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Development of Yellow Mosaic Virus (YMV) resistance linked DNA marker in *Vigna mungo* from populations segregating for YMV-reaction

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Abstract

Yellow mosaic virus, YMV, causes one of the most severe of biotic stresses in Vignas, an important group of pulse crops. The viral disease is transmitted through the white fly, *Bemicia tabaci*, and the yield of the plants is affected drastically. YMV-tolerant lines, generated from a single YMV-tolerant plant identified in the field within a large population of the susceptible cultivar T-9, were crossed with T-9, and F_1, F_2 and F_3 progenies raised. The different generations were phenotyped for YMV-reaction by forced inoculation using viruliferous white flies. A monogenic recessive control of YMV-tolerance was revealed from the F_2 segregation ratio of 3:1 (susceptible: tolerant), which was confirmed by the segregation ratio of the F_3 families. Of 24 pairs of resistance gene analog (RGA) primers screened, only one pair, RGA 1F-CG/RGA 1R, was found to be polymorphic among the parents. Selected F_2 individuals and F_3 families were genotyped with the polymorphic RGA primer pair and the polymorphism was found to be linked with YMV-reaction. This primer pair amplified a 445bp DNA fragment only from homozygous tolerant and the heterozygous lines. The 445bp marker band was sequenced and named '*VMYR1*'. The predicted amino acid sequence showed highly significant homology with the NB-ARC domain present in several gene products involved in plant disease resistance, nematode cell death and human apoptotic signaling. To the best of our knowledge, this is the first report of YMV-resistance linked DNA marker development in any crop species using segregating populations. This YMV-resistance linked marker is of potential commercial importance in resistance breeding of plants.

Introduction

In South East Asia, pulses are a major source of dietary protein and *Vigna mungo* (black gram, urd bean) is one of the most important cash crops. Yellow mosaic virus (YMV) transmitted through the white fly, *Bemisia tabaci* Genn. (Nariani 1960; Nene 1972, 1973), is one of the most devastating types of biotic stresses that can cause up to 100% damage to a large number of leguminous crops. The most seriously affected grain legumes are mung bean, black gram and soybean, which are excellent sources of easily digestible protein of low flatulence. YMV infection to the leguminous crops, including these three, causes an annual loss of US$300 million (Varma et al. 1992). YMV belongs to the Gemini virus group and the bipartite genome consists of two circular single-stranded DNA molecules, commonly referred to as DNA-A and DNA-B (Varma et al. 1992). Both molecules are required for infectivity.

Despite the severity of the damage caused by YMV, development of a sustainable resistance against YMV through conventional breeding has not yet been successful in this part of the globe. However, considering the importance of *Vignas* as pulse crop, development of YMV-resistant varieties is of prime

importance for stabilizing the yield levels. Introduction of YMV-resistant pulses in farmer's field may also reduce insecticide application.

The advent of DNA-marker technology in the 1980s has dramatically enhanced the efficiency of plant breeding. Tanksley (1983) was the proponent of marker assisted genotype selection. Later, Tanksley and his associates (1989) established that plant breeders can use molecular markers to select, indirectly, individuals in segregating populations that carry a gene for a desirable trait provided a tight linkage exists between the marker and the genetic locus that controls the trait. These environmentally insensitive molecular markers are being widely and efficiently used to follow the pattern of inheritance of a number of desirable agronomic traits adequately (Peleman and van der Voort 2003), while selection for resistance genes on a phenotypic scale is time consuming (Procunier et al. 1997). It is therefore an ideal strategy to search for DNA marker/s linked with YMV-resistance, which is of significance as the presence of the resistance (R) gene, can be rapidly detected to screen populations for YMV-reaction. Similar approaches have been undertaken to screen *Lr9* leaf rust resistance and bunt resistance genes in wheat (Scachermayr et al. 1994; Demeke et al. 1996). Among the various molecular markers that are being used to screen resistance genes, use of RGA-markers is comparatively recent (Chen et al. 1998) and can be conveniently designed from the diagnostic motifs of known disease resistance genes (Kanazin et al. 1996; Huang and Gill 2001; Yan et al. 2003).

