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ASSESSMENT OF GENETIC DIVERSITY OF OKRA (*ABELMOSCHUS ESCULENTUS* L.) FOR YVMV USING RAPD AND SSR MARKERS

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ABSTRACT
Okra is an important vegetable crop in India and grown in the tropical and subtropical region of the world. Okra plays significant role in the human diet by supplying carbohydrates, proteins. Present study was carried out for assessment of genetic diversity of okra for YVMV using RAPD and SSR markers. Thirteen RAPD primers produced total 228 loci among which 214 are polymorphic with 93.36% polymorphism. Four SSR primers produced total 60 bands with average 0.72 PIC value. On the basis of clustering pattern, RAPD markers useful for genetic diversity while SSR markers from cross species not useful for varietal screening of okra against YVMV.

KEY WORDS: okra, RAPD, SSR, whitefly, YVMV**.**

INTRODUCTION

Okra is an important vegetable crop in India, West Africa, South-East, Asia, U.S.A, Brazil, Australia and Turkey. It is belongs to *Malvaceae* family. It is a very for tropical and subtropical region of the world. Okra is broadly cultivated for its young fruits as well as leaves, petioles, stems, shoots, rhizomes, inflorescence and seeds. Okra plays significant role in the human diet by supplying carbohydrates, proteins, fats, minerals and vitamins that are generally lacking in the staple foods (Akhter *et al.*, 2014). There are significant variations in the chromosome numbers of okra and at ploidy levels of different species in the genus *Abelmoschus*. The lowest number reported is 2n=56 for *A. angulosus* (Ford, 1938) whereas the highest chromosome number reported are close to 200 for *A. manihot* var. caillei (Singh and Bhatnagar, 1975; Siemonsma, 1982).

The crop is prone to damage by various insects, fungi, nematodes and viruses. Its cultivation in India is challenged by severe incidence of viral disease, such as Yellow Vein Mosaic Virus (YVMV) disease spread by an insect vector, namely whitefly (*Bemisia tabaci* Gen). YVMV cause mosaic, degradation of chlorophyll in vein, banding of vein and plant stunting. If plants are infected within 20 days after germination, their growth is retarded with few leaves and malformed fruits resulting in loss ranging from 94% to 100% (Ndunguru and Rajabu, 2004).

Morphological markers are those characters of plant which are influenced by surrounding environment, while the major limitation of biochemical markers is present in small number so genetic information obtained from such markers may not be very representative of genes throughout the genome. The limitation in the number of markers was overcome beginning in the 1980s with the development of molecular or DNA based genetic markers. The use of various DNA based molecular marker tools like Randomly 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 the evaluation of genetic diversity (Lal *et al.*, 2012). They have large number of applications like characterization of gene pool, DNA fingerprinting, phylogenetic analysis, molecular dissection of complex traits and characterization of genome organization. Because of high mucilage content of okra it is very difficult to extract nucleic acid. Very little work has been done in this crop. Keeping in view the above facts the present experiment is planned to study the genetic diversity among genotypes of okra using RAPD and SSR markers.

MATERIALS & METHODS

Plant Material and DNA Isolation

Experimental material consisted 10-12 days old leaves of ten genotypes of okra (AOL-09-2, AOL-09-17, AOL-10-22, AOL-11-34, AOL-11-37, AOL-11-39, AOL-11-49, GAO-5, Parbhani-krani and Pusa-sawani). DNA was extracted from young, healthy seedling as described by Patel *et al.*, (2014) with some modifications. Purified DNA was quantified by running on 0.8% agarose gel at 60V using 1X TBE buffer and stained with ethidium bromide (3µL/100 mL). The total genomic DNA was diluted to 20 ng/µl for polymerase chain reaction (PCR) analysis.

