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SCREENING OF OKRA GENOTYPES FOR YELLOW VEIN MOSAIC VIRUS DISEASE USING ISSR MARKERS

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Abstract

Okra (*Abelmoschus esculentus*L. Monech) is an important vegetable crop in India. The major yield loss of crop up to 100% is due to Yellow mosaic virus. Molecular identification against Yellow mosaic virus in Bhendi has been observed in thirteen genotypes for resistance and susceptible genotypes.. This was detected by using two ISSR markers. PCR amplification by these markers showed six genotypes are resistance to YVMV.

***Keywords*:** Okra, Markers, ISSR, Resistance, Susceptible.

Introduction

Okra is an important vegetable crop in India, West Africa, South-East Asia, U.S.A., Brazil, Australia and Turkey. It is a very important crop for tropical and subtropical region of the world. (Akhter *et al*., 2014). The crop is attacked by a number of fungi, bacteria, phytoplasma, virus, nematodes and insect pests (Ali *et al*., 2000). Okra yellow vein mosaic virus is important constraint in the production of okra by infecting all growth stages of the plant resulting in losses through reduced growth and yield, distortion and mottling of fruit which are unmarketable (Capoor and Varma 1950). The first report of this virus was from Bombay in India indicating (Kulkarni G.S., 1924) indicating that OYVMV might have originated from India. Uppal *et al*. 1940 studied the viral nature of the disease and gave the name "Yellow vein mosaic". The yield loss of vegetable due to crop pests has been estimated up to 20-30 percent, and may increase upto 80-90 percent in case of a severe infestation (Ali *et al.*). Yellow mosaic virus is very difficult to control properly by chemical means the only practical remedy of this problem is to develop resistant varieties (Sanwal *et al*., 2016). The OYVMV is one of the major limiting factors in okra production in India. Nowadays, application of chemical pesticides is limited because of hazards environmental pollutions and health risks. So, using genetic resistance and cultivating resistant genotypes is the most suitable and practical method for management of OYVMV. The phenotyping selection of the resistant genotypes is time consuming and complicated. So, using DNA based molecular marker which is tool for the selection of the resistant genotypes (Lindhout, 2000; Tanksley *et al*. 1992). The use of various DNA based molecular marker tools like Random Amplified Polymorphic DNA (RAPD), Inter simple sequence repeats (ISSR), Simple Sequence Repeats (SSR) and Sequence Related Amplified Polymorphism (SRAP) are receiving much attention than morphological characterization for evaluation of genetic diversity (Lal at al., 2012).High mucilage content of okra makes it difficult to isolate nucleic acid. Very few work has been done in this crop. The present study is planned to study the genetic diversity of thirteen genotypes of Okra for Yellow vein mosaic virus using ISSR markers.

Materials and Methods

Plant material

The samples were collected from the Pot culture yard at the Department of genetics and plant breeding, Faculty of Agriculture, Annamalai Nagar. The genotypes used for the study is as follows.

S.No.	Name of the genotype
1.	Okra Elephant Tusk
2.	Okra Red
3.	Okra Bommidi
4.	Okra multi branch white
5.	Okra red long
6.	Co1
7.	Avinashi local
8.	PKM 1
9.	ArkaAnamika
10.	Okra multi branch red
11.	Hill Okra
12.	Okra Short
13.	Okra short white

DNA isolation

Fresh immature leaves of 10 days old seedling was taken and DNA was isolated. The fresh leaves were washed with sterile distilled water. CTAB method (Doyle and Doyle, 1987) was used for the extraction of genomic DNA. 200 mg of fresh leaf was gring into fine powder using liquid nitrogen and extraction buffer was added and transferred to 2 ml eppendorf tubes and was mixed by inversion and mixture was incubated for 1 hr at 65^0C in hot water bath with intermittent shaking. Then the tubes containing homogeneous were centrifuged at 12000 rpm for 15 min at 4^0C. Then supernatant was transferred into new 2 ml eppendorf tubes without disturbing the pellet cell debris and supernatant was treated with RNAse at 37^0C for 15 min. Then 1 ml of phenol:chloroform : isoamyl alcohol (25:24:1) was added

and mixed gently then upper aqueous phase was transferred into a new 2ml eppendorf tubes with a wide bore pipette. Equal volume of ice-cold isopropanol was added and mixed by inversion. The samples were kept for 1 hr 4^0C Temperature and then centrifuged at 12000 rpm at 4^0C for 15 min then pellet was formed at the bottom of the eppendorf tubes. The suppernatant was removed and the pellet was washed with 500 µl chilled 70% ethanol and centrifuge at 800 rpm for 5-10 min at 4^0C. The pellet was air dried for 10-15 min and then dissolved in 100 µl TE buffer; pellets were allowed to dissolve completely overnight at 4^0C without agitation. The DNA obtained after extraction was confirmed by running it on 0.8% agarose gel (containing ethidium bromide @ 0.5 mg/ml) in a gel electrophoresis system. Quantification of DNA was done by Nano drop spectrophotometer.

