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Research Article

Identification of MYMV resistant donors through agroinoculation and validation of linked marker(s) in black gram [*Vigna mungo (L.)*Hepper]

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Abstract
Black gram is highly prone to Mungbean yellow mosaic virus (MYMV) disease which affects its production and productivity. Agroinoculation for MYMV resistance/ susceptibility on six genotypes of black gram revealed that lines ADT (Bg) 3, CO (Bg) 6 were susceptible and the lines CO – BG 10-5, VBN (Bg) 6, VBN (Bg) 8, VBN (Bg) 4 were resistant to MYMV infection. The lines were also confirmed for resistance / susceptibility using MYMV gene specific coat protein primer by PCR analysis . For marker validation four molecular markers namely CEDG180, VMYR1, YR4 and CYR1which were previously identified as MYMV resistance linked in black gram were chosen and amplified. Among the four linked markers, CEDG180 was found polymorphic between resistance and susceptible black gram genotypes. The resistant lines were further substantiated using whitefly transmission and no symptoms were observed in resistant lines CO – BG 10-5, VBN (Bg) 6, VBN (Bg) 8, VBN (Bg) 4. Therefore, it is concluded that obtained markers could be effectively used for marker assisted selection of YMV resistance and resistant donors would act as a valuable donor in black gram breeding programs for incorporating YMV resistance.

Key words
Black gram, MYMV resistance, markers

Introduction

Black gram is a rich source of quality protein comes under the family *Fabaceae*. Proteins acquired from them serves a major part in vegetarian diet. The crop is prone to several pests and diseases among them the widely devastating viral diseases cause heavy yield losses (Paul *et al.*, 2013) and the most important damage amongst the virus is caused by *Mungbean yellow mosaic virus* (MYMV).The disease causing yellow mosaic virus belongs to *Begomovirus*, the largest genus of the family *Geminiviridae* (Dhakar *et al.*, 2010) and transmitted by whitefly in a circulative persistent manner (Sidhu *et al.*, 2009). In India, the annual pecuniary losses in legumes caused by yellow mosaic virus have been estimated to be approximately US $300 million per year (Varma and Malathi, 2003).Several genotypes resistant to YMV have been identified in black gram germplasms and development of MYMV resistant cultivars has long been a major objective in disease resistance breeding. However, MYMV field screening procedures for identification of resistance may often fails due to various external factors that alters transmission behavior, viral load and host nature. Considering above facts two major methods was found to overcome the inaccuracy in field evaluation. With a view towards developing a reliable laboratory screening protocol for assessing resistance/susceptibility against MYMV, Rogers *et al.* (1986) developed a new innovative technique called “Agroinfection” using the Ti plasmid of *Agrobacterium* for viral infection and demonstrated on tomato golden mosaic virus. The infectious viral clones are introduced into plants using *A. tumefacians* in this method which results in the production of symptoms in fifteen to twenty days after infection and interestingly molecular markers can also be used as a diagnostic tool to predict the presence of a specific gene with high accuracy in order to efficiently transfer the genes in different genetic backgrounds. The use of molecular markers for resistance genes is particularly powerful as it may avoid the delay in breeding programme associated with the phenotypic analysis (Paran and Michelmore, 1993). With the above mentioned facts , the study was conducted with the objectives of , i) Screening of black gram germplasm and TNAU released varieties through Agroinoculation, ii) Molecular characterization of MYMV in identified resistant lines of black gram, iii) Validation of already reported markers linked to YMV resistance in black gram.

Materials and Methods

A total of six diverse black gram TNAU released varieties namely CO – BG 10-5, VBN (Bg) 6, ADT (Bg) 3, CO (Bg) 6, VBN (Bg) 8, VBN (Bg) 4 differing for their response to YMV disease were used in the study for agroinoculation and marker validation. Agroinoculation was done on 2 days old sprouted seeds of selected germplasms by protocol suggested by Jacob *et al.* (2003) through MYMV infectious clone VA 239 (KA30 DNA A + KA27 DNA B) constructed by Balaji *et al.* (2004) and the protocol were as follows.

