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RESEARCH **P**APER

Assessment of genetic purity in hybrid lines by field grow-out test and molecular markers

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Tomato is a significant vegetable crop of special economic importance, ranking second to potato in the world. As genetic composition of a variety is its ultimate identity, achieving and maintaining genetic purity in crops is an important both from agronomic as well as breeding point of view. The present investigation was carried out to identify polymorphic primers where it can distinguish the hybrid parental line and utilization of this marker for further genotyping in the hybrid population. Towards this, 18 random decamer primers (RAPD), were screened between hybrid 2 parental lines, out of which only one primer (OPAO9) showed polymorphism and also study the tomato hybrid 2 parental lines consisted of 42 plants were analysed by using SSR Marker and only one primer (TGS0100) was found to be polymorphic which showed difference in banding pattern with hybrid 2 parental lines (male and female) and to check the genetic purity through protein analysis in the given tomato hybrid 2 parental lines (male and female). It was successfully reproduced in the hybrids. Thus, the present investigation shows the efficiency of RAPD and SSR marker as a molecular tool for genetic purity analysis. Hence, this marker was employed in the hybrid population. Furthermore DUS characters were also taken in the hybrids and parental lines. The results were correlating with the molecular data. Therefore, molecular GOT can be effectively employed in hybrid lots for purity checking. This could be better and fast technology than field GOT and it will be more useful for the quality assessment in the seed industries.

Key words : Parental line, Genetic purity, RAPD, SSR marker, Biochemical marker

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INTRODUCTION

Tomato (*Solanum lycopersicum* L., formerly Lycopersicum esculentum Mill.) is the second most important vegetable crop in the world next to potato, having a worldwide commercial distribution. It ranks third in priority after potato and onion in India. It is a member of the family Solanaceae and significant vegetable crop of special economic importance in the horticultural industry worldwide. The world area under tomato is 4,615,000 ha with production of 1, 27, 993, 000 tones and with productivity of 27.6 tones/hectare (IHD, 2006). China is the largest producer of tomato followed by United States and Turkey. India is the fourth largest and third in area with a production of 10,303,000 tones (FAOSTAT Database, August 2010) and area of 5.72 lakh hectares in 2007-08 (NHB Database). Tomato is native to Western South American and Central America. It is typically cultivated for its edible fruit. The plant grows up to 1-3 meters (3-10 ft) in height and has a weak stem that often sprawls over the ground and vines over other plants. The leaves are 10-25 centimeters (4-10 in) long, odd pinnate, with 5-9 leaflets on petioles, each leaflets up to 8 centimeters (3 in) long, with a serrated margin; both the

stem and leaves are densely glandular hairy. The flowers are 1-2 centimeters (0.4-0.8 in) across, yellow, with five pointed lobes in the corolla. They are borne in cymes of 3-12 together. It is perennial, often grown outdoors in temperate climates as an annual. Tomato is rich source of several nutrients. Per 100g of fruit contains 4.64g carbohydrates, 0.33g fat, 0.85g protein and 1.1g. fibre. Apart from this it is good source of potassium, phosphorus, magnesium, calcium, vitamin C, vitamin A, vitamin E and niacin (USDA Nutrient Database). Tomato has been recently gaining attention in relation to the prevention of some human diseases. This interest is due to the presence of carotenoids and particularly lycopene, which is an unsaturated alkylic compound, that appears to be an active compound in the prevention of cancer, cardiovascular risk and in slowing down cellular aging (Gerster, 1997; Di Cesare *et al.,* 2012 and Abdel-Monaim, 2012). Lycopene is found in fresh, red-ripe tomatoes as all-trans (79–91%) and cis- (9–21%) isomers (Shi *et al*., 1999; Boileau *et al*., 2002; Abdel-Fattah and Al-Amri, 2012).

RESEARCH METHODOLOGY

Plant material :

Tomato Hybrid-2 (male hybrid lines) plants were supplied by Indo American Hybrid Seeds (India) Pvt. Ltd. Bangalore.

Distinctness, uniformity and stability (DUS) characterization in tomato :

Tomato Hybrid-2 and parental line seeds were sown in the field to take down the DUS characteristics. The seeds which were sown had the germination capacity (98%), moisture content (<6%) and genetic purity (70%). For the assessment of distinctness and stability, observation was made on 10 plants or parts of 10 plants. For the assessment of uniformity of characteristics on the plot as a whole, was done by a single visual observation on a group of plants or parts of plants.

Determinate and indeterminate :

Tomatoes are determinate if they eventually form a flower cluster at the terminal growing point, causing the plant to stop growing in height. Plants that never set terminal flower clusters, but only lateral ones and continue indefinitely to grow taller are called indeterminate.

