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Research Article

Molecular marker for screening yellow mosaic disease resistance in blackgram [*Vigna mungo* (L.) Hepper]

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Abstract
Yellow mosaic disease is a serious disease in blackgram which causes severe yield losses. Screening of 45 lines along with PU-31 and PU-19 resistant checks for YMD under field conditions with artificial inoculation, identified 19 lines having 1 score with no disease symptoms. PCR reactions using SCAR marker for screening the disease reaction with genomic DNA of these lines resulted in identification of 19 resistant sources with specific amplification for resistance to YMV at 532bp with SCAR 20F/20R developed from OPQ1 RARD primer linked to YMV disease. Considering the YMV reaction and resistance linked SCAR marker, it is possible to identify the new resistance sources in a short time and they can be utilized in breeding programme or for direct release.

Key words: Blackgram, YMD disease screening, SCAR marker

Introduction

Blackgram or Urdbean (*vigna mungo* (L.) Hepper) is an excellent source of easily digestible good quality protein with low flatulence. There are many factors responsible for low productivity ranging from plant ideotype to biotic and abiotic stresses. Among the various diseases, the Mungbean Yellow Mosaic Disease (MYMD) disease was given special attention because of its severity and ability to cause yield loss up to 85%.(Nene ,1972; Verma and Malathi, 2003). Mungbean Yellow mosaic virus belongs to the genus begomovirus causes the YMV in a number of economically important edible grain legumes including mung bean, urd bean and soybean . In India, mungbean occupies about 3.15 mha. The average yield of urdbean is 341 Kg ha^{-1} in India despite its yield potential of more than 1.5 t $ha^{-1.}$ (Anon., 2012). Mungbean yellow mosaic virus, a whitefly (*Bemisia tabaci*) - transmitted Gemini virus, causes one of the most serious diseases of blackgram in South Asia. In blackgram, MYMV causes irregular yellow green patches on older leaves and complete yellowing of young leaves of susceptible varieties (Singh and De, 2006).

The disease can occur at early stage of the crop transmitted by white fly vector leading to progressive yellowing of leaves, and whole plant. It spreads rapidly to new areas in India and other countries. PU-31, PU-35, M1-1 TU94-2 and PU-19 lines with high degree of resistance may not be adaptable to all situations in India because of low yield and small dull seeded nature. Screening of large number of lines needs endemic areas, artificial inoculation and time taking as the disease occurs at any stage of crop and rapid screening may not be possible. As different needs and opportunities surface, blackgram breeders need to incorporate new genetic sources using various breeding methods aided with modern tools such as biotechnology. An approach with more perspective is marker assisted selection (MAS), which emerged in recent years due to developments in molecular marker technology especially those based on the Polymerase chain reaction (PCR) (Basak *et al.*, 2004).

Therefore, to facilitate research programme on breeding for disease resistance, it was considered important to screen and identify the sources of resistance against YMV in blackgram. Screening for new resistance sources by one of the genetically linked molecular markers could facilitate marker assisted selection for rapid evaluation. This method of genotyping would save time and labour. Molecular breeding as a method of phenotyping against begomoviruses is tedious, labour and time intensive. In the present study for screening we used SCAR 20f/20r designed from DNA nucleotide sequence amplified from RAPD OPQ1 primer closely linked to the resistant gene of yellow mosaic disease using resistant source PU-31 (Prasanthi *et al.*, 2011)

Material and Methods

Forty five lines obtained from different sources(RARS and LAM farm, Guntur, Andhra Pradesh ,G.B Pant University of Agriculture and Technology, Panth Nagar, Baba Atomic Research Centre, Mumbai) were screened during late *kharif* season of 2012 by artificial inoculation. Susceptible culture (LBG-623) was grown after every10 test lines. Plants were free from disease up to 20days after sowing. At 20days of crop white flies were collected from YMD affected susceptible plants maintained in green house using

a small, transparent glass trapper with spring cap. The same trapper with the trapped flies was next attached to a healthy plant and the viruliferous insects were allowed to feed on the leaf for 24 hrs. The disease symptoms was observed by 20-25 days after inoculation. In the forced inoculation, YMV reaction was observed after 20-25 days in PBG 32, LBG 623 initially and after 60 days there was spectacular increase in YMV infection in all other susceptible lines whereas resistant varieties were free from disease symptoms. For YMV, the observations on number of plants infected with the virus were recorded at weekly interval. Disease score was recorded at 70DAS when the disease incidence was maximum (90%) in the susceptible check (LBG-623). Disease score was recorded at maturity stage by counting number of infected plants in each line and the per cent disease incidence was calculated. The disease intensity was scored adopting the following 1-9 score (Saikat Gantait and Prakash Kantidas, 2009) (Table-1).

