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Molecular Screening for MYMV Resistance in Interspecific Hybrids of *Vigna raidiata* with Wild *Vigna* sp. using Tobacco N Gene Primers

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A B S T R A C T

Interspecific hybridization was made between mungbean yellow mosaic virus (MYMV) susceptible greengram variety CO 5 with MYMV resistant wild *Vigna sp*. Crossed seeds were raised in field condition for testing the MYMV incidence. F_1 showed dominant resistance reaction against MYMV disease incidence. The field growing F_2 populations of C0 5 x wild *Vigna* sp, cross produced a wide range of segregant *viz.*, susceptible, parental and intermediate type. F $_2$progenies were subjected to PCR amplification using tobacco N gene primers designed based on the tobacco N-gene and *Arabidopsis* RPS gene sequences. The PCR reaction was carried out and expected product was amplified at annealing temperature at 40°C. In parental polymorphisms, a detectable single band of 500bp being present in MYMV resistant Wild *Vigna* species, which was absent in MYMV susceptible CO 5 greengram variety. All the F_2 resistant individuals showed 500bp DNA band with use of the same tobacco N gene primers.

Introduction

Greengram, *Vigna radiata* (L) Wilczek is one of the important, ancient and well-known grain legume in almost all states of India. It is an excellent source of easily digestable and good quality protein for the growing population in many developing countries of south Asia and south-east Asia (Surashi *et al.*, 2017). Greengram seeds are favoured for their digestible protein with low flatulence, rich in phosphorus, provitamin-A and are relatively free from anti-nutritional factors (Adsule *et al.*, 1986). Greengram seed contains high amount of protein and lysine and or low amount of methonine amino-acid

profile compliment to cereals to form a balanced amino acid diet (Shahrajabian *et al.*, 2019). Greengram is widely grown in India and other countries like Pakistan, USA and Australia. India accounts for more than two-third of total production of greengram, for example annual production of greengram in Tamil Nadu is 1.21 lakhs tonnes cultivated in 1.89 lakhs ha with an average yield of 642 kg/ha (Mariyammal *et al.*, 2019). There are several biotic and abiotic factors are responsible for low productivity in greengram crop. Among the biotic stresses, the MYMV disease cause serious yield loss up to 85%. MYMV disease can be controlled by different methods (Paul *et al.*, 2013). Among the different methods, the host resistance alone is a best method of choice to control the MYMV diseases for long period.

Utilization of wild species for improving their cultivated counterparts is steadily increasing in various crops where genetic diversity in the primary gene pool is limited. The wild relatives of greengram such as *V. umbellata*, *V. vexillata*, *V. trilobata* and other wild *V. spp.* carrying desirable genes for many yield components coupled with resistance to MYMV. The use of wild *Vigna* species in greengram breeding programme has been difficult because of problems encountered in obtaining successful F_1 hybrids between cultivated and wild *Vigna* spp. due to crossability barriers. In spite of these difficulties, interspecific hybridization between *V. radiata* and its wild relatives of *Vigna* was successfully accomplished by many researchers (Renganayaki, 1985; Pandae *et al.*, 1990; Ganeshram 1993; Subramanian and Muthiah, 2000; Umamaheswari, 2002; Sidhu and Satija, 2003; Pandiyan *et al.*, 2010; Pandiyan *et al.*, 2012; Sudha *et al.*, 2013; Chitra *et al.*, 2018; Basavaraja *et al.*, 2019). Developing molecular markers or cloning genes of traits known only phenotypically is not an easy task; mungbean yellow mosaic disease is one such category. However, a handful of plant disease resistance (*R*) genes have been recently cloned by transposon tagging or map based cloning. These approaches are, on the other hand, time consuming and cumbersome. However, many laboratories followed Polymerase Chain Reaction (PCR)-based approach for isolating and using R genes and or Resistant Gene Analogous (RGA) for identification of MYMV resistance in greengram genotypes (Kabi *et al.*, 2017; Sai *et al.*, 2017; Patel *et al.*, 2018; Sing *et al.*, 2018). In the present study the occurrence of *R* gene (tobacco N gene) sequences was tested in the progenies derived from greengram with wild *Vigna* species for MYMV resistance.