Determination of genetic purity in tomato :

Material used:

The experimental material of present study comprised of tomato hybrids and their parental lines. For genomic DNA isolation young leaves of parental line and hybrids were used. Quality of DNA was assessed with 0.8 per cent agarose gel electrophoresis.

PCR amplification :

Different dilution concentration of template DNA with double distilled water was used for the optimization of PCR to obtain bright and reproducible RAPD patterns. (Williams *et al.*, 1990).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Quality assessment in tomatoes :

After taking the DUS characteristics to check the quality and purity of tomato hybrid 2 parental lines it was found that tomato hybrid 2 parental lines were phenotypically observed to check their purity based on 47 DUS characterization descriptors, out of 47 descriptors only 8 important descriptors are shown in Table 1.

Tomato hybrid-2 parental line seeds were sown in the field to take down the DUS characteristics. The seeds which were sown had the germination capacity (98%) moisture content (<6%) and genetic purity (70%). For assessment of distinctness and stability, observations were made on 47 individual characteristics of DUS, which is shown in Table 2.

Table 1: Details of morphological DUS descriptors

Variety	Anthocyanin Colouaration of hypocotyls	Plant growth type	Leaflet serration	Number of inflorescence on main stem	Flower stigma	Fruit shape in longitudinal section	Fruit shape at blossom end	Fruit total soluble solids (Brix)
Hyb-2 male	Present	Determinate	Highly serrated	4	Unilobe	Flat	Flat	4
Hyb-2female	Present	Determinate	Highly serrated	4	Unilobe	Flat	Flat	4
Hyb-2	Present	Determinate	Highly serrated	4	Unilobe	Slightly flattened	Flat	4

Table 2: Individual DUS characteristics

Sr. No.	Characteristics	States	Note	Example verities	Stage of observation	Type of assessment
1	2	3	4	5	6	7
1.	Seedling:Anthocyanin	Absent	1	--	10	VS
	Colouaration of hypocotyl	Present	9	Kashi Amrit		
2.	Leaf: intensity of green colour	light	3	P.Chhuhara	20	VG
		Medium	5	Kasi Amrit		
		Dark	7	Kashi Sharad		
3.	Plant: Growth type	Determinate	1	Hisar Arun	50	VG
		Indeterminate	2	Arka Vikas		
4.	Stem: Pubescence	Absent	1	--	30	VS
		Present	9	Roma		
5.	Stem: Anthocyanin	Absent	1	Arka Abha	30	VG
	colouaration of upper third portion	Weak	3	SwarnaNaveen		
		Medium	5	Kashi Vishesh		
		Strong	7	Kalyanpur Selection-118		
6.	Stem: Length of internodes between 1st	Short(<25)	3	DT-10	30	MS
	and4th inflorence(for indeterminate	Medium(25-40)	5	Arka Vikas		
	varieties) (cm)	Long(>40)	7	Kashi Shared		
7.	Stem: Length of internodes between 1st	Short(<20)	3	Hisar Arun	30	
	and4th inflorence(for indeterminate	Medium(20-30)	5	Kashi Vishesh		MS
	varieties) (cm)	Long(>30)	7	Flora Dade		
8.	Leaf: Length(cm)	Short(<25)	3	Azad T-3	40	MS
		Medium(25-30)	5	Roma		
		Long(>30)	7	Flora Dade		
9.	Leaflet: Length (cm)	Short(<5)	3	PS-1	40	MS
		Medium(5-10)	5	Swarna Naveen		
		Long(>10)	7	Pusa Selection-120		
10.	Leaf: Width (cm)	Narrow(<15)	3	Kashi Sharad	40	MS
		Medium(15-20)	5	Azad Type-1		
		Broad(>20)	7	Flora Dade		
11.	Leaflet: Width (cm)	Narrow(<4)	3	P. Chhuhara	40	MS
		Medium(4-6)	5	Arka Abha		
		Broad(>6)	7	-		
12.	Leaflet: Serration	Absent(potato type)	1	DT-10	40	VS
		Less serrated	3	Kashi sharad		
		Highly serrated	7	Hissar Arun		
13.	Leaf: Structure	Open	3	Kashi sharad	40	VG
		Intermediate	5	Kashi Anupam		
		Closed	7	DT-10		
14.	Leaf: Attitude in relation to main stem	Semi-erect	3	DT-10	40	VG
	(in middle third of plant)	Horizontal	5	Kashi Anupam		
		Drooping	7	Hisar Arun		
15.	Leaf: Attitude of petioles of leaflets in	Semi-errect	3	DT-10	40	VG
	relation to main axis	Horizontal	5	Kashi sharad		
		Semi-drooping	7	Kalyanpur Angoorlata		
16.	Influences: Type	Uniparous	1	--	40	VS
	(2nd and 3rd truss)	Intermediate	2	Swarna Lalima		
		Multiparous	3	Roma		
17.	Plant: Number of inflorescence on	Few(<4)	3	Swarn Lalima	50	MS
	main stem (side shoots to be	Medium(4-8)	3	P.Kesari		
	ignored)(for determinate varieties only)	Many(>8)	7	--		
18.	Flower: Fasciation	Absent	1	Pant T-3	40	VG
	(1st flower of inflorescence)	Present	9	--		
19.	Flower: Pubescence of style	Absent	1	--	40	VG
		Present	9	Pusa Ruby		
20.	Flower: Colour	Yellow	1	Kashi Amrit	40	VG
		Orange	2	--		
21.	Flower: Anther colour	Green	1	--	40	VG
		Yellow	2	Kashi Anupam		
22.	Flower: Nature of stigma	Non-exserted	1	Kashi Amrit	40	VS
		Exserted	2			
23.	Flower: Stigma	Unilobe	1	Roma	40	VS
		Bilobe	2	GujaratTomato2		
		Multilobe	3	Kashi Anupam		