DNA extraction**:** Genomic DNA was extracted from 20 days old leaves of parents before forced inoculation through C-TAB method (Murray and Thompson, 1986). The quality and quantity of DNA were checked through 1% agarose gel by electrophoresis. The DNA obtained was quantified using Nanodrop spectrophotometer.

SCAR marker analysis**:** Genomic DNA was used as template for PCR amplification as described by Williams *et al*., 1990. Amplification was carried out with 20µL reaction volume containing 1X Assay buffer, 2mM $MgCl_2$, 0.2mM dNTP, 10 picomole primer SCAR primer 20F/20R designed from the sequence of cloned RAPD product for specific amplification of the loci identified by selected RAPD marker, OPQ1,(Prasanthi *et al.*, 2011), 50-100ng of genomic DNA and 1 U of Taq DNA polymerase. Amplification was performed in 0.2ml thin walled tubes using a thermocycler (Corbett, Australia) programmed for initial denaturation at 94ºC for 2 minutes, followed by 35 cycles of denaturation at 94ºC for 1 minute, annealing at 67.4ºC for 1 minute, primer extension at 72ºC for 2 minutes and a final extension at 72ºC for 7 minutes. PCR amplified products were subjected to electrophoresis on 1% agarose gel in 1XTBE buffer. The electronic image of ethidium bromide stained gels were captured using Alpha imager system.

Results and discussion

The incidence of disease in the present study ranged form 0 – 92% among the test entries (Table-2). The data revealed that among the lines screened 19 lines were free from disease with one score and 12 lines showed 3-4 score with 5-15 % disease intensity and rest of the test lines showed susceptible reaction with the disease incidence ranging from 16 to 92%. Among the resistant lines, PDBG-10,PU-35,PU-30, BG-104 with high per plant yield can be utilized for direct cultivation, whereas other resistant lines can be used in varietal improvement programme as resistant sources because of their low yield and small dull seed type. Among the 12 moderately resistant lines, LBG-752, LBG-775, WBG-26, and LBG-784 are bold and shiny types and can be improved for YMV resistance through introgression or backcross breeding approaches. These varieties could be used further in blackgram varietal improvement programme.

In the absence of genome sequence information and other marker systems, SCAR marker designed from RAPD (OPQ1) primer was chosen for the present study though the parents are morphologically similar and shared ancestry, the level of polymorphism among the parents were moderate to high. This marker was utilized to test the lines that showed resistance to YMV in artificial inoculation.19 resistant lines which were free from disease incidence with disease grade of 1 have amplification as that of resistant parent PU-31 and PU-19 at 532 bp (Fig-1). Four MR lines WBG-26,LBG-775,LBG-784 and T9 also shown amplification as that of resistant parent. All Susceptible lines with disease incidence above 15% have shown absence of SCAR marker there by indicating the validation of marker for screening YMV disease in blackgram. The SCAR marker linked with this disease would hasten the introgression of the resistance gene and may be useful in mapping resistant gene and development of high yielding YMD resistant genotypes through MAS without the need of artificial inoculation.

Development of PCR based SCAR developed from RAPD markers is a method of choice to test YMV resistance in blackgram because it is simple and rapid. The marker was consistently associated with the genotypes resistant to YMV but susceptible genotypes without the resistance gene lacked the marker. These results are to be expected because of the linkage of the marker to the resistance gene. With the closely linked marker, quick assessment of susceptibility or resistance at early crop stage, it will eliminate the need for maintaining disease for artificial screening techniques.

