	3-5	0	0	0	44.1	44.1	11.8	73.53	HS
Line # 108	100	3-4	0	0	0	38.5	61.5	0	72.31	HS
Line # 109	100	3-5	0	0	0	55.2	34.5	10.3	75.17	HS
Line # 110	100	3-4	0	0	0	46.4	53.6	0	70.71	HS
Line # 111	100	4-5	0	0	0	0	71.4	28.8	85.71	HS
Line # 112	100	3-5	0	0	0	35.7	50.0	14.7	75.71	HS
Line # 113	100	3-5	0	0	0	48.4	45.1	6.5	71.61	HS
Line # 114	100	5	0	0	0	0	0	100.0	100.0	HS
Line # 115	100	3-4	0	0	0	56.5	43.5	0	68.70	HS
Line # 116	100	3-5	0	0	0	23.1	73.1	3.8	76.15	HS
Line # 117	100	3-4	0	0	0	31.0	69.0	0	73.79	HS
Line # 118	100	3-5	0	0	0	27.3	68.1	4.6	75.46	HS
Line # 119	100	3-5	0	0	0	20.0	76.0	4.0	76.80	HS
Line # 120	100	4-5	0	0	0	0	83.3	16.7	83.33	HS
Line # 121	100	4-5	0	0	0	0	77.4	22.6	84.52	HS
Line # 122	100	4-5	0	0	0	0	74.1	25.9	85.19	HS
No.1 MYMV (G)	100	4-5	0	0	0	0	81.8	18.2	83.64	HS
No.3 MYMV (G)	100	3-4	0	0	0	52.4	47.6	0	69.52	HS
No.5 MYMV (G)	100	3-4	0	0	0	76.9	23.1	0	64.62	HS
No.6 MYMV (G)	100	2-4	0	0	4.4	78.3	17.4	0	62.61	HS
No.7 MYMV (P)	100	3-4	0	0	0	71.8	28.2	0	65.71	HS
No.18 MYMV (P)	100	3-5	0	0	0	52.2	39.1	8.7	71.30	HS
No.19 MYMV (G)	100	4-5	0	0	0	0	61.9	38.1	87.00	HS
No.21 MYMV (P)	100	4-5	0	0	0	0	60.0	40.0	88.00	HS
No.22 MYMV (P)	100	3-4	0	0	0	51.8	48.2	0	69.63	HS
No.23 MYMV (G)	100	3-4	0	0	0	50.0	50.0	0	70.00	HS
No.25 MYMV (P)	100	4-5	0	0	0	0	50.0	50.0	90.00	HS
No.26 MYMV (G)	100	4-5	0	0	0	0	47.1	52.9	90.59	HS
No.27 MYMV (G)	100	4-5	0	0	0	0	50.0	50.0	90.00	HS
No.28 MYMV (P)	100	4-5	0	0	0	0	80.0	20.0	84.00	HS
No.30 MYMV (G)	100	3-4	0	0	0	60.0	40.0	0	68.00	HS
No.31 MYMV (P)	100	3-5	0	0	0	56.0	40.0	4.0	69.60	HS
No.38 MYMV (G)	100	3-5	0	0	0	36.8	52.6	10.6	74.74	HS
No.39 MYMV (P)	100	3-5	0	0	0	33.3	61.9	4.8	74.29	HS
No.40 MYMV (G)	100	3-5	0	0	0	16.7	50.0	33.3	83.33	HS
No.43 MYMV (P)	100	3-5	0	0	0	8.0	64.0	28.0	84.00	HS
No.44 MYMV (G)	100	3-5	0	0	0	54.5	36.4	9.1	70.91	HS
No.45 MYMV (P)	100	4-5	0	0	0	0	40.0	60.0	92.00	HS

R=Resistant; T=Tolerant; S=Susceptible; HS=Highly susceptible

used method for evaluation of resistance/susceptibility, so to exert maximum inoculum pressure. The crop was sown late, about 10 days after the sowing of other surrounding field with mungbean experiments to receive maximum inoculum. Our strategy was successful and we got 100% infection in all the test material when the crop was about 30-35 days old. The high inoculum pressure together with the new disease severity scale allowed only one genotype NM-10-12-1 to be rated as resistant and three RILs as tolerant and 12 RILs as susceptible. Tolerant RILs, viz. Line no. 30, 100 and 101 showed 31.89, 40.0 and 39.26 %DI respectively, which is seemed to be high. This is the case because the DSSR were ranged from 1-3 irrespective of the time of disease appearance as 100% infection was observed in early stage, i.e. 30-35 days after germination. Most of the infected plants for tolerant RILs viz; line no 30, 100, and 101 ranged in ITR2 (62.2, 65.6 and 66.7%, respectively) but some ranged in ITR1 (21.6, 17. 2 and 18.5%) and ITR3 (16.2, 17.2 and 14.8%, respectively). Our findings showed that these RILs have good resistance and can be used to manage the disease in the areas with high incidence of MYMD. Additionally, 12 RILs were rated as susceptible, they are Line No-21, 26, 28, 35, 36, 38, 39, 48, 72, 82, 102, 103, while 6 RILs, viz; Line No-27, 68, 71, 81, 89 were rated as highly susceptible, they can also be considered further because they showed ITR 2-4 with the maximum percentage of plants in DSSR 2 and 3. Our results showed harmony with earlier findings of Ahmad (1975), Pandya et al. (1977), Gill et al. (1983), Naqvi et al. (1995), Singh et al. (1996), Saleem et al. (1998), Bashir (2005) and Shad et al. (2006) who demonstrated that resistance in mungbean against MYMD is rare.

In northern Thailand a severe outbreak of MYMD occurred in 1977, which caused major production losses and since this time MYMD has remained a problem (Morinaga et al., 1993). However, the present findings showed that good sources of resistance to MYMD are available and these could be used to manage and to minimize the MYMD severe outbreaks in Thaialand in future after evaluation for high yield, other suitable agronomic characteristics and resistance to other economically important diseases and insect pests. From the present investigations it can also be assumed that determination of the resistance only based on infection percentage could be misleading and consideration should also be given to the DSSR following % DI as well.

Acknowledgement

A part of this work was supported by the Thailand's National Science and Technology Development Agency.

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