RAPD and SSR amplification

Thirty primers from OPA, OPC, OPK, OPN and OPO series were used for initial screening. The primers were selected based on the polymorphism information provided in earlier reference (Saifullah *et al.*, 2010). Based on these results, 13 primers that generated distinct and polymorphic banding patterns were selected for further analysis. PCR conditions were optimized for uniform clearly visible and dense bands. The PCR reaction mixture contained Taq

buffer A (10 x), dNTPs mix (2.5 mM each), Primer, Taq DNA polymerase (3 U/µl), Template DNA (20 ng) and 1.5 mM of $MgCl_2$ in a final volume of 25µL. The PCR amplification was performed for 38 cycles: Initial denaturation at 94ºC for 7 min, Denaturation at 94ºC for 1 min, Primer annealing at 36-39ºC for 1 min, Primer extension at 72ºC for 1.5 min, Final extension step at 72ºC for 7 min. Amplification products were resolved on 1.5% agarose gel. One kb ladder was used for approximate sizing of the products. In earlier study Gutierrez *et al.*, (2005) have reported that SSR of *Medicago truncatula* can be used in cross species amplification of major pulses, so here we have tried to amplify SSR of *Medicago truncatula* in different YVMV susceptible and resistant genotypes. For SSR analysis total 10 primers were selected from earlier research reported by Sawadogo *et al.* (2009). Primer annealing temperature was set to 55-60 ºC. The amplified products of SSR were analyzed electro phoretically using 2.5% agarose gel (Latha 2012 & Chander *et al.*, 2017). The molecular weight marker, 100bp ladders were used for band sizing. The amplification was carried out in Bio-Rad master cycler (PCR) for RAPD and SSR. Amplified products were stored at -20ºC till further use. Ten micro litter PCR products were run on electrophoresis at a constant voltage of 100 V in 1X Tris-Boric acid-EDTA (TBE) buffer stained with ethidium bromide and photographed to a Gel DocTMEZ Imager (BIO- RAD).

Data analysis

For statistical analysis of data, clearly resolved bands on gel were scored as 0 for absence and 1 for presence, in all the 10 genotypes of okra (Fougat *et al.*, 2015). Estimates of genetic similarity were calculated according to Jaccard's similarity index; while the matrix obtained was used to evaluate the genetic relationship among okra with an Unweighted Pair Group Method with Arithmetic averages (UPGMA). All statistical analyses were performed with aid NTSYSPC computer genotypes with cluster analysis using the SHAN module of NTSY sp. version 2.0.

RESULTS & DISCUSSION

The phenomenon of resistance and susceptible has been attributed to the existence of genetic variation among the genotypes which can be easily identified through molecular markers like RAPD, which detects variation at the DNA level. The RAPD analysis was carried out in order to analyze molecular characterization of all okra genotypes for YVMV.

Polymorphism Pattern of RAPD

The data from random amplification of polymorphic DNA with 13 arbitrary primers produced 228 total loci and total bands produced were 670. Out of the 228 loci produced, 214 were polymorphic and hence the total polymorphism percentage was found to be 93.36% (Table 1). Samarajeewa and Rathnayaka, (2004) obtained 73.84% polymorphism in okra by using RAPD markers. The average Polymorphism Information content (PIC) value for RAPD was 0.914. Average number of loci per primer was found to be 17.54 average number of polymorphic loci obtained per primer (Assay Efficiency index) was found to be 16.46. The molecular size of the amplified PCR products ranged from 80bp (OPK-20) to 3472bp (OPC-06) (Table 1). Results were agreement with previous studies by Prakash *et al.* (2011) in okra.

The highest similarity index value 0.640 was found between AOL 11-34 and AOL 11-37, while the least similarity index value 0.114 was found between AOL 09-17 and AOL 11-49. The average similarity coefficient among genotypes was 0.388 (Appendix 1).