In 1984, a single YMV-tolerant plant, probably a natural mutant, was identified in a large population of the susceptible cultivar T-9 in the field. A total of six progeny lines were obtained from this YMV-tolerant plant through selfing. In this investigation, *V. mungo* populations segregating for tolerance/susceptibility were raised by crossing two of the YMV-tolerant lines, VM-1 and VM-4, with T-9 and a simple method was adapted for phenotyping the populations segregating for YMV-reaction to determine the genetic nature of the YMV-resistance. Here we report the development of a RGA-based polymorphic marker, '*VMYR1'*, linked with YMV-resistance. The sequence of the marker and the predicted amino acid sequence indicate that '*VMYR1'* is a part of a candidate gene for YMV-resistance.

Materials and methods

Genesis of the YMV-tolerant lines

Vigna mungo cv. T-9, a YMV-susceptible cultivar, was collected from Baharampur Pulse and Oil Research Centre, West Bengal, India. Six YMV-tolerant lines, (VM-1 to VM-6), were developed from an apparently YMV-tolerant *V. mungo* plant, by selfing, which was identified in a large population of the susceptible (ca. 5000 plants) but agronomically superior cultivar T-9, at the Madhyamgram Experimental Farm, Bose Institute, Kolkata, India. The seeds of these six lines were inbred for five generations prior to any experimentation. Of these six lines VM-1, VM-4, and VM-6 were tested at the Indian Agricultural Research Institute (New Delhi, India) by Dr V.G. Malathi through the Agroinfection method (by infecting with T-DNA containing coat protein gene of YMV; Jacob et al. 2003) and the YMV-tolerant nature of these three lines confirmed.

Development of populations segregating for YMV-reaction

The susceptible cultivar T-9 (female) was crossed with the two best performing tolerant lines, VM-1 and VM-4 (males), during October to December 1999. Out of a total of 1063 individual flowers crossed, 233 putative F_1 plants from the combination T9/VM-1 and 37 putative F_1 plants from the combination T9/VM-4 were grown during February to May 2000 along with the parents. Seeds from selfed individual F_1 plants were collected, half of the seeds from each F_1 plants were stored, and the other half sown to raise F_2 populations along with the parental lines and a known resistant check (cv. 'Sarada').

Phenotyping the populations segregating for YMV-reaction by forced inoculation method

The parental lines and the F_2 populations were screened from July to September 2000, by forced inoculation and under natural field epiphytotic condition. For forced inoculation, white flies were collected from the plants and confined in a susceptible plant showing typical YMV symptoms for 24 hrs using a small, transparent glass trapper with a spring cap. The same trapper with the trapped flies was next attached to an apparently healthy plant and the viruliferous insects were allowed to feed on the leaf for 24 h. After

Table 1. Resistance gene analog primers and the respective annealing temperatures.