The RAPD marker converted into simple SCAR marker SCAR20F/R is expected to increase efficiency and precision for selecting YMD resistant lines and for large-scale application in marker- assisted breeding . The conversion of a linked marker to SCAR has been applied successfully in a number of crops, like common bean (Alzate- Marin , *et al.,* 1999), greengram (Dhole and Reddy 2012) and soybean(Zheng *et al* 2003). The objective of the present study is to identify resistant sources through artificial screening and validating disease linked SCAR

marker. This facilitates plant breeders to carry out repeated genotyping throughout the growing season in the absence of any disease incidence. This method of genotyping would save time and labour during the introgression of MYMIV resistance through molecular breeding as methods of phenotyping against begomoviruses.

Many commercial blackgram varieties are susceptible to YMV and there is a need to identify other closely linked SSR and EST markers that could facilitate the transfer of the resistant genes to popular cultivars using marker-assisted breeding. High performing specific genotypic selection against YMD infection would be more rewarding as a positive step towards recombination breeding.

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Fig-1 Amplification profile of YMV SCAR 20F/20R in Blackgram genotypes for screening YMV resistance

M:1KB ladder 1)LBG-623 2)PU-31 3)LBG-770 4)LBG788 5)LBG772 6)LBG775 7)LBG771 8) LBG 784 9)LBG765 10)LBG776 11)IC1704 12)IC 11668 13)LBG-685 14)IC 37978 15)IC 49203 16)LBG-645 17)MASH-1-1 18)IC 6110 19)PDBG-10 20)PU-30 21)PBG-32 22)MASH-114 23)PU-35

Table1. Rating scale for YMV reaction on blackgram

% infection	Score	Disease reaction
0.1-5	1-2	resistant
5.1 -15	2.1- 4	Moderately Resistant
15.1 -30	4.1-5	Moderately susceptible
30.1 -75	5.1-7	Susceptible
75.1 -100	7.1-9	Highly susceptible

Table 2. Reaction of Blackgram lines to YMD under disease stress conditions in field during late *kharif*- 2012

Entry	SCAR Marker Status	YMV disease %	Score/ Rate	Disease reaction	Seed yield/plant (g)
PBG-1	A	36	6	S	2.0
LBG-752	A	9	3	MR	6.0
LBG-709	A	50	6	S	2.0
LBG-623	A	92	8	HS	0.0
LBG-780	A	23	5	MS	3.5
LBG-767	A	16	5	MS	3.0
LBG-782	A	30	5	S	3.0
LBG-763	A	5	2	MR	3.2
LBG-786	A	17	5	MS	4.0
LBG-733	A	8	3	MR	3.3
LBG-783	A	15	4	MR	3.5
LBG-770	A	22	5	MS	3.0
LBG-788	A	12	4	MR	3.1
LBG-772	A	5	3	MR	3.0
LBG-771	A	18	5	MS	4.0
LBG-765	A	20	6	S	2.8
LBG-776	A	15	4	MR	2.0
LBG-685	A	50	6	S	2.5
LBG-645	A	44	6	S	2.0
PBG-32	A	80	8	HS	0.0
PBG-107	A	55	6	S	2.0
PU-31	P	-	1	R	3.0
PU-205	P	-	1	R	3.5
PU1075	P	-	1	R	3.0
WBG-26	P	9	3	MR	6.5
T-9	P	15	4	MR	5.0
LBG-777	P	4	2	MR	3.0
LBG-775	P	13	4	MR	5.0
LBG-784	P	13	4	MR	2.6
IC-1704	P	-	1	R	3.0
IC-11668	P	-	1	R	3.5
IC-37978	P	-	1	R	4.0
IC-49203	P	-	1	R	3.8
MASH-1-1	P	-	1	R	3.8
IC -6110	P	-	1	R	4.2
PDBG-10	P	-	1	R	6.1
PU-30	P	-	1	R	6.5
MASH-114	P	-	1	R	4.8
PU-35	P	-	1	R	6.0
IC-59702	P	-	1	R	3.2
TBG-104	P	-	1	R	6.2
PU-19	P	-	1	R	3.2
TU94-2	P	-	1	R	2.5
MASH338	P	-	1	R	3.5
IC-14691	P	-	1	R	3.0

A: Absent; P: Present ; R: Resistant ; MR: Moderately resistant: MS: Moderately susceptible ; S: Susceptible; HS: Highly susceptible