Briefly, *A. tumefaciens* harboring VA 239 (KA30 DNA A + KA27 DNA B) was grown in AB minimal medium (pH 7.0) at 28°C to an optical density of 1 at 600 nm. Later the grown cultures were centrifuged at 1,100 × g for 10 min at 25°C. The pellet was re-suspended in AB minimal medium (pH 5.6) supplemented with 100 µM acetosyringone. Two day old germinated *Vigna* seeds were immersed in the respective culture after puncturing the hypocotyl region with a 30-G needle. The infection was carried out at 25°C for 12 h in the dark. Subsequently, the seedlings were washed with sterile single distilled water and sown. After agroinoculation, the plants were maintained in a growth chamber at 25 ^{0}C with proper relative humidity of 60–70% and photoperiod of 16/18 h. Hoagland's solution was sprayed twice a week for proper growth and development of the plants. The symptom observation in the trifoliate leaves was recorded after 15th day from inoculation and the plants were identified as susceptible or resistance based on the presence or absence of yellow mosaic symptoms in a given time. The uninoculated plants of each accession without agroinoculation were maintained as control. The leaves with yellow mosaic symptoms were harvested 24 days after inoculation for DNA analysis was done as per the protocol of Sudha (2009) and were PCR confirmed for the presence of MYMV using CP gene specific primer (Maheswari, 2008).

A total of four molecular markers which were reported to be linked to MYMV resistance in black gram namely CEDG 180 (SSR marker), VMYR1 (RGA marker), YR4 (RGA marker), CYR1 (RGA marker) were used for the validation study. Reactions were performed in 20 µl mixture containing approximately 50 ng of genomic DNA, 50µM each dNTPs, 20 pmol of each forward and reverse primer and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The reaction was carried out in an Eppendorf gradient S Master Cycler (Eppendorf, Hamburg, Germany) programmed with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 10 min. Amplified products were analyzed by gel electrophoresis in TBE buffer and visualized by staining with ethidium bromide and recorded with an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA).

Whiteflies *(Bemisia tabaci)* were collected from different locations and were reared in closed insect free cages in different hosts. After PCR confirmation, new adults after three generations were collected using nylon clip cages from the whitefly maintained chamber by aspirator and were allowed for a period of starvation. After starvation, the clip cages containing whiteflies were clipped on to the MYMV agroinoculated symptomatic plants and allowed to feed for an acquisition access period (AAP) of 24h. After 24h AAP, *B. tabaci* adults were removed from symptomatic plants and are transferred into a separate insect free chamber for inoculation access period (IAP) of 24 h on resistant plants which were confirmed through agroinoculation for further substantiation. After 24 h of IAP, the *B. tabaci* adults were removed and the plants were sprayed with an insecticide and were evaluated for resistance.

Results and Discussion

Many commercial black gram varieties are susceptible to YMV and there is a need to identify the resistant donors and molecular markers linked to MYMV that could facilitate the relocation of the resistant genes in to popular cultivars using marker assisted breeding. In this study, screening of six black gram released varieties of TNAU through agroinoculation was done. Among the six lines, the varieties namely ADT (Bg) 3 and CO (Bg) 6 were found to be susceptible to MYMV infection. The lines CO – BG 10-5, VBN (Bg) 6, VBN (Bg) 8 and VBN (Bg) 4 were observed to be resistant (Fig 1). In all the germplasms, symptoms were observed 15 – 17 days of post agroinoculation and the data was scored for the presence of resistance/susceptibility and were confirmed through PCR (Fig 2). Similar to the above results, Jacob *et al.* (2003) co delivered MYMV DNA A and DNA B using single strain strategy and obtained 100% agroinfection. Usharani *et al.* (2005) conducted infectivity analysis of MYMV through agroinoculation in soybean isolate and obtained infectivity of about 70–95 percent. Similarly, Sudha *et al.* (2013) and Madhumitha *et al.* (2019) observed for 0–100 percent level of MYMV infectivity on agroinoculated plants and also even for the field-resistant genotypes. On this context, to prove infectivity and to identify the virus-resistant lines, agroinoculation method was found to be effective to deliver viral constructs into the plant.