Code	Nucleotide Sequence	Annealing temperature
RGA-1- F-CC	: 5′-AGT TTA TAA TTC CAT TGC T-3′	45 °C
RGA-1- F-CG	: 5′-AGT TTA TAA TTC GAT TGC T-3′	45 °C
RGA-1- F-TC	: 5′-AGT TTA TAA TTT CAT TGC T-3′	45 °C
RGA-1- F-TG	: 5′-AGT TTA TAA TTT GAT TGC T-3′	45 °C
RGA-1- R	: 5′-ACT ACG ATT CAA GAC GTC CT-3′	45 °C
RGA-2- F	: Same as RGA-1-Fs	
RGA-2- R	: 5′-CAC ACG GTT TAA AAT TCT CA-3′	As given in respective forward primer
RGA-3- F	: Same as RGA-1-Fs	
RGA-3- R	: 5′-CTC TCG ATT CAA AAT ATC AT-3′	As given in respective forward primer
RGA-4- F	: 5′-TGT TAC TGC TTT GTT TGG TA-3′	50 °C
RGA-4- R	: 5′-TAC ATC ATG TGT TAC CTC T-3′	50 °C
RGA-5- F	: 5′-TGC TAG AAA AGT CTA TGA AG-3′	50 °C
RGA-5- R	: 5′-TCA ATC ATT TCT TTG CAC AA-3′	50 °C
RGA-6- F	: 5′-AGC CAA AGC CAT CTA CAG T-3′	50 °C
RGA-6- R	: 5′-AAC TAC ATT TCT TGC AAG T-3′	50 °C
RGA-7- F	: Same as RGA-1-Fs	
RGA-7- R	: 5′-CCG AAG CAT AAG TTG CTG-3′	As given in respective forward primer
RGA-8- F	: 5′-AGC GAG AGT TGT ATT TAA G-3′	50 °C
RGA-8- R	: 5′-AGC CAC TTT TGA CAA CTG C-3’	50 °C
RGA-9- F	: Same as RGA-1-Fs	
RGA-9-R	: 5′-GTC-TTG-AAA-GCA-GCG-GGA-GC-3′	As given in respective forward primer

Table 2. Segregation of YMV-reaction on forced inoculated F_2 individuals.

Cross combination	No. of plants	YMV-reaction		Expected ratio	Degree of freedom	χ^2	P
		Susceptible	Tolerant				
T-9 X VM-1	191	138	53	3:1	1	0.77	<0.50
				15:1	1	136.49	<0.001
T-9 X VM-4	278	203	75	3:1	1	0.58	<0.50
				15:1	1	184.38	<0.001

Table 3. Segregation of F_2 population for YMV-reaction under natural condition.

Cross combination	No. of plants	YMV-reaction		Expected ratio	Degree of freedom	χ^2	P
		Susceptible	Tolerant				
T-9 X VM-1	245	177	68	3:1	1	0.992	>0.30
T-9 X VM-4	619	410	209	3:1	1	25.357	<0.001

acquisition feeding, the flies were used for 3–5 transfers for inoculation feeding, thereby allowing the viruliferous flies to transmit YMV into the plant. In the forced inoculation experiment, YMV-reaction was observed on the parental lines, 191 F_2 plants obtained from 12 putative F_1s of the cross T9/VM-1 and on 278 F_2 plants obtained from 18 putative F_1s of the cross T9/VM-4. Data on YMV-reaction under natural epiphytotic condition was obtained from 245 F_2 plants derived from 15 putative F_1s in the cross T9/VM-1 and on 619 F_2 plants derived from 30 putative F_1s in the cross T9/VM-4.

In March 2001, 200 F_3 families (25 plants/family) from the cross T9/VM-1 and 100 F_3 families from the cross T9/VM-4 were sown. The YMV-reactions of F_3 plants were assessed by forced inoculation and under field epiphytotic condition to confirm the inheritance pattern of YMV-tolerance.

Isolation of PCR- compatible genomic DNA

Genomic DNA was isolated from a number of source tissues including whole mature seeds, imbibed seeds (without seed coat), mature field grown leaves and etiolated seedlings using a modified protocol (Dellaporta et al. 1983). Briefly, tissues were ground to a fine powder using mortar and pestle in liquid nitrogen. The powder was extracted for 30 min at 60 °C using a high salt extraction buffer (50mM Tris pH 8.0, 100mM EDTA pH 8.0, 150mM NaCl, 1.8% SDS, 1.0% PVP). The supernatant was extracted 3-4 times with chloroform-isoamyl alcohol (24:1), 2.5 volume of chilled alcohol added, stored at − 20 °C overnight and centrifuged at 10,000g for 10min. The pellet was suspended in TE buffer (10mM Tris and 1mM EDTA, pH 8.0). RNA was removed by RNase treatment. Nuclear DNA was isolated from *V. mungo* cv. T-9, the two tolerant lines, VM-1 and VM-4, and from F_2 and F_3 individuals showing YMV-tolerance and susceptibility.