TABLE 1: Polymorphism pattern of amplified RAPD primers of 10 okra genotypes

Sr. No	Locus Name	Molecular size range (bp)	Total no of bands	No. of Loci	No. of Polymor-phic loci	Polymor-phism (%)	PIC Value
1	OPA-01	349-1214bp	45	16	15	93.75	0.898
2	OPA-03	403-1638bp	67	16	14	87.50	0.918
3	OPC-02	144-1769bp	60	17	15	88.24	0.911
4	OPC-06	327-3472bp	57	23	22	95.65	0.934
5	OPC-11	192-1074bp	43	12	11	91.67	0.888
6	OPC-18	237-817bp	47	15	14	93.33	0.907
7	OPK-16	123-1459bp	52	22	22	100.00	0.931
8	OPK-18	235-1441bp	31	18	18	100.00	0.932
9	OPK-20	80-3363bp	51	23	23	100.00	0.938
10	OPN-04	228-3000bp	64	17	15	88.24	0.916
11	OPN-06	215-1258bp	58	20	18	90.00	0.913
12	OPO-05	330-1265bp	40	11	10	90.91	0.876
13	OPL-18	200-1279bp	55	18	17	94.44	0.925
Total		-	670	228	214	-	-
Average		235-1773bp	51.54	17.54	16.46	93.36	0.914

The minimum (31) and maximum (67) number of bands were produced by OPK-18 and OPA-03 respectively. The highest polymorphism (100%) was produced by OPK-16, OPK-18 and OPK-20 (Table 1). The highest PIC value obtained was 0.934 for OPC-06 and lowest PIC value obtained was 0.876 for OPO-05. Marker Index values were calculated according to the formulas described in section 3.8.3.5 Marker Index value for polled RAPD data was found to be 11.16. The high value of Marker Index can be justified due to high value of Effective Multiplex Ratio (EMR) component. EMR value is affected by the number of polymorphic and total number of loci

amplified. Based on the RAPD data, cluster analysis was performed using genetic similarity values and a dendogram was generated showing the grouping of genotypes according to their resistance and susceptibility reaction to YVMV.

Clustering Pattern for RAPD

The Clustering pattern of 10 okra genotypes was generated based on UPGMA-dendogram using the pooled molecular data of 13 RAPD loci and presented in Fig 1. The results indicated that two clusters namely A and B were formed at a similarity coefficient of 0.17. Cluster A was divided into two sub-cluster A_1 and A_2. Grouping of four genotypes that are susceptible to YVMV were in one major cluster 'A_1'. Sub-cluster of A_1included two minor clusters in which one consisted alone of AOL 09-2 and another minor cluster included genotypes *viz.*, AOL 11-34, AOL 11-37 and Pusa sawani which all susceptible genotypes to YVMV. Sub-cluster A_2 consisted two minor clusters of resistant genotypes AOL 10-22, AOL 11-49 and GAO-5. Cluster B represented for moderately resistant genotype which was divided in to two sub-clusters B_1 and B_2. Sub-cluster B_1 consist only genotype AOL 09-17, whereas Sub-cluster B_2 consist genotype AOL 11-39 and Parbhani kranti.

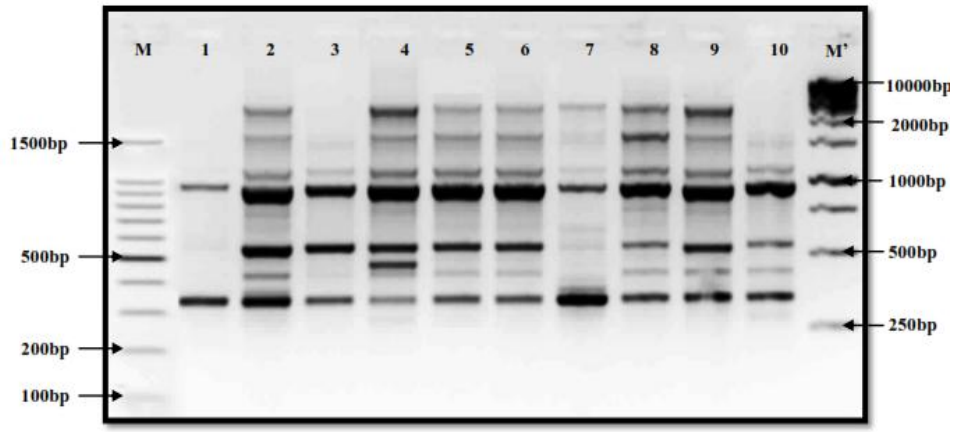

PLATE 1: RAPD profile of primer OPC-06

M = 100bp DNA ladder M'= 1kb DNA ladder

(1) AOL 09-2, (2) AOL 09-17, (3) AOL 10-22, (4) AOL 11-34, (5) AOL 11-37, (6) AOL 11-39, (7) AOL 11-49, (8) GAO-5, (9) Parbhani kranti, (10) Pusa sawani