Materials and Methods

The parental greengram (Var. CO 5) seed material was obtained from Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, and *Vigna* wild species was collected from Top slip area of Tamil Nadu. The ovule parents Var.C0 5 and tester parent (wild *Vigna* sp.) was raised in rows to accommodate 10 plants per row with an intra row 30 cm. The female parent was crossed with pollen parent and the crossed seeds were collected carefully. The emasculation and crossing in greengram were exercised following the procedure developed by Boling *et al.*, (1961). F_1 seeds were raised in the field for assessing the MYMV resistance based on morphological characters. The infector row method was followed for assessing the MYMV reaction in both F_1 and F_2 populations. The susceptible greengram variety CO 5 was planted every 10^{th} row of planting as the check (Singh, 1980).

The present study was also carried out with an aim to tag the MYMV resistance gene by using tobacco N gene primers. The amino

acid sequence homology between these two gene was 100% restricted to two short regions one in the kinase-1a or P-loof motif (GGVGKTT; Sarste *et al.,* 1990) of the nucleotide-binding site (NBS) and the second (GLPLAL; Mindrinos *et al.,* 1994) approximately 160 amino acids further downstream. The consensus sequences and possible primers are noted below.

Extraction of plant genomic DNA

A modified CTAB (Cetyl trimethyl ammonium bromide) DNA extraction technique of Gawel and Jarret, (1991) was followed to extract the genomic DNA from leaf tissue.

Primer sequences used for PCR

N' terminal sequence G G V G K T T
Forward 5' –GGI GGI GTI GGI AAI ACI AC- 3'
C' terminal sequence G L P L A L
Reverse 5' –IAG IGC IAG IGG IAG ICC - 3'

The PCR was performed for both parents and progenies The présence and absence désirable band of through agarose gel electrophoresis (Sambrook *et al.,* 1989)

Results and Discussion

Expeditions and evaluation of wild *Vigna sp*

The expeditions and collection of wild *Vigna* sp. in resistance to yellow mosaic virus disease was carried out at two different places *viz*., top slip area of Tamil Nadu and Kotaimaidan (Western Ghat) area of Kerala. Among the expeditions, one wild *Vigna* species was collected from Top slip area of Tamil Nadu. The collected wild *Vigna* species was raised in the field for assessing the yellow mosaic virus incidence using susceptible variety CO 5 as check. The collected wild *Vigna* species was phenotypically similar to cultivated *Vigna* species (Fig.1- P_2). The flower buds, pod and seed colours were closely resembles the cultivated *Vigna* species. The collected wild *Vigna* species was phenotypically resistant to yellow mosaic viral disease.