Screening RAPD and RGA primers for polymorphism amongst the parents

To detect polymorphism between the YMV-susceptible cultivar, T-9, and the tolerant lines, VM-1 and VM-4, 50 RAPD (Operon) primers and 24 pairs of resistance gene analog (RGA) primers designed from 'R' genes of soybean (Kanazin et al. 1996; Table 1) were used in PCR. Amplification was carried out in 50μl reaction mixture containing 100ng of genomic DNA, 100ng of each primer, 1X PCR buffer containing 1.5mM $MgCl_2$, 250μM of each dNTPs and 1unit of Taq DNA polymerase (Bangalore Genei, India), using a Perkin Elmer Cetus 2400 thermal cycler. The following cycling programme was used: a first cycle of 5min at 94 °C, followed by 35 cycles of 45s at 94 °C, 45s at the annealing temperature, followed by 1min at 72 °C, and a final extension of 10min at 72 °C. The annealing temperature for RAPD primers was 37 °C and that for RGA primer pairs was 45 °C with some exceptions as given in Table 1. The amplified PCR products were separated in 1.5% agarose gels and visualized by ethidium bromide staining.

Genotyping of phenotyped segregating F_2 and F_3 individuals

The primer pair, RGA 1F-CG/RGA 1R, polymorphic amongst the parents, was used to genotype the F_2 and F_3 plants of known phenotype for detecting linkage, if any, with YMV-reaction.

Sequence Analysis

The 445bp marker band amplified from three sources, VM-1, one screened tolerant F_3 individual and one heterozygous susceptible F_3 individual, was cloned separately in pGEM-T vector using a kit following the supplied protocol and sequenced at the University of Delhi, South campus, India, using a ABI Prism 3100 automated DNA sequencer. The marker band was named '*Vigna mungo* yellow mosaic virus resistance 1' (*VMYR1*). Nucleotide and peptide-sequence similarities between the '*VMYR1'* and other published sequences were determined by searches through the GenBank non-redundant database using the computer program BLAST (Basic Local Alignment Search Tool; Altschul et al. 1997) at the website http://www.ncbi.nlm.nih.gov/BLAST/. To ascertain the presence of conserved domain(s) in '*VMYR1'*, the NCBI conserved domain database was also searched.

Results

Phenotyping the populations segregating for YMV-reaction

The lines involved in the crosses and the F_1's were screened for YMV-reaction under field epiphytotic conditions with abundant white fly population during the months of February to May, 2000. The F_1 plants were susceptible to YMV signifying the recessive nature of YMV-tolerance.

The YMV-reaction data were collected from the parental lines and from the F_2 population by forced inoculation and under natural field conditions. A total of 469 F_2 plants, derived from the two crosses, T9/VM-1 and T9/VM-4, were forced inoculated and the results are given in Table 2. The observed segregating pattern fits almost perfectly a 3 susceptible: 1 tolerant ratio, which is in agreement with the presence of a single recessive locus in the YMV-tolerant lines VM-1 and VM-4. The assumption of 15:1 was rejected by the χ^2 tests (Table 2). Although the segregation pattern of YMV-reaction under natural conditions in one F_2 population (T9/VM-1; 245 plants) corroborated with monogenic recessive control of YMV-tolerance, data on the other F_2 population (T9/VM-4; 619 plants) did not (Table 3). Most probably

this was due to susceptible F_2 plants escaping natural inoculation in the later population.

In March 2001, YMV-reaction of F_3 plants and families from the two cross combinations was assessed by forced inoculation and under natural field conditions. None of the F_3 families derived from tolerant F_2 plants, categorized as tolerant upon forced inoculation, segregated for YMV-reaction. The F_3 families, derived from phenotyped susceptible F_2 plants, segregated in a ratio of 2 (families segregating for YMV-reaction): 1(families non-segregating susceptible). Thus the F_3 progeny test confirmed the monogenic recessive control of YMV- tolerance.