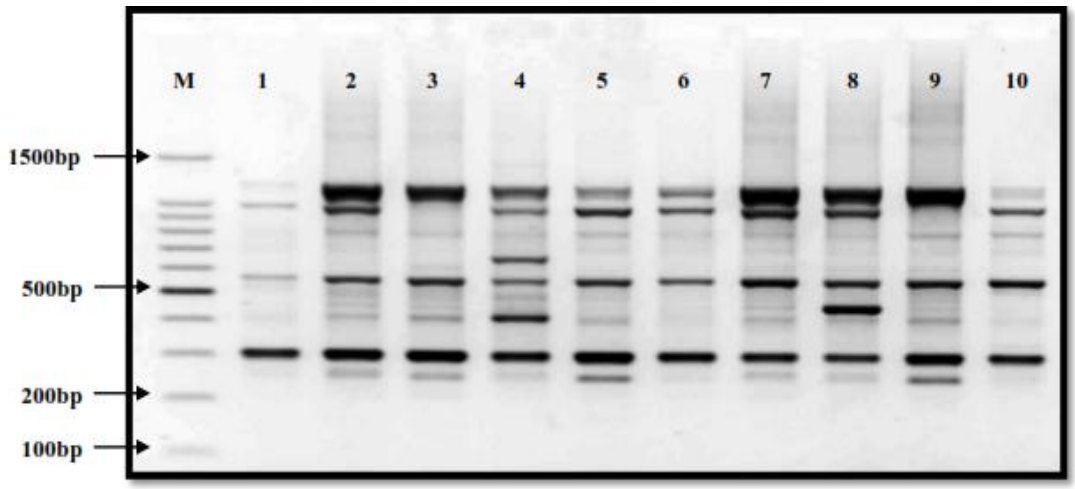

PLATE 2: RAPD profile of primer OPN-06

M = 100bp DNA ladder

(1) AOL 09-2, (2) AOL 09-17, (3) AOL 10-22, (4) AOL 11-34, (5) AOL 11-37, (6) AOL 11-39, (7) AOL 11-49, (8) GAO-5, (9) Parbhani kranti, (10) Pusa sawani

Since disease reactions are generally controlled by dominant and recessive genes; not many QTLs reported. RAPD markers can very well serve the purpose of identifying resistance from susceptibility. The results of RAPD analysis of 10 okra genotypes with 13 random primers revealed that this dominant marker can efficiently differentiate susceptible genotypes from resistant ones.

SSR MARKER ANALYSIS

In present investigation, ten okra genotypes were subjected to SSR analysis using different primers of MTIC series. From those 4 primers such as MTIC-7, MTIC-55, MTIC-82 and MTIC-95 were amplified. Various bio-statistical parameters were calculated which are efficient for diversity analysis like PIC value and similarity index.

Polymorphism Pattern of SSR

The data obtained in the present investigation are presented in Table 2 and Table 3. In the present SSR analysis 18 alleles were produced by 4 amplified markers. The average number of alleles per locus was found to be 4.5, while effective number of alleles was found 4.25. The maximum numbers of alleles (6) were recorded for markers MTIC 82 as well as MTIC 95. MTIC 7 and MTIC 55 generated 3 alleles. The highest allele frequency found by marker MTIC 7 and MTIC 55 was 0.40. The highest PIC value (0.82) was recorded for MTIC 82 and MTIC 95. The average PIC value and number of alleles were 0.72 and 4.5 respectively. The molecular weight of the amplified PCR products ranged from 85 (MTIC 82) to 326 (MTIC 7) (Table 3). The SSR regions of *Medicago truncatula* were used to amplify genomic DNA samples of different okra. Two okra accessions were different from other 18 based on molecular markers as well as morphological features of their fruits.