In the present study four molecular markers namely CEDG180 (Gupta *et al*., 2013), VMYR1 (Basak*et al*., 2004), YR4 and CYR1 **(**Maiti*et al*., 2011) which were previously identified as MYMV resistance linked in black gram were chosen for validation and amplified. Among the four linked markers, CEDG180 was found polymorphic between resistante and susceptible black gram genotypes and other than that three markers failed to discriminate the resistance / susceptibility studied (Fig 3). Similar to our results Jegadeesan (2015) observed polymorphism between resistant and susceptible black gram genotypes for SSR marker CEDG180. In other studies also, it has been observed that molecular markers reported as linked to target resistance genes in specific mapping populations may not be polymorphic in other genetic backgrounds and hence may not be suitable for MAS (Prabhu *et al*., 2004; Bernardo *et al*., 2013). Transmission experiment for further substantiation using artificially reared *B. tabaci* revealed that 24 hr AAP and 24 hr IAP were observed to cause no infectivity to the resistant lines (CO – BG 10-5, VBN (Bg) 6, VBN (Bg) 8, VBN (Bg) 4). In the present study, to prove the Koch's postulates for MYMV using whitefly transmission under controlled conditions agroinoculation was carried out, which is an alternative method to introduce viral nucleic acids in to the plants. Several studies in the past have been carried out to define the host range of MYMIV using both agroinoculation and whitefly transmission (Maheshwari *et al.*, 2014; Usharani *et al.*, 2004). In the present investigation, it was observed that the vector transmits the virus from agroinoculated plants to the healthy plants. Further, PCR amplification using gene specific primer of YMV confirmed the presence of this virus in the agroinoculated plants. YMV is not sap transmissible, hence, vector transmission studies proved that the whitefly as a vector had transmitted the virus from the agroinoculated plants to healthy plants. In concordance with the earlier studies (Wege *et al.*, 2000), where agroinoculation is routinely used to prove Koch's postulates, this study also strengthen this assumption.

The molecular markers obtained in the present study are identified specific for the particular line. Therefore it could be beneficial for plant breeders for the utilization and improvement of the genotypes.

References

Balaji, V., Vanitharani, R., Karthikeyan, A., Anbalagan, S., and Veluthambi, K. (2004). Infectivity analysis of two variable DNA B components of Mungbean yellow mosaic virus-Vigna in Vigna mungo and Vigna radiata. *Journal of biosciences*, **29**(3), 297-308.

Dhakar, K., Gupta, V., Rathore, M., and Gaur, R. (2010). Virus resistance and gene silencing in plants infected with begomovirus. *Journal Applied Sciences*, **10**, 1787-1791.

Jacob, S., Vanitharani, R., Karthikeyan, A., Chinchore, Y., Thillaichidambaram, P., and Veluthambi, K. (2003). Mungbean yellow mosaic virus-Vi agroinfection by codelivery of DNA A and DNA B from one Agrobacterium strain. *Plant disease*, **87**(3), 247-251.

Jegadeesan, S. (2015). Validation of molecular markers linked to yellow mosaic virus disease resistance in diverse genetic background of black gram [Vigna mungo (L.) Hepper] (Vol. 6).

Maheshwari, R., Panigrahi, G., and Kathithachalam, A. (2014). Molecular characterization of distinct YMV (Yellow mosaic virus) isolates affecting pulses in India with the aid of coat protein gene as a marker for identification (Vol. 41).

Maheswari, R. (2008). DNA fingerprinting of Mungbean yellow mosaic virus (MYMV) affecting pulses. (M.Sc Thesis), TamilNadu Agricultural University.

Paul, P. C., Biswas, M. K., Mandal, D., and Pal, P. (2013). Studies on host resistance of mungbean against *mungbean yellow mosaic virus* in the agro-ecological condition of lateritic zone of West Bengal. *The Bioscan***, 8**(2), 583-587.