Isolation of PCR-compatible genomic DNA from V. mungo

Isolation of PCR-compatible genomic DNA from leaf tissues of *V. mungo* was found to be difficult with two commonly used protocols; the CTAB (Doyle and Doyle 1990) and the Benzyl chloride (Zhu et al. 1993) methods; the problem was circumvented by using a modified Dellaporta et al. (1983) protocol as given in materials and methods. The best nuclear DNA yield and quality was obtained from etiolated seedlings and green leaf tissues using this protocol.

Screening RAPD and RGA primers for polymorphism amongst the parents

A total of 50 RAPD primers were used to detect polymorphism amongst the parental tolerant lines, VM-1 and VM-4, and the susceptible T-9. All of the amplified DNA profiles (on an average 8 bands/ primer; data not shown) were monomorphic amongst the three lines signifying a high degree of sequence similarity amongst the genomes. Of the 24 RGA primer pair combinations tested, only one (RGA 1F-CG/RGA 1R) produced detectable polymorphism with a single band of 445bp being present in VM-1 and VM-4, which was absent in T-9 (Figure 1). All other RGA primer pairs generated monomorphic bands with an average of 4.63 bands per primer pair (data not shown).

Development of a DNA- based marker linked with YMV-tolerance and genotyping of the F_2 and F_3 segregating populations

The polymorphic primer pair, RGA 1F-CG/RGA 1R, was used to genotype the individuals of known phenotypes from the F_2 (Figure 2 A) and F_3 populations (data not shown). RGA 1F-CG/RGA 1R amplified a 445bp DNA band from all the phenotyped YMV-tolerant individuals originating from the two crosses (T9/VM-1 and T9/VM-4); 300 from F_2 and 75 from F_3. None of the F_3 families raised from screened tolerant F_2 individuals segregated, i.e. all the individuals of these F_3 families remained YMV-tolerant and all of them produced the 445bp band.

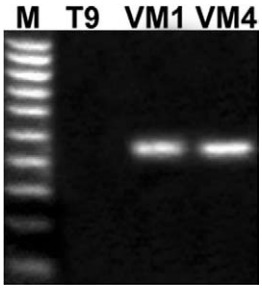

Figure 1. PCR amplification of a single 445 band in the two tolerant parents, VM-1 and VM-4. The band is absent in the susceptible parent T-9. M = Molecular weight marker (100bp ladder).

This primer pair generated the 445bp band in 144 out of 220-screened susceptible F_2 individuals, which followed the segregation ratio of 2:1 ($\chi 2 = 0.1458$; $p = > 0.70$), for the presence or absence of the DNA band, expected from the monogenic recessive control for YMV-tolerance. The genotypes of YMV-susceptible F_2 individuals are expected to be in the ratio of 2 heterozygous: 1 homozygous, and the heterozygous individuals are expected to produce the 445bp band.

A total of 140 forced inoculated, susceptible F_3 individuals (obtained from forced inoculated susceptible F_2 individuals) were also genotyped with the RGA 1F-CG/RGA 1R. These individuals segregated for the presence or absence of the 445bp band at the ratio of 1:1 ($\chi 2 = 1.028$; $p = > 0.30$), 76 individuals producing the 445bp band while the rest of the 64 individuals did not produce any band. The ratio of 1:1 for the presence or absence of the 445bp band is expected when one susceptible F_3 individual per F_3 family derived from a susceptible F_2 plant is genotyped at random.

Combining the results, that all the screened tolerant F_2 and F_3 individuals produce the 445bp band with RGA 1F-CG/RGA 1R without any segregation, that the screened susceptible F_2 individuals segregate at the expected ratio of 2:1 for the presence or absence of the 445bp band, and that the screened susceptible F_3 individuals segregate at the expected ratio

