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Characterization of okra genotypes by molecular markers against yellow vein mosaic virus

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Abstract
Molecular characterization against yellow vein mosaic virus resistance has been observed in 18 genotypes okra genotypes. The virus associated with yellow vein mosaic virus were detected by PCR amplification of specific Primer Pair 1 and Primer Pair 3 were used for characterizing different genotypes with yellow vein mosaic virus which is linked with detection of presence or absence of okra yellow vein mosaic virus. PCR analysis revealed that all 18 genotypes screened for presence of okra yellow vein mosaic virus, were linked to susceptibility by Primer Pair 1 and Primer Pair 3 yielding band size 750bp except Phule Vimukta shows resistance and absence band in PCR analysis.

Keywords: Orka, resistance, Phule Vimukta, vein mosaic virus

Introduction
Okra (*Abelmoschus esculentus* L. Moench) belonging to the family Malvaceae is an important and extensively consumed vegetable crop of the tropical and subtropical region of the world (Wammanda *et al.*, 2010). The crop is attacked by a number of fungi, bacteria, phytoplasma, viruses, nematodes and insect pests (Ali *et al*., 2000) [1]. 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) [2]. The first report of this virus was from Bombay in India (Kulkarni G. S., 1924) [5] indicating that OYVMV might have originated from India. (Uppal *et al.* 1940) [11] 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 per cent, and may increase up to 80-90 Per cent in case of a severe infestation (Ali *et al*., 2005a). Yellow vein mosaic disease 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) [8]. The OYVMV management requires fast and accurate detection system due to complex nature of the virus-plant-environment interactions. 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 phenotypical 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 cultivars at an early stage facilitating the process of selection of resistant genotypes (Lindhout, 2002; Tanksley *et al*. 1992) [6, 10].

Materials and Methods
Plant material
The samples were collected from the field of chilli and vegetable research unit, Dr. PDKV, Akola. The materials used during the present investigation are as given in table number 1.

DNA isolation
Total genomic DNA was isolated from the young leaves following the CTAB method (Murray and Thompson 1980). 200 mg of fresh leaf sample was grind in liquid nitrogen to obtain the fine powder then powder was immediately homogenized by adding pre-warmed (60 ºC) extraction buffer and transferred to 2 ml eppendorf tubes and was mixed by inversion and

mixture was incubated for 1 hr at 65 ºC in hot water bath with intermittent shaking every after 10min after that tubes containing homogenate were centrifuged at 12000 rpm for 15 min at 4 ºC. Then supernatant was transferred into new 2 ml eppendorf tubes without disturbing the pellet cell debris and supernatant was treated with RNAse at 37 ˚C for 15 min. Then 1 ml of phenol:chloroform : isoamyl alcohol (25:24:1) was added and mixed gently but throughly to emulsify both the components for 15 min after centrifugation it at 12000 rpm for 15 min at 4 ºC 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. CTAB-DNA complexes formed a fibrous network after mixing with isopropanol, the samples were kept for 1 hr 4 ^{0}C Temperature and then centrifuged at 12000 rpm at 4 ºC for15 min then pellet was formed at the bottom of the eppendorf tubes. The supernatant was removed and the pellet was washed with 500 µl chilled 70% ethanol and centrifuge at 8000 rpm for 5-10 min at 4 ºC. 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 ºC 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 horizontal gel electrophoresis system.

Characterization by primers

Primer 1 and Primer 3 were selected to study for characterization of okra genotypes against yellow vein mosaic. The detailed of primers given in Table 2.

PCR amplification and electrophoresis

The PCR was carried out in small reaction tubes, containing a reaction volume typically of 20µl that was inserted into a thermal cycle (Eppendorf) that heated and cooled the reaction tubes within it to the precise temperature required for each step of the reaction. PCRs were run on the programmable thermal cycle 94 ^{0}C Denaturation, 50 ^{0}C Annealing, and 72 ^{0}C Extension. Annealing temperature varied from primer to primer. Electrophoresis of Primer 1 and Primer 3 PCR amplified analysis on 1.2% agarose gel was carried out in 1x TBE buffer in horizontal gel electrophoresis.