Rogers, S. G., Bisaro, D. M., Horsch, R. B., Fraley, R. T., Hoffmann, N. L., Brand, L., Elmer, J. S., and Lloyd, A. M. (1986). *Tomato golden mosaic virus* A component DNA replicates autonomously in transgenic plants. *Cell*, **45**(4), 593-600.

Sidhu, J., Mann, R., and Butter, N. (2009). Deleterious effects of *Cotton leaf curl virus* on longevity and fecundity of whitefly, *Bemisia tabaci* (Gennadius). *Journal of Entomology*, **6**(1), 62-66.

Sudha, M. (2009). DNA isolation protocol for *Vigna radiata* with free of phenolics. *Protocol Exchange*. doi: 10.5281/zenodo.15918

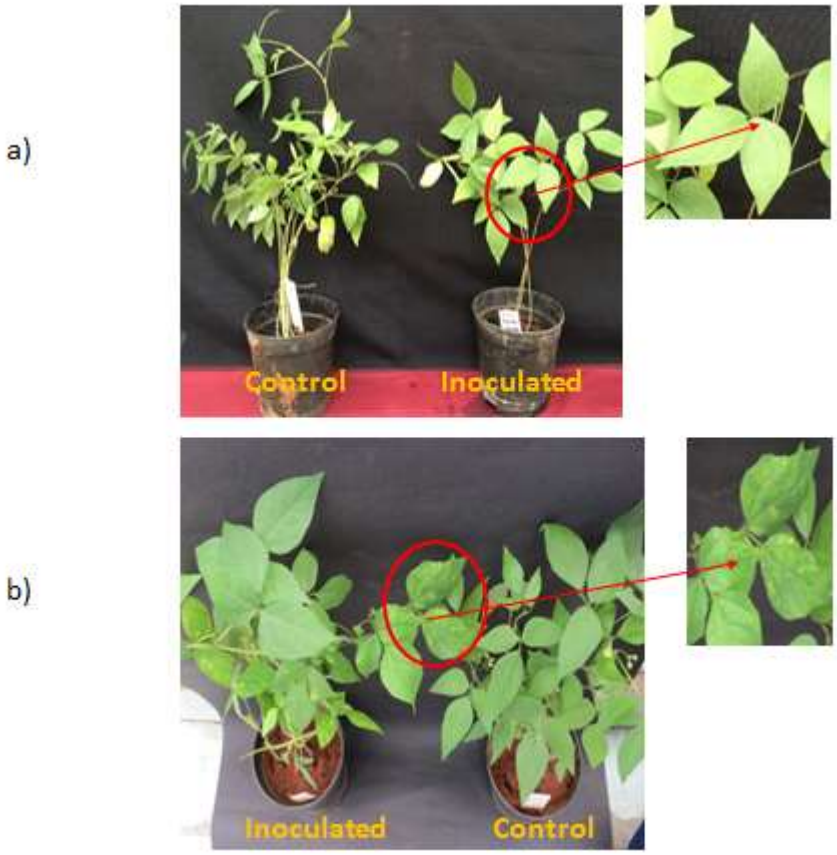
Sudha, M., Karthikeyan, A., Nagarajan, P., Raveendran, M., Senthil, N., Pandiyan, M., Angappan, K., Ramalingam, J., Bharathi, M., and Rabindran, R. (2013). Screening of mungbean (*Vigna radiata*) germplasm for resistance to *Mungbean yellow mosaic virus* using agroinoculation. *Canadian journal of plant pathology*, **35**(3), 424-430.

Usharani, K., Surendranath, B., Haq, Q., and Malathi, V. (2004). *Yellow mosaic virus* infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. *Current Science*, 845-850.

Usharani, K. S., Surendranath, B., Haq, Q. M., and Malathi, V. G. (2005). Infectivity analysis of a soybean isolate of *Mungbean yellow mosaic India virus* by agroinoculation. *Journal of General Plant Pathology*, **71**(3), 230-237.

Varma, A., and Malathi, V. (2003). Emerging geminivirus problems: a serious threat to crop production. *Annals of Applied Biology*, **142**(2), 145-164.