TABLE 2: No. of bands, Molecular size, total no. of allels and PIC value of SSR analysis of okra seedlings

Sr. No	Locus Name	No. of bands amplified	Molecular size range (bp)	Total no of Alleles	PIC
1	MTIC 7	10	300-326 bp	3	0.56
2	MTIC 55	10	129-138 bp	3	0.66
3	MTIC 82	20	85-144 bp	6	0.82
4	MTIC 95	20	217-293 bp	6	0.82
Total		60	-	18	2.86
Average		15	183-225bp	4.5	0.72

TABLE 3: Results of SSR analysis of okra seedlings

Sr. No	Locus Name	Na	Ne	Ho	He
1	MTIC 7	3	2.94	0.0	0.66
2	MTIC 55	3	2.94	0.0	0.66
3	MTIC 82	6	5.56	1.0	0.82
4	MTIC 95	6	5.56	1.0	0.82
Total		18	16.99	2.0	2.96
Average		4.5	4.25	0.5	0.74

Na = No. of Different Alleles, **Ne** = No. of effective Alleles,
Ho = Observed Heterozygosity, **He** = Expected Heterozygosity,

Based on the SSR data, cluster analysis was performed using genetic similarity values and a dendogram was generated showing the grouping of genotypes. The highest similarity index value of 0.715 was found between genotypes GAO-5 and AOL 10-22 (Appendix - 2), whereas the least similarity index value 0.001 was found between genotypes GAO-5 and Pusa sawani (Appendix - 2). Marker Index value for pooled SSR data was found to be 12.96. The high value of Marker Index can be justified due to high value of effective multiplex ratio (EMR) component. EMR value is affected by the number of polymorphic and total number of loci amplified.

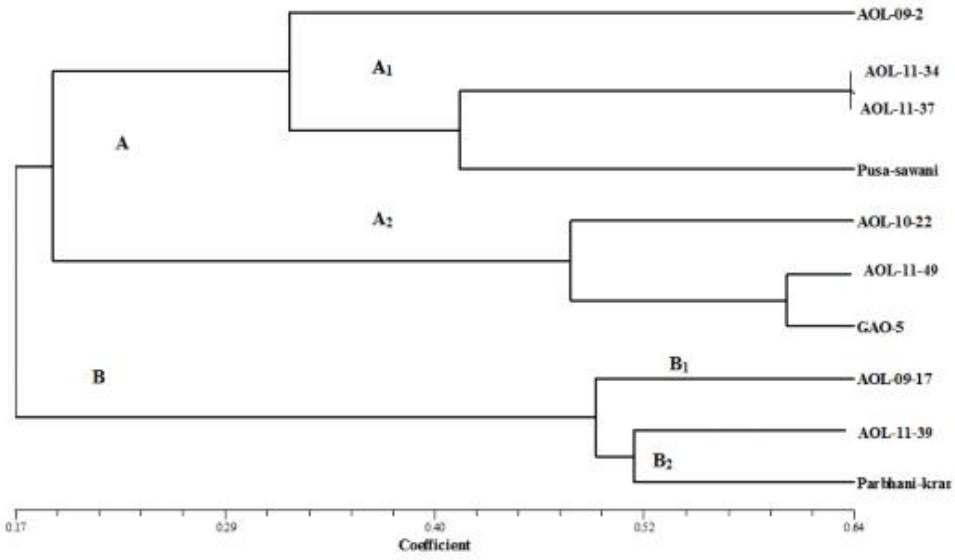

FIGURE 1: Dendogram showing clustering of 10 okra genotypes constructed using UPGMA based on Jaccard's coefficient obtained from RAPD analysis