Table 2 : Contd.……..…

Table 2 : Contd……

24.	Flower: Calyx size	Short(<1cm)	3	Swarna Naveen	40	VS
		Medium(1-1.5cm)	5	Co-3		
		Large(>1.5cm)	7	Flora Dade		
25.	Peduncle: Abscission layer	Absent (jointless)	1	F-6050	40	VS
		Present (jointed)	9	Pusa Ruby		
26.	Jointed peduncle: Length (from abscission layer to calyx) (cm)	Short(<1.5cm)	3	--	40	MS
		Mediun(1.5-2.0cm)	5	Utkal Urbashi		
		Long(>2.0cm)	7	--		
27.	Time of flowering(50% of the plants with at least one open flower from seed sowing)	Early(<65days)	3	Hisar Arun	40	VG
		Medium(65-80days)	5	Kashi Amrit		
		Late(>80days)	7	Kashi Sharad		
28.	Fruit: Intensity of green colour (before maturity)	Light	3	Co-3	60	VG
		Medium	5	Kashi Amrit		
		Dark	7	--		
29.	Fruit: Green shoulder (before maturity)	Absent	1	Kashi Anupam	60	VS
		Present	9	Flora Dade		
30.	Fruit: Size (average weight of 10 fruit) (g)	Very small(<100)	1	--	70	MG
		Small(100-200)	3	--		
		Medium(201-700)	5	DT-10		
		Large(701-1000)	7	Hisar Arun		
		Very large(>1000)	9	Kashi Anupam		
31.	Fruit: Length (cm)	Very short(>3.0)	1	--	70	MS
		Small(3.0-5.0)	3	Kalyanpur		
		Medium(5.1-7.0)	5	Pusa-120		
		Large(7.1-9.0)	7	Roma		
		Very large (>9.0)	9	--		
32.	Fruit: Width (cm)	Very short(<3.0)	1	--	70	MS
		Small (3.0-5.0)	3	Swarna nave		
		Medium(5.1-7.0)	5	Kashi vishes		
		Large(7.1-9.0)	7	Kashi Anipa		
		Very large(>9.0)	9	--		
33.	Fruit: Shape in longitudinal section	Flattened	1	Hisar Lalima	70	VS
		Slightly flattened	2	Kashi Anupam		
		Circular	3	Kashi Vishes		
		Rectangular	4	--		
		Cylindrical	5	Roma		
		Heart shaped	6	--		
		Obovoid	7	Kashi Sharad		
		Ovoid	8	--		
		Pear shaped	9	Punjab chhuhara		
34.	Fruit: Ribbing at peduncle end	Absent	1	Kalyanpur	70	VS
		Weak	3	Kashi vishesh		
		Medium	5	Hisar Arun		
		Strong	7	Kashi Anupam		
35.	Fruit: Cross section	Not round	1	Hisar Lalima	70	VS
		Round	2	Pusa Ruby		
36.	Fruit: Depression at peduncle end	Absent	1	P.Chhuahara	70	VS
		Shallow	3	Kalyanpur Angootlata		
		Medium	5	Flora Dde		
		Deep	7	Kashi Anupam		
37.	Fruit: Size of scar around peduncle(diameter)	Small(<1.0cm)	3	P.Chhuhara	70	MS
		Medium(1.1-2.0cm)	5	Kashi Anupam		
		Large(>2.0cm)	7	--		
38.	Fruit: Size of blossom scar	Small	1	--	70	MS
		Medium	5	Kashi Sharad		
		Large	7	Kashi Anupam		
39.	Fruit: Shape at blossom end	Indented	1	Kashi Anupam	70	VS
		Indented to flat	2	Hisar Arun		
		Flat to pointed	3	Kashi Vishesh		
		Pointed	4	--		
			5	DT-10		
40.	Fruit: Size of core in cross section (in relation to total diameter)	Small	3	Swarna Naveen	70	MG
		Medium	5	Punjab kesari		
		Large	7	Kashi Sharad		
41.	Fruit: Thickness of the per carp	Thin(<0.3cm)	3	Hisar Arun	70	MG
		Medium(0.3 to 0.6)	5	Kashi Amrit		
		Thick (>0.6cm)	7	Kashi Sharad		

