CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1318621

Available online at: http://www.iajps.com Research Article

SCREENING AND IDENTIFICATION OF MOLECULAR MARKER ASSOCIATED TO YELLOW VEIN MOSAIC VIRUS (YVMV) DISEASE RESISTANCE AND TOLERANCE IN OKRA

SumanSahu[1], Dr.Pulak Das[2] and Dr.Kuldeep Dwivedi[3]

[1]Department of Biotechnology, ITM University Gwalior, Madhya Pradesh, India
Email- sumansahu174@gmail.comWhatsApp- 7693041704

[2]SR. Scientist at Adithya Biotech Lab & Research PVT LTD, Chandandih, Nandanban Road
Email- pulak16@gmail.com

[3]Assistant professor, Department of Life Science, ITM University, Gwalior, M.P, India
Email: kuldipdwivedi@itmuniversity.ac.in

Abstract:

Okra, botanical name is Abelmoschusesculentus(L.) is a flowering plant which belongs to Malvaceae family. It is also known as Ladies' finger. This plant is best known for its edible green seed pods.Okra is also vulnerable to the attack of many disease-causing pathogens affecting leaves, flowers, and fruits. The yellow vein mosaic virus causes a disease known as yellow vein mosaic disease. The YVM virus is transmitted by the whitefly, namely Bemisiatabaci Gen. The yellow vein mosaic virus causes a disease in the okra plant, known as yellow vein mosaic disease, resulting in the massive loss of crop especially if the condition occurs in the early stage of the crop growth. Yellow vein mosaic virus (YVMV) belongs to Geminiviridae family. The present study focuses on the identification and development of the molecular marker, associated with the yellow vein mosaic virus resistance and tolerance in the okra. It is difficult to isolate genomic DNA from the green leaves of the okra because the tissue of okra contains high mucilaginous acidic polysaccharides content. This study exhibited the new methods for the extraction of genomic DNA. For this experiment sodium citrate were used, Sodium causes the cell walls and membranes to burst and concentration of 100mM sodium citrate add to the suspension in the lysis step did result in the highest yields of total genomic DNA. The Salt use during precipitation of DNA increases the solubility of polysaccharides in ethanol thus preventing co-precipitation with DNA. Results of this study were observed by using RAPD marker.

Keywords: *Okra, Molecular marker, Yellow vein mosaic virus, Germiniviridae, Abelmoschusesculentus(L.)*

Corresponding author:

SumanSahu,
Quarter number 19/B
Street 1, sector 1
Bhilai CG 490001

QR code

Please cite this article in press SumanSahu et al., ***Screening and Identification of Molecular Marker Associated To Yellow Vein Mosaic Virus (YVMV) Disease Resistance and Tolerance in Okra.,*** *Indo Am. J. P. Sci, 2018; 05(07).*

INTRODUCTION:

Abelmoschusesculentus (L.), also known as Bhindi or Lady's finger, belongs to the Malvaceae family. The most common name of this plant is okra. Okra has comparatively high nutrition value and it is a good component in developing country [1]. Okra plant is the most popularly grown in Aisa, among the different species of genus *Abelmoschus*. This plant has great commercial value due to its great nutritional value[2].

The yellow vein mosaic virus causes a disease in the okra plant, known as yellow vein mosaic disease is the most common disease of the okra plant [3]. The YVM virus is transmitted by the whitefly, namely *Bemisiatabaci Gen* [4]. Yellow vein mosaic of okra or vein clearing of okra is a ruinous disease in all the okra growing regions of India. If the disease occurs at the early stage of development it causes about 80% loss of the crop[5].

The molecular marker is defined as the sequence of DNA which is associated with the particular place within a genome. A molecular marker is mainly used to find out a particle sequence of DNA in a puddle of unknown DNA. The best example of a naturally occurring molecular marker is a protein which is essential for the diagnosis of complex diseases like Alzheimer disease[5].

MATERIALS AND METHODS:

Plants Materials

For this experiment, plants materials were collected from Adithya Biotech Lab and Research Pvt Ltd, Raipur, Chhattisgarh. The study was based on the screening and identification of molecular marker associated to yellow vein mosaic virus. All the experiments were conducted in triplicates. Young and fresh leaves of hybrids and their parental lines were collected for DNA isolation.