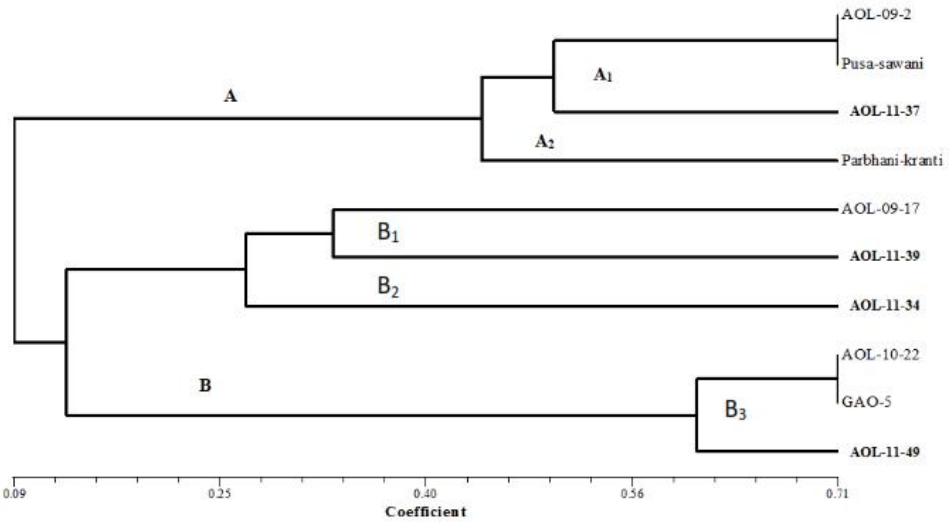

FIGURE 2: Dendogram showing clustering of 10 okra genotypes constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis

Clustering Pattern of Different SSR Primers Used

Clustering pattern of dendogram was generated by using the pooled molecular data of four SSR loci indicated that two clusters namely A and B were formed at a similarity coefficient of 0.09 (Fig 2). Cluster A was divided into two sub-cluster A_1 and A_2. Grouping of four genotypes that are susceptible to YVMV were in one major cluster 'A_1'. Sub-cluster of A_1 included two minor clusters in which one consisted AOL 09-2 and Pusa sawani, while another minor cluster included alone genotypes AOL 11-37. Sub-cluster A_2 consist alone genotypes Parbhani kranti which is moderately resistant to YVMV. Cluster B represented for moderately resistant and resistant genotype which was divided in to three sub-clusters B_1, B_2 and B_3. Sub-cluster B_1 contain AOL 09-17 and AOL 11-39 which are moderately resistant genotypes, while another minor cluster contain only genotype AOL 11-34 which is susceptible genotype to YVMV. Sub-cluster B3 consist resistant genotype AOL 10-22, GAO-5 and AOL 11-49. The presence of moderately resistant genotype (Parbhani kranti) in cluster in which all others genotypes are susceptible and susceptible (AOL 11-34) in cluster in which all others genotypes are moderately resistant indicate that SSR markers from cross species are not much useful for genetic diversity of okra against YVMV.