Results and Discussion

OYMV affected sample of okra collected from chilli and vegetable research unit, Dr. PDKV, Akola and investigation was carried out in the molecular plant pathology laboratory of the Department of Plant Pathology, Dr. PDKV, Akola. The Characterization of 19 okra genotypes for OYVMV was carried out in the present study; Primer Pair 1 and Primer Pair 3 detect presence or absence of YVMV virus. These two primers amplified against 19 genotypes of okra. Primer Pair 1 and Primer Pair 3 show amplification in 18 genotypes of size 750bp. Only Phule Vimukta variety shows no amplification. Hence it is resistant variety as these markers detect presence or absence of Yellow Vein Mosaic Virus.

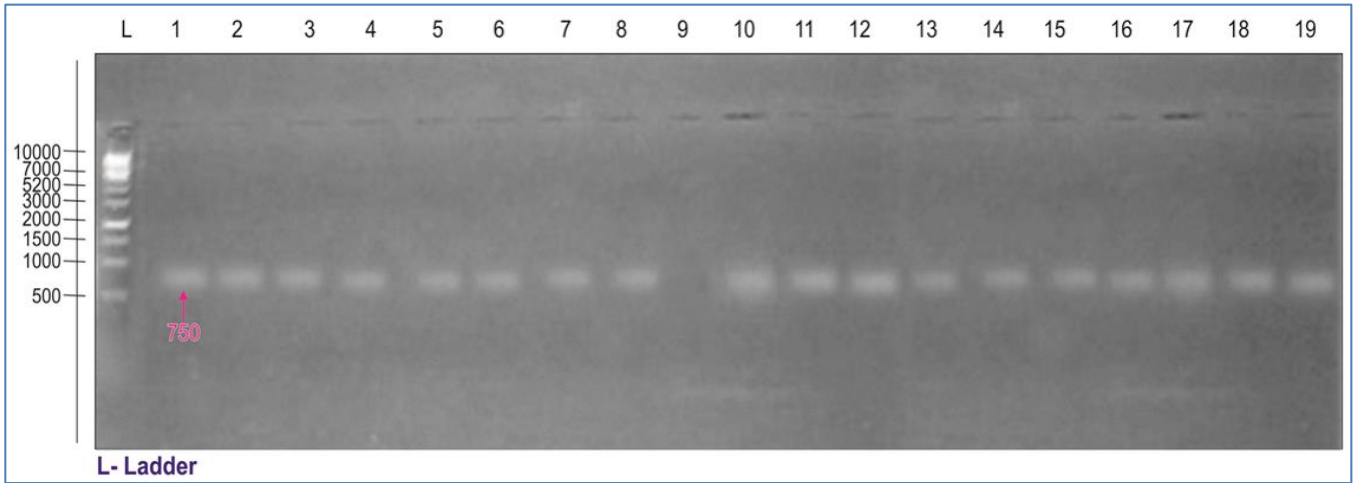

Plate 1: PCR amplification of DNA using primer 1 in Okra genotypes

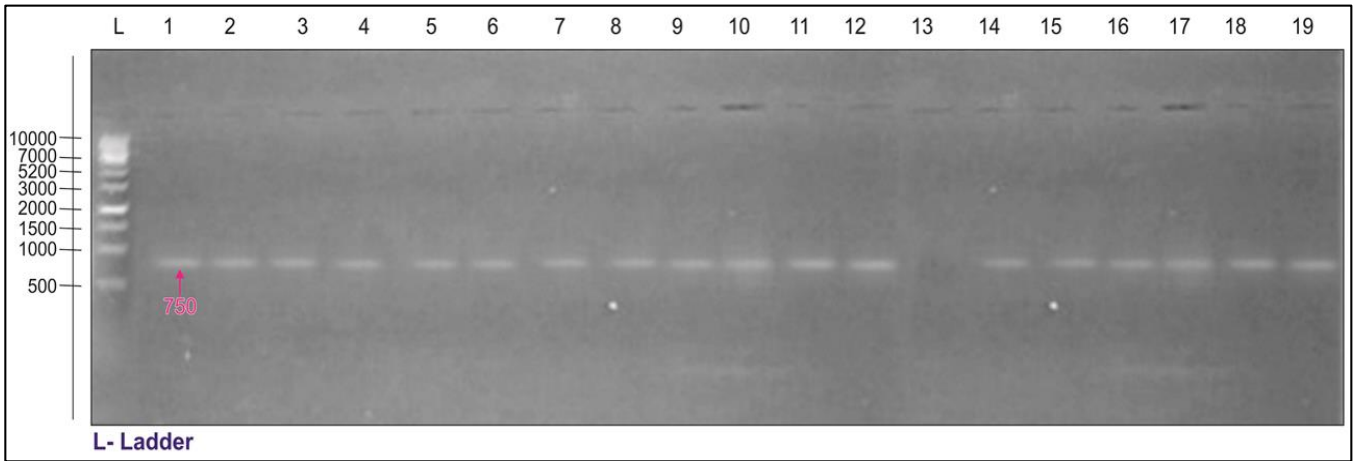

Plate 2: PCR amplification of DNA using primer 3 in Okra genotypes

Table 1: The list of Genotypes utilized for study

Sr. No.	Name of Genotype
1.	AKOV-145
2.	AKOV-111
3.	AKOV-107
4.	AKOV-154
5.	AKOV-102
6.	AKOV-160
7.	AKOV-117
8.	AKOV-108
9.	AKOV-153
10.	AKOV-131
11.	AKOV-97-16-7-2
12.	Arka Anamika
13.	Parbhani Kranti
14.	Pusa A-4
15.	Arka Abhay
16	Phule Vimukta
17.	Akola Bahar
18.	Tanvi F1 hybrid
19.	Mahyco hybrid 10

Table 2: List of primers used with their sequences

Sr. No.	Primer	Primer sequence	Expected fragment size (bp)	Annealing Temp. (˚C)
1.	Primer pair 1	F- TTCGTCGTGAATGGACGGTT R- ATCTTTCAGGAGCCACCAGC	750	55.3
2.	Primer pair 3	F- GTGGAGTCGGTTTGACCCAT R- AACCGTCCATTCACGACGAA	750	55.3

Table 3: Reaction of different genotypes of YVMV with Primer Pair 1

Sr. No.	Name of Genotype	Primer Pair 1 Banding pattern	YVMV Reaction
1.	Akola Bahar	Present	Susceptible
2.	AKOV-153	Present	Susceptible
3.	Parbhani Kranti	Present	Susceptible
4.	AKOV-108	Present	Susceptible
5.	AKOV-160	Present	Susceptible
6.	AKOV-107	Present	Susceptible
7.	Mahyco hybrid10	Present	Susceptible