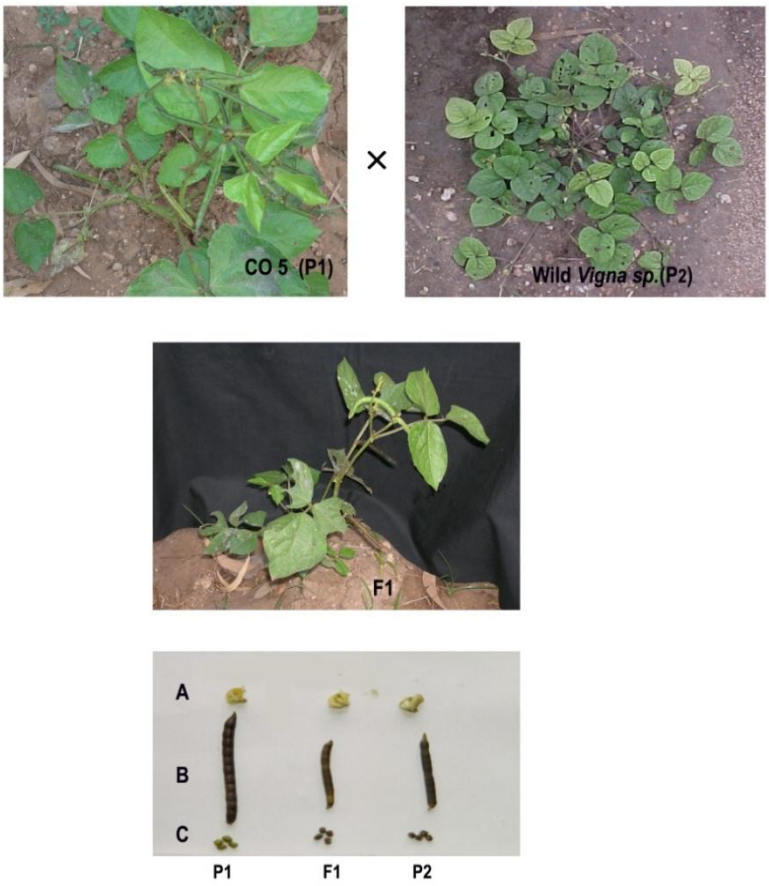
Evaluation of F_1 and F_2 segregants

Interspecific hybrdization has been made between MYMV susceptible greengram variety CO with MYMV resistant wild *Vigna sp* and F1 plants were produced (Fig. 1). The hybridization was done with more than 500 flowers and obtained only few pods (12-15 seeds). The occurrence of low pod and seed set is mainly due occurrence of pre and post fertilization barriers. These results are agree with the findings of Gopinathan *et al.,* (1986), Mendioro and Ramirez, (1994), James *et al.,* (1999), Uma Maheshwari (2002), Pandiyan *et al.,* (2010) and Chitra *et al.,* (2018). The field raised F_1 plants showed dominant resistance reaction against MYMV disease incidence. A similar finding was also reported by Jain *et al.,* (2013) in mungbean varietal crosses. The selfed seeds of the F_1 hybrid was collected and raised in the field for evaluating the MYMV incidence. The segregating population produced a wide range of segregant *viz.,* susceptible, parental and intermediate type (Fig 2). F_2 population produced a wide range of segregants *viz.,* susceptible, parental and intermediate types (Fig. 2). Similarly, Satija and Ravi (1996) reported that hybrids of the cross *V. radiata* x *V. umbellata* were either transgressive or intermediate compared to their parents. Earlier same findings also reported for the production of fertile hybrids with intermediate phenotypes compared to both the parents (Monika *et al.,* 2001; Uma Maheswari, 2002; Sidhu and Satija, 2003; Chitra *et al.,* 2018;

Basavaraja *et al.*, 2019). These results are also agree with the results of Parida *et al.*, (1985), Subramanian and Muthiah, (2000), Premkumar *et al.*, (2007), Pandiyan *et al.*, (2010), Sudha *et al.*, (2013) and Chitra *et al.*, (2018) in interspecific hybridization of *Vigna* species.

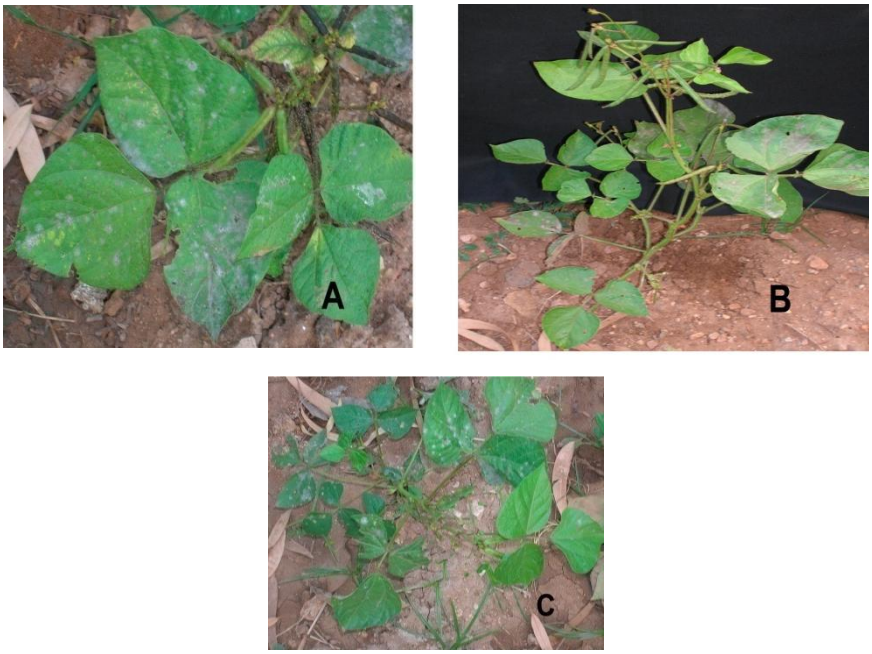
Molecular screening F_2 population by using tobacco N gene primers

In order to understand whether there are R Gene sequences whose products contain NBS and LRR motifs in mungbean, the F_2 progenies DNA subjected to PCR amplification using tobacco N gene primers designed based on the tobacco N-gene and Arabidopsis RPS gene sequences. The PCR reaction was carried out and expected product was amplified at annealing temperature at 40°C. In parental polymorphisms, a detectable single band of 500bp being present in MYMV resistant Wild *Vigna* species, which was absent in MYMV susceptible CO 5 greengram variety. All the F_2 resistant individuals showed 500bp DNA band with use of the same tobacco N gene primers (Fig. 3). This band corresponds to the product size expected from the primers designed as evidenced in studies on potato (Leister *et al.*, 1996) and soybean (Yu *et al.*, 1996). Whitham *et al.*, (1996) demonstrated that the tobacco N gene confers a hypersensitive responsive to tobacco mosaic virus (TMV) disease in transgenic tomato. He also found the expression of N gene is effectively inhibit and systemic movement of TMV in transgenic tomato.