Wege, C., Gotthardt, R.-D., Frischmuth, T., and Jeske, H. (2000). Fulfilling Koch's postulates for *Abutilon mosaic virus*. *Archives of virology*, **145**(10), 2217-2225.

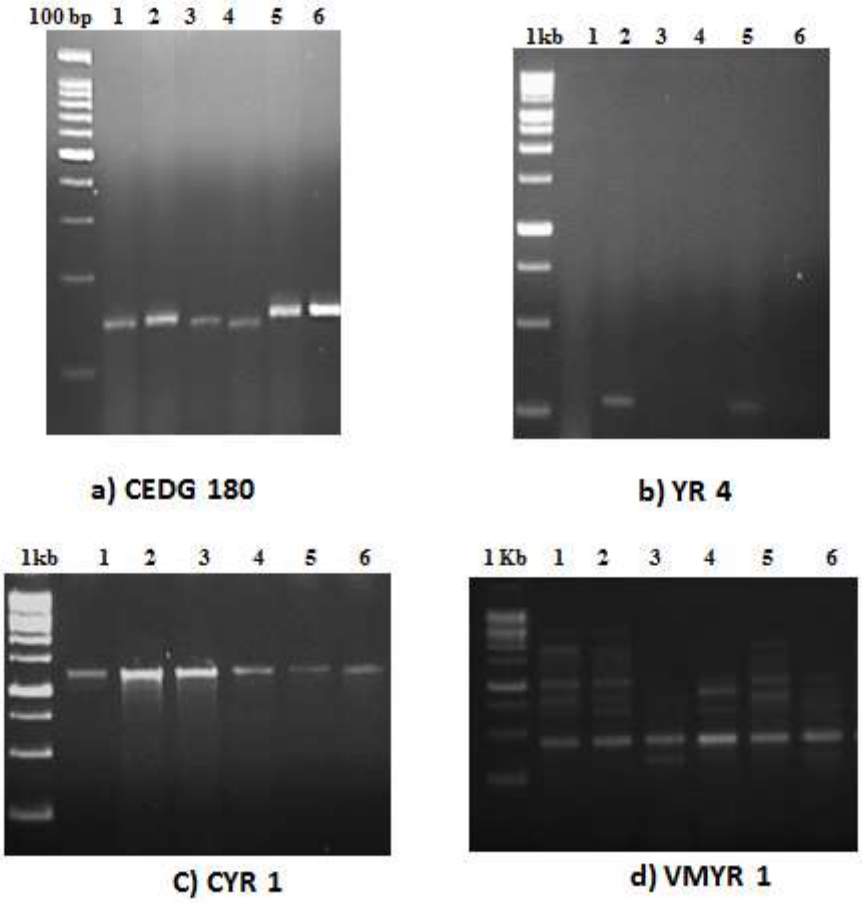


a) Resistant genotype – VBN (Bg)6
b) Susceptible genotype – CO (Bg) 6

Fig. 1. Mungbean yellow mosaic virus agroinoculated and control plants of resistant and susceptible black gram plants

lane M – 1 kb ladder, lane 1 - VBN (Bg) 6 control , lane 2 -VBN (Bg) 6 inoculated , lane 3 -CO –BG 10-5 control , lane 4 - CO –BG 10-5 inoculated, lane 5 -VBN (Bg) 8 control, lane 6 -VBN (Bg) 8 inoculated, lane 7 -VBN (Bg) 4 control, lane 8 - VBN (Bg) 4 inoculated, lane 9 -ADT (Bg) 3 control, lane 10 -ADT (Bg) 3 inoculated, lane 11 -CO (Bg) 6 control, lane 12 - CO (Bg) 6 inoculated.

Fig. 2. PCR confirmation of MYMV agroinoculated and controlplants of resistant and susceptible black gram plants



Lane 1 Co(Bg) 10 -0 - 5, Lane 2:VBN(Bg)4, Lane 3:VBN(Bg)6, Lane 4:VBN(Bg)8, Lane 5:ADT(Bg)3, Lane 6:Co(Bg)6

Fig. 3.Amplification of MYMV linked markers on resistant and susceptible black gram plants

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