Amplification of DNA

The isolated samples were amplified by using PCR. It was carried out in a reaction tube containing reaction volume of 15µl. The reaction mixture contains template DNA, assay buffer, MgCl2, dNTPs, and Primers. It was transferred to PCR tubes and the temperature for denaturation, annealing and extension was set and the desired number of cycles was also set. The amplified products was analysed in Electrophoresis system on 1.2% agarosegel. Three ISSR markers was used to screen the YVMV resistance and susceptible genotypes. The details of ISSR markers is presented in Table 1.

S. No.	ISSR Primers used	Nucleotide sequence	Annealing Temperature
1.	ISSR 8	5"- ATTATTATTATTATTGTA-3"	49.9 C
2.	UBC 873	5"- GACAGACAGACAGACA-3	59 C

Results

Two primers used for the study showed the polymorphic pattern for resistance and susceptible genotypes. The marker ISSR 8 yielded eleven amplicons. Amplicons obtained at 1 kb was distinctly polymorphic for resistance to YVMV. Only six genotypes yielded amplicons at 1kb. The marker UBC 873 yielded twelve amplicons. Amplicons obtained at 800 kb was polymorphic to YVMV. Only six genotypes yielded amplicons at 800 kb. The six genotypes are Okra Elephant Tusk, Okra Red long, Okra Bommidi, ArkaAnamika, Hill Okra, Avinashi local.

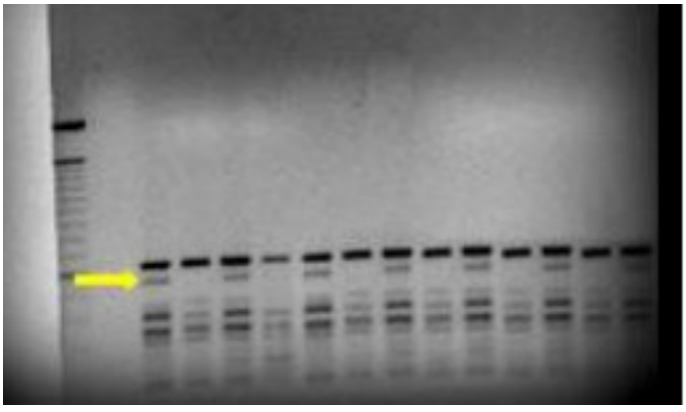

Fig. 1 : PCR amplification of DNA using ISSR 8 in Okra genotypes

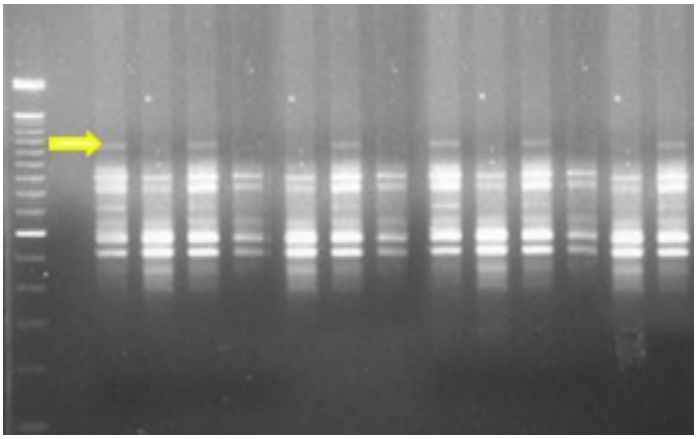

Fig. 2 : PCR amplification of DNA using UBC 873 in Okra genotypes

Conclusion

The ISSR markers are good tools in the detection of resistance and susceptible genotypes and shows polymorphism. The screening of 13 Okra genotypes by ISSR markers showed susceptibility and resistance for YVMV. Out of 13 genotypes screened only six were resistance to YVMV. They are Okra Elephant Tusk, Okra Red long, Okra Bommidi, ArkaAnamika, Hill Okra, Avinashi local.

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