Genomic DNA extraction and Quantification

Sodium citrate methods of DNA extraction

Extraction of genomic DNA was done by Modified CTAB (CetylTrimethyl Ammonium Bromide) method. It is difficult to isolate genomic DNA from the green leaves of the okra because the tissue of okra contains high mucilaginous acidic polysaccharides content. The DNA was quantified by using 1% agarose gel in 1X TAE buffer and Photograph was observed under UV light of Bio-Rad gel documentation system.

PCR amplification and electrophoresis

RAPD marker(Random Amplified Polymorphic DNA) primers were used to detect the polymorphism among parental lines.Polymerase chain reaction (PCR) amplifications were carried out in thermo cycler (Life). PCR reactions were performed on each DNA sample in a 20-µl reaction mixture containing 1µl of DNA sample, 2µl of 10X PCR buffer, 0.5µl MgCl2 (25mM), 1 µldNTP's (5mM), 1µl BSA(10mg), 0.3µl Tween 20, Primer (ISSR/RAPD) 2.0µl/ 1.5µl (3-5 pM), 0.2µl(1Unit) of Taq polymerase. PCR thermal profile consist of initial denaturation at 94ºC for 4min, followed by 38 cycles at 94ºC for 20sec, annealing at different tm of primer for 30sec, initial extension at 72ºC for 1min 50sec and final extension step at 72ºC for 5min. The PCR product was analyzed in 1.8% agarose gel in 1X TBE buffer and photographed under UV light of Bio-Rad gel documentation system.

RESULT AND DISCUSSION:

The results of this study were observed with RAPD marker. The significant bands appear during amplification of DNA through PCR with RAPD primers. Resistance lines were observed using OPX 17 and OPA 12whereas tolerant lines were observed by using OPY 18.

The sample1, sample2, sample3, and sample5 were observed by using OPX17, showing bands for resistance lines at 900bp, OPA 12 was used to observe the sample1, sample2 and sample 3, showing band for resistance 1820bp whereas the sample4, sample6, sample7, and sample8 were observed by using OPY 18, showing bands for tolerance at 370bp.

Table 2 : Details of RAPD Primer Sequence

S.No	Name of marker	Sequence	Tm (ºC)
1	OPA 12	TCGGCGATAG	36.7
2	OPX 17	GACACGGACC	34.0
3	OPY 18	AGACGATGGG	32.2