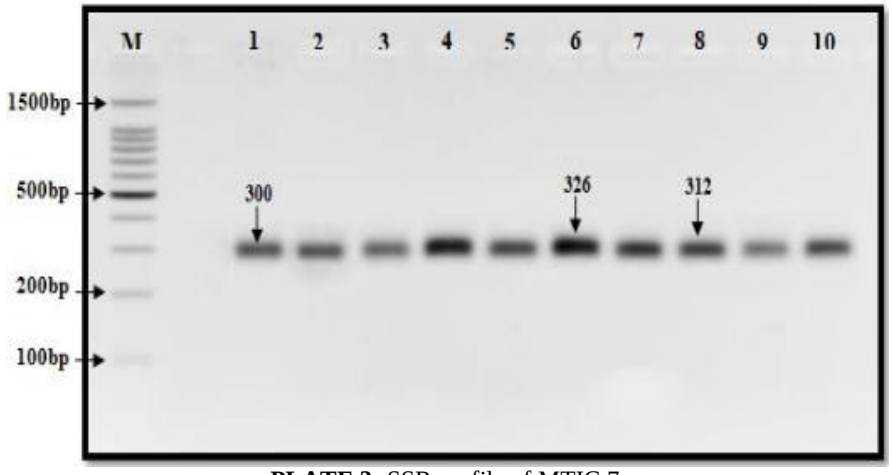

PLATE 3: SSR profile of MTIC 7

M = 100bp DNA ladder

(1) AOL 09-2, (2) AOL 09-17, (3) AOL 10-22, (4) AOL 11-34, (5) AOL 11-37, (6) AOL 11-39, (7) AOL 11-49, (8) GAO-5, (9) Parbhani kranti, (10) Pusa sawani

APPENDIX-1: Genetic similarity matrix of pooled RAPD data based on Jaccard's similarity coefficient

	AOL-09-2	AOL-09-17	AOL-10-22	AOL-11-34	AOL-11-37	AOL-11-39	AOL-11-49	GAO-5	Parbhani-kranti	Pusa-sawani
AOL-09-2	1									
AOL-09-17	0.173	1								
AOL-10-22	0.213	0.161	1							
AOL-11-34	0.303	0.143	0.221	1						
AOL-11-37	0.291	0.157	0.243	0.64	1					
AOL-11-39	0.16	0.505	0.19	0.207	0.195	1				
AOL-11-49	0.168	0.114	0.462	0.156	0.153	0.175	1			
GAO-5	0.16	0.143	0.5	0.177	0.195	0.227	0.602	1		
Parbhani-kranti	0.144	0.485	0.177	0.146	0.172	0.516	0.153	0.195	1	
Pusa-sawani	0.373	0.149	0.19	0.446	0.391	0.167	0.219	0.188	0.208	1

APPENDIX-2: Genetic similarity matrix of pooled SSR data based on Jaccard's similarity coefficient

	AOL-09-2	AOL-09-17	AOL-10-22	AOL-11-34	AOL-11-37	AOL-11-39	AOL-11-49	GAO-5	Parbhani-kranti	Pusa-sawani
AOL-09-2	1.000									
AOL-09-17	0.090	1.000								
AOL-10-22	0.090	0.200	1.000							
AOL-11-34	0.090	0.200	0.200	1.00						
AOL-11-37	0.500	0.090	0.090	0.09	1.000					
AOL-11-39	0.002	0.330	0.002	0.33	0.200	1.000				
AOL-11-49	0.002	0.200	0.500	0.09	0.002	0.090	1.000			
GAO-5	0.001	0.200	0.715	0.20	0.002	0.002	0.710	1.000		
Parbhani-kranti	0.330	0.090	0.200	0.09	0.500	0.200	0.200	0.090	1.000	
Pusa-sawani	0.710	0.200	0.200	0.00	0.500	0.002	0.090	0.090	0.500	1.00

CONCLUSION

The RAPD analysis with 13 arbitrary primers produced 228 total loci and total bands produced were 670. Out of the 228 loci produced, 214 were polymorphic and hence the total polymorphism percentage was found to be 93.36%. Clustering pattern in 10 okra genotypes was generated using the pooled molecular data of 13 RAPD loci indicated that two clusters were formed at a similarity coefficient of 0.17. RAPD analysis revealed that this dominant marker can efficiently distinguish susceptible genotypes from resistance ones. RAPD markers can very well serve the purpose of identifying resistancy from susceptibility. In the SSR analysis 4 markers were amplified and produced 18 alleles. Clustering pattern of dendogram in was generated by using the pooled molecular data of 4 SSR loci indicated that two clusters were formed at a similarity coefficient of 0.09. Grouping of four genotypes that are susceptible to YVMV were in one Sub-cluster of which included AOL 09-2, Pusa sawani, AOL 11-37. Another Sub-cluster consist alone genotypes Parbhani kranti which is moderately resistance genotype, which suggested that SSR primers from cross species did not help for screening of okra against YVMV.

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