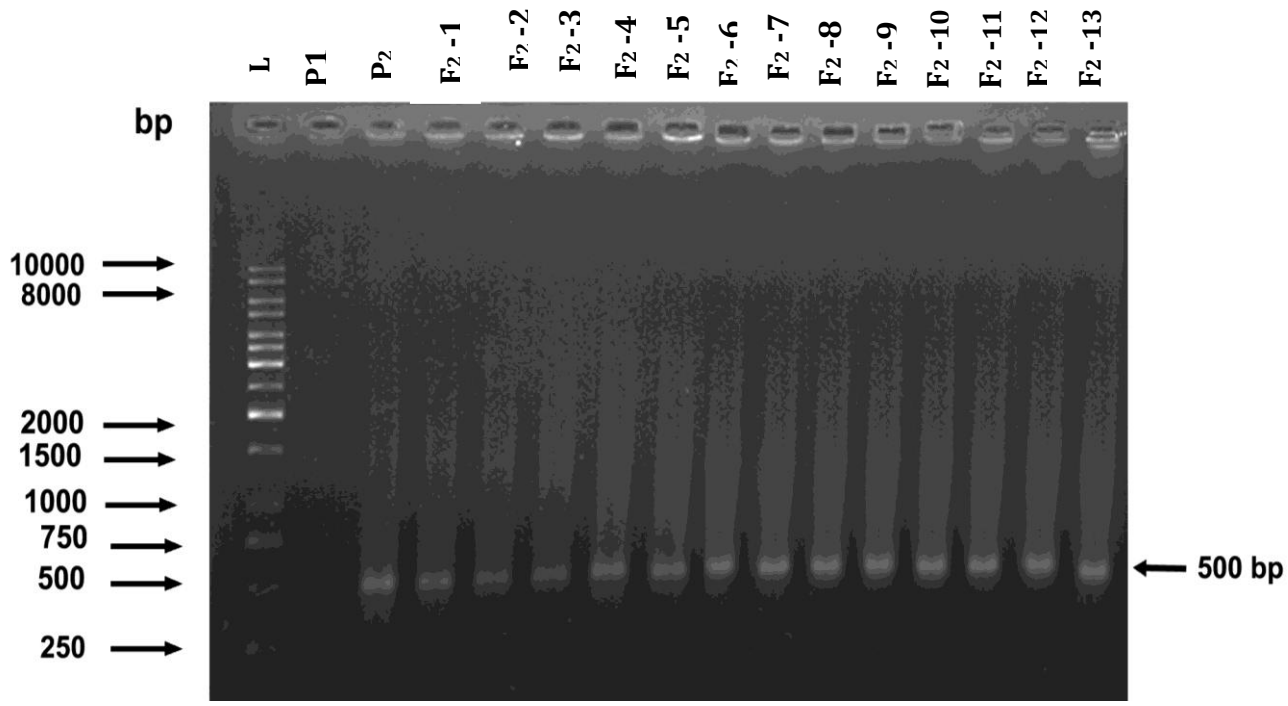
(A- Flower bud, B- Pod, C-Seed)

Fig.1 F_1 cross of CO5 greengram × Wild *Vigna sp.*



(A- Susceptible B- Resemble to CO 5 parent C- Intermediate -Hybrid)

Fig.2 F_2 sergeants of CO 5 × wild *Vigna* sp.



(L-DNA ladder; P_1 – Co5; P_2- Wild *Vigna* sp.)

Fig.3 PCR profile of parental and F_2 resistant individuals using Tobacco N gene primers

In the present study, the expeditions and search for MYMV resistant wild *Vigna* species was undertaken and collected one wild *Vigna* species. The *Vigna* species was phenotypically resistant to MYMV. Interspecific hybridization was made between elite, MYMV susceptible greengram Var. CO 5 × wild *Vigna sp.* and progenies followed up

to F_2 generation. In molecular analysis, all F_2 Progenies, wild *Vigna sp*. amplified a 500bp DNA products and susceptible parent CO 5 didn't yield any characteristic PCR products using tobacco N gene-primer sequences.

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