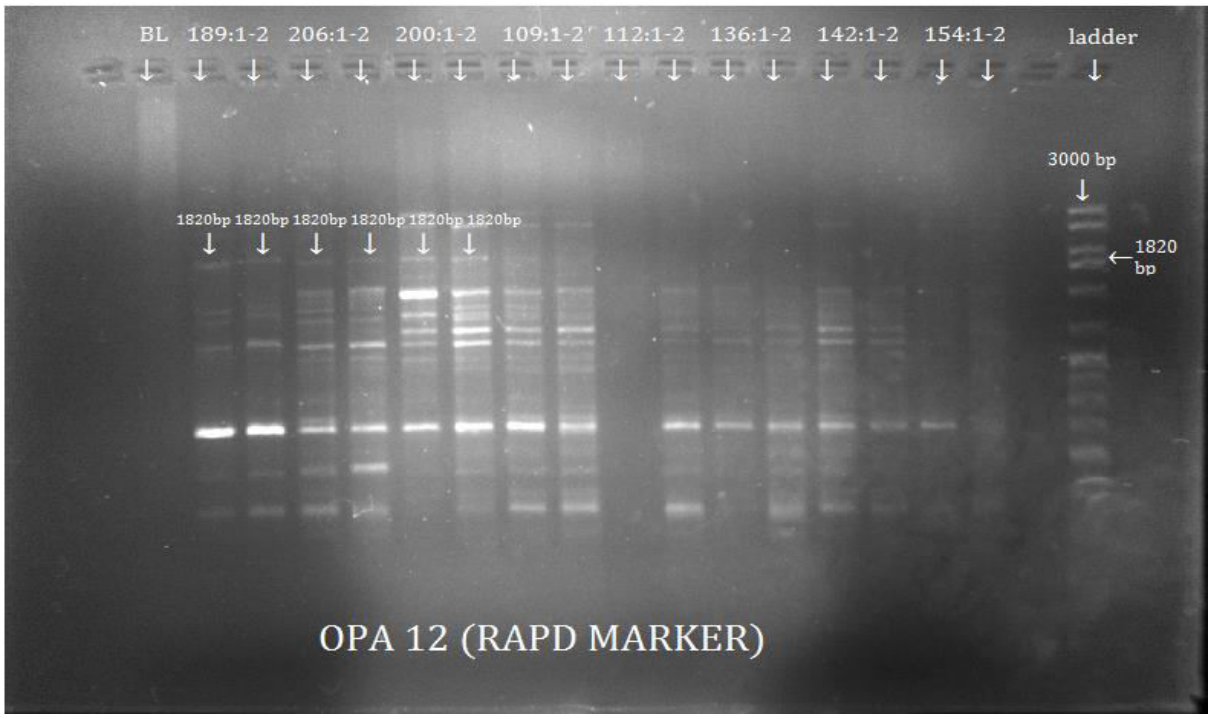

Figure 1: RAPD analysis of Okra genotypes of primer OPA12. Lane 2,3 represent okra genotype 189, Lane 4,5 represent okra genotype 206, Lane 6,7 represent okra genotype 200, which showed polymorphic band of 1820bp.This lines are resistance for OYVMV.

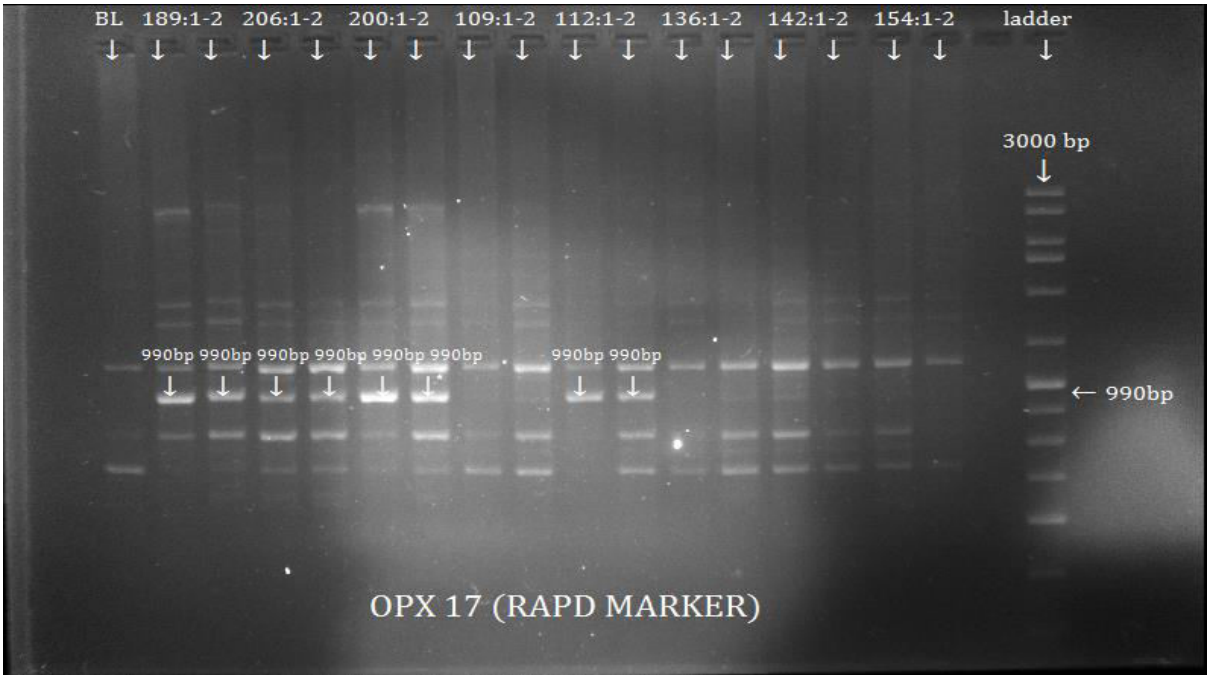

Figure 2: RAPD analysis of Okra genotypes of primer OPA17. Lane 2,3 represent okra genotype 189, Lane 4,5 represent okra genotype 206, Lane 6,7 represent okra genotype 200, which showed polymorphic band of

900bp.This lines are resistance for OYVMV.