8.	AKOV-131	Present	Susceptible
9.	Phule Vimukta	Absent	Resistant
10.	Arka Anamika	Present	Susceptible
11.	AKOV-145	Present	Susceptible
12.	AKOV-117	Present	Susceptible
13.	AKOV-102	Present	Susceptible
14.	AKOV-154	Present	Susceptible
15.	AKOV-111	Present	Susceptible
16	Tanvi F1 hybrid	Present	Susceptible
17.	AKOV-97-16-7-2	Present	Susceptible
18.	Arka abhay	Present	Susceptible
19.	Pusa A-4	Present	Susceptible

Table 4: Reaction of different genotypes of YVMV with Primer Pair 3

Sr. No.	Name of Genotype	Primer Pair 1 Banding pattern	YVMV Reaction
1.	AKOV-117	Present	Susceptible
2.	Akola Bahar	Present	Susceptible
3.	AKOV-131	Present	Susceptible
4.	Pusa A-4	Present	Susceptible
5.	Parbhani Kranti	Present	Susceptible
6.	AKOV-153	Present	Susceptible
7.	AKOV-154	Present	Susceptible
8.	AKOV-111	Present	Susceptible
9.	AKOV-145	Present	Susceptible
10.	AKOV-117	Present	Susceptible
11.	AKOV-102	Present	Susceptible
12.	AKOV-160	Present	Susceptible
13.	Phule Vimukta	Absent	Resistant
14.	Mahyco hybrid10	Present	Susceptible

15.	AKOV-97-16-7-2	Present	Susceptible
16	AKOV-107	Present	Susceptible
17.	Akola Anamika	Present	Susceptible
18.	Tanvi F1 hybrid	Present	Susceptible
19.	AKOV-131	Present	Susceptible

Conclusion

The Molecular identification markers are good tools in detection of resistant and susceptible genotypes. The confirmation of resistance and susceptibility by this marker shows variation against different okra genotypes against yellow vein mosaic virus. The screening of 19 genotypes by molecular markers showed variation in their susceptibility and resistance from these 18 genotypes shows susceptibility over 1 genotypes. In present investigation banding patterns of yellow vein mosaic virus was found to be 750 bp by these specific Primer Pair 1 and Primer Pair 3 in 18 different genotypes. Only Phule Vimukta showed absence of Yellow Vein Mosaic Virus by this specific Primer Pair 1 and Primer Pair 3.

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