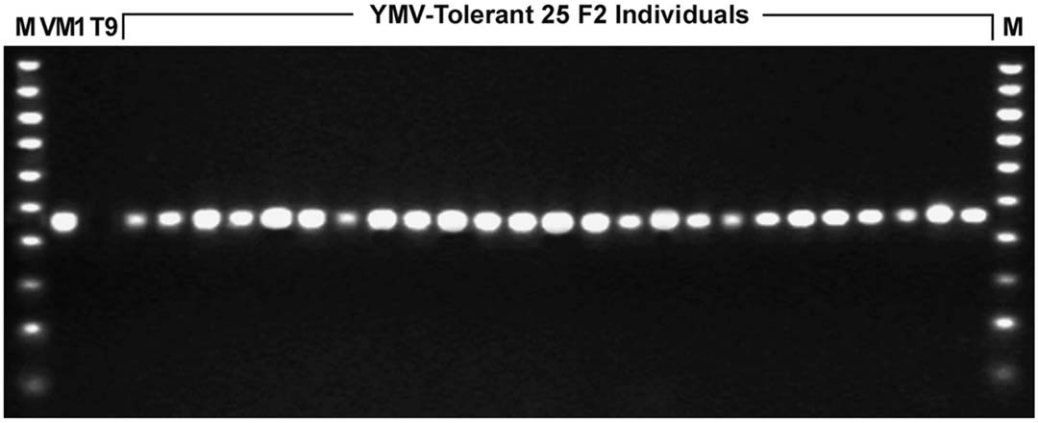

Figure 2. Detection of PCR amplified 445bp marker band, linked with YMV resistance in selected individuals of F_2 segregating population of *Vigna mungo*. Genomic DNA from tolerant and susceptible parents, VM-1 and T-9, respectively, along with YMV- tolerant F_2 individuals, amplified with RGA 1F-CG/RGA 1R primer pair, resolved in 1.5% agarose gel and stained with ethidium bromide. M = Molecular weight marker (100bp ladder).

of 1:1 for the presence or absence of the 445bp band, confirm that the YMV-tolerance in this cross combination is a monogenic recessive trait and that the 445bp band is linked with YMV-tolerance. The 445bp marker band was named '*VMYR1'* (*Vigna mungo* yellow mosaic virus resistance 1).

Sequence Analysis

The '*VMYR1'* was found to have sequence similarity with more than 500 GenBank accessions. Majority of these accessions were either plant 'R' genes or putative or partial 'R' gene sequences. The highest homology (84%) with '*VMYR1'*, of both nucleotide and predicted amino acid sequences, was obtained with a resistance gene *R1* from *Glycine max* with an Expectation value of 4e–60.

The BLAST search with '*VMYR1'* sequence to ascertain the presence of any conserved domain revealed that it is within a NB-ARC (Nucleotide Binding, *APAF-1* in humans for apoptotic signaling, 'R' genes in plants and *CED-4* in nematode for cell death) domain, a novel signaling-motif shared by plant 'R' gene products and regulators of cell death in animals. The multiple alignment of the predicted amino acid sequence of '*VMYR1'* with few selected 'R' genes from plants containing NB-ARC domain, *CED-4* and *APAF-1* is shown in Figure 3. '*VMYR1'* has been given the GenBank accession No. AY297425.

Discussion

In India, breeders have attempted to generate YMV-resistant *Vigna mungo* genotypes through classical breeding and the genetic studies revealed two recessive genes for resistance to the disease (Singh 1980, 1981; Shukla 1978).

In this report, the inheritance of YMV-resistance in crosses of *V. mungo* was studied. Segregation into tolerant and susceptible individuals in progenies of crosses between two tolerant lines, VM-1 and VM-4, with the susceptible cultivar T-9 showed expression of one 'R' gene in the tolerant individuals studied both under natural and artificial screening conditions. Assuming Mendellian inheritance, the almost perfect fit to a ratio of 3:1 (susceptible: resistance) for segregating progenies suggest the action of a single recessive gene in homozygous state conferring YMV-resistance in *V. mungo* (Table 2). The deviation from the expected 3:1 segregation for YMV-reaction in the second F_2 population (T9/VM-4) under natural condition was probably due to escapes.