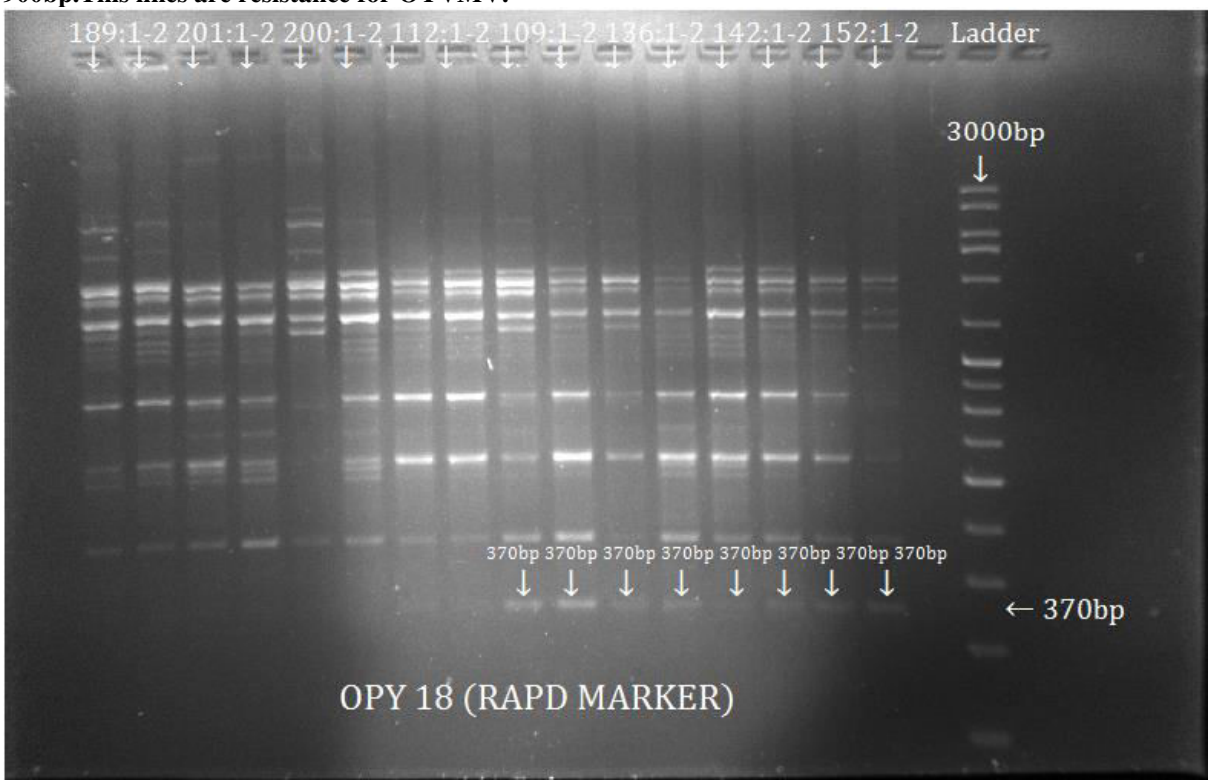

Figure 3: RAPD analysis of Okra genotypes of primer OPA18. Lane 9,10 represent okra genotype 109, Lane 11,12 represent okra genotype 136, Lane 13,14 represent okra genotype 142, Lane 15,16 represent okra genotype 152, which showed polymorphic band of 370bp.This lines are tolerance for OYVMV.

CONCLUSION:

Okra is also vulnerable to the attack of many disease-causing pathogens affecting leaves, flowers, and fruits. The yellow vein mosaic virus causes a disease known as yellow vein mosaic disease. The YVM virus is transmitted by the whitefly, namely *BemisiatabaciGen*.It is difficult to isolate genomic DNA from the green leaves of the okra because the tissue of okra contains high mucilaginous acidic polysaccharides content. This study exhibited the new methods for the extraction of genomic DNA. For this the this experiment sodium citrate were used, Sodium causes the cell walls and membranes to burst and concentration of 100mM sodium citrate add to the suspension in the lysis step did result in the highest yields of total genomic DNA. This protocol provides a high amount of DNA, therefore, higher content of DNA template for PCR reactions despite the fact that the concentration of total template DNA was slightly higher when using a standard protocol. The results of this study were observed with RAPD marker. The significant bands appear during amplification of DNA through PCR with RAPD primers. Resistance lines were observed using OPX 17 and OPA 12whereas tolerant lines were observed by using OPY 18.The sample1, sample2, sample3, and sample5 were observed by using OPX17, showing bands for resistance lines at 900bp, OPA 12 was used to observe the sample1, sample2 and sample 3, showing band for resistance 1820bp, where as the sample4, sample6, sample7, and sample8 were observed by using OPY 18, showing bands for tolerance at 370bp.

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