By understanding the genetic basis of the YMV-reaction trait and the allelic variation at the locus, the breeder would be able to design superior genotypes of *V. mungo*.

In our experiments, YMV-resistance of *V. mungo* is controlled by a single recessive gene as determined by classical genetical analysis, which was confirmed by the development of a linked DNA marker of 445bp named '*VMYR1'*. To the best of our knowledge, this is the first report of YMV-resistance linked DNA

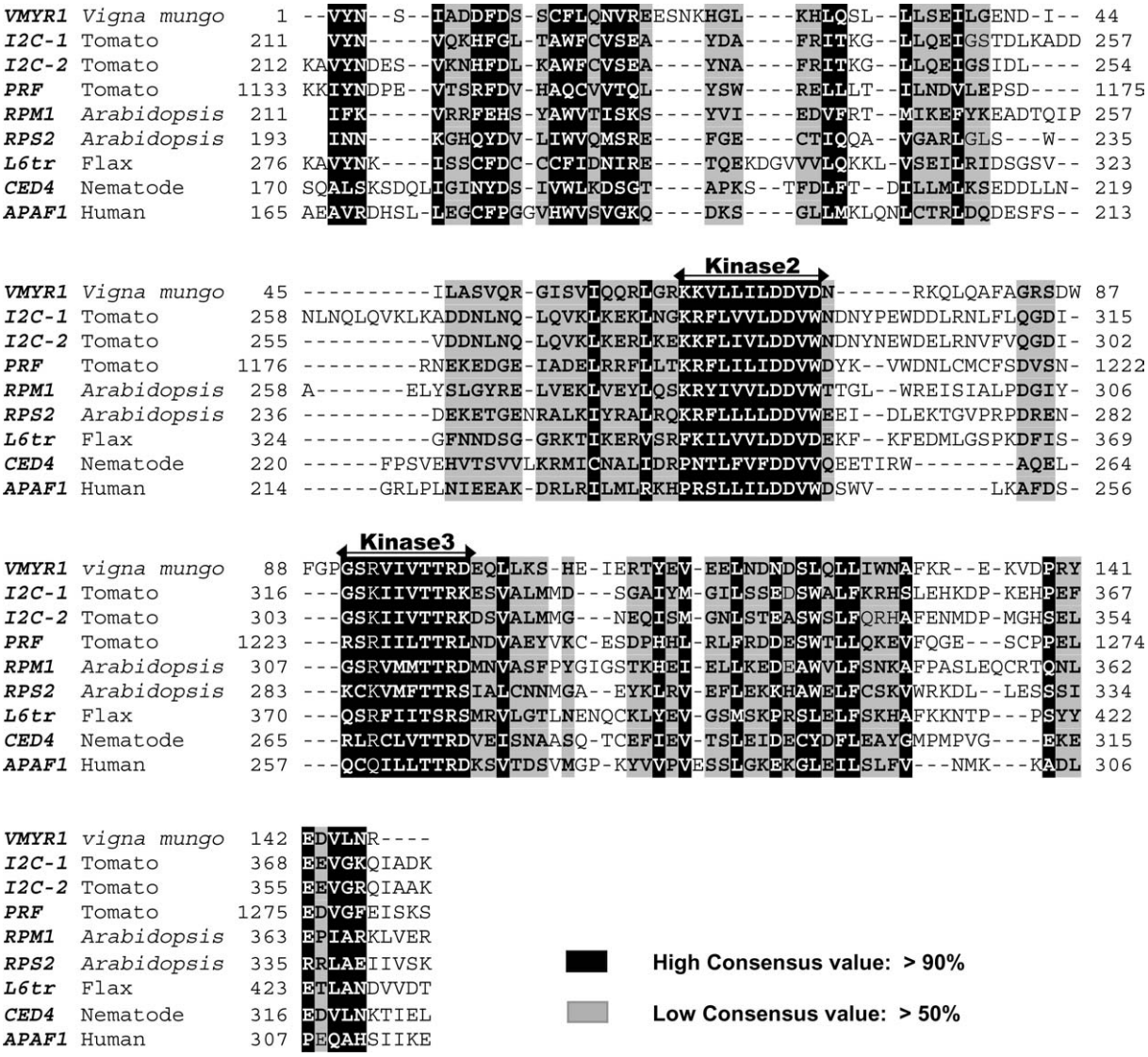

Figure 3. Multiple alignment of the translated amino acid sequence of the marker '*VMYR1*' linked with YMV-resistance and the NB-ARC domain from six plant 'R' gene products; '*12 C-1*', '*12 C-2*' and '*PRF*' from tomato; '*RPM1*' and '*RPS2*' from *Arabidopsis*, '*L6*' from flax, '*CED-4*' from nematode and '*APAF-1*' from human. Presence of conserved motifs of kinase 2 and kinase 3 are shown within all the sequences and the other conserved regions are represented in black (consensus value $>90\%$) and gray (consensus value $>50\%$).

marker development in any crop species using segregating populations. In most other studies, it was resolved by mutant or transgenic component analysis (Michelmore and Meyers 1998). The 445bp DNA marker developed during this study, always segregated with the screened YMV-tolerant individuals of F_2 population and F_3 families confirming its linkage with YMV-resistance. The proportion, 2:1 in F_2 and 1:1 in F_3, in which the 445bp DNA marker was amplified from YMV-susceptible heterozygous individuals, further confirms the monogenic recessive nature of YMV-tolerance in the experimental populations.

Many important genes in breeding for resistance and quality traits are inherited recessively (Fritsch and Melchinger 2001). In conventional backcross programs for introgression of a recessive target gene, that gene's presence or absence among the individuals of a backcross is determined by a phenotypic assay (Allard 1960). Here we have adapted a forced inoculation method that can be conveniently used to

phenotype the population segregating for YMV-reaction. However, as an alternative to this time-consuming, laborious method, the use of linked molecular markers is more appropriate. Young (1999) was of the opinion that the opportunity to select desirable lines based on genotyping rather than analysis of phenotype was extremely attractive to plant breeders.

Due to the conserved nature of 'R' genes in plant taxa and the presence of diagnostic motifs within the 'R' genes, it is easy to identify 'R' genes simply by specific amplification using aptly designed RGA primers (Yan et al. 2003). The significance of the present study over most of the resistance gene candidates isolated simply by PCR amplification with degenerate primers is that the presence of the 445bp DNA marker is linked with the YMV-tolerant phenotypes; whereas, in a number of reports no linkage with the respective resistance phenotypes was assigned.

Plant disease resistance genes are comprised of diverse groups of related sequences and appear to be members of an ancient gene family (Young 2000). Majority of 'R' genes encode the conserved NBS motif in the protein that has recently been designated as NB-ARC (van der Beizen and Jones 1998). The predicted protein sequence of the 445bp DNA marker, '*VMYR1*', is a part of the NB-ARC domain, has an ORF-signature, and a kinase 2 and a kinase 3 motif (Figure 3). These motifs are components, which are usually associated with plant disease resistance proteins. The NB-ARC domain has been reported from several 'R' gene products, including *12C-1*, *12C-2* (Ori et al. 1997) and *Prf* (Salmeron et al. 1996) in tomato, *RPM1* (Grant et al. 1995) and *RPS2* (Bent et al. 1994) in *Arabidopsis thaliana* for resistance to *Pseudomonas* spot, respectively, and *L6* in flax for resistance to *Fusarium* wilt (Lawrence et al. 1995). This conserved domain has been expanded to include homology to animal cell death effectors, *CED-4* and *APAF-1*, reported from nematodes and mammals, respectively, which have been implicated in protease-mediated apoptosis (Li et al. 1997).

In conclusion, the tightly linked marker developed in this study, endowed with features of resistance gene candidates, may be useful for generating superior genotypes with durable YMV-resistance. The marker will be of use in marker-assisted selection and will hopefully aid in the development of resistant cultivars in relatively shorter time span.

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