Table 2 : Contd……..

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42.	Fruit: Number of locales	2	1	Punjab Keshari	70	VS
		3-4	2	Roma		
		>4	3	Kashi Anupam		
43.	Fruit: Colour at maturity	Yellow	1	--	70	VG
		Orange	2	--		
		Pink	3	--		
		Red	4	Kashi Vishesh		
44.	Fruit: Colour of flesh at maturity	Yellow	1	--	70	VG
		Orange	2	--		
		Pink	3	--		
		Red	4	Kashi Vishesh		
45.	Fruit: Firmness 9kg/cm^2	Soft(<3)	1	--	70	
		Medium(3-6)	2	--		
		Firm(>6)	3	--		
46.	Time of maturity(from seed sowing)	Early(110days)	3	Hisar Arun	70	MG
		Medium(110-	5	Kashi Amrit		
		130days)	7	Kashi Vishesh		
		Late(>130days)				
47.	Fruit: Total soluble solids (Brix)	Low(>3)	3	--	70	MG
		Medium (3.1-4)	5	Hisar Arun		
		High (4.1-5.0)	7	Pant T-3		
		Very high (>5)	9	--		

Genetic purity analysis in tomatoes (Tomato hybrid 2 male parental lines) isolation of genomic DNA:

The DNA was isolated from tomato seedlings samples, using CTAB method. And sample underwent agarose gel electrophoresis for genomic DNA analysis. The gel documentation showed good concentration of genomic DNA in the observed sample, which is shown in Fig. 1. This isolated genomic DNA was used for MAS using PCR for evaluation.

RAPD analysis of tomato hybrid 2 male parental lines:

The (OPC02) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 2.

The (OPD18) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 3.

The (OPD05) primer of RAPD marker was used

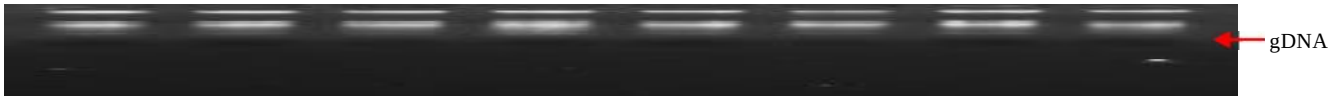

Fig. 1: Bands of genomic DNA extraction using CTAB method, run on 0.8 per cent agarose gel

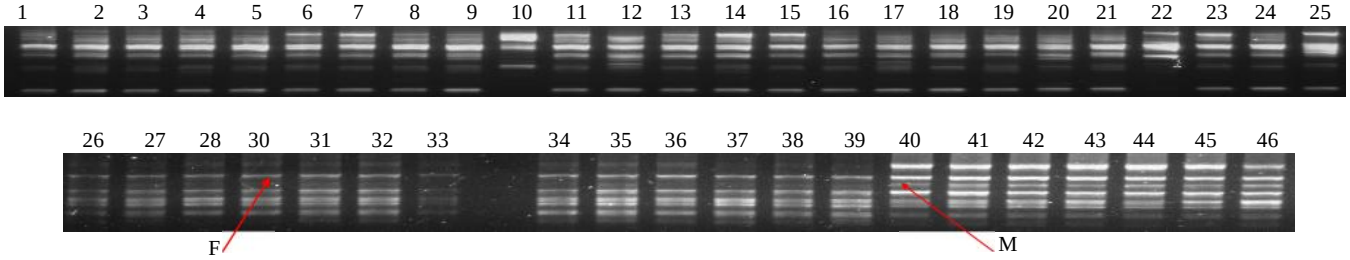

Fig. 2: RAPD profile of tomato hybrid 2 male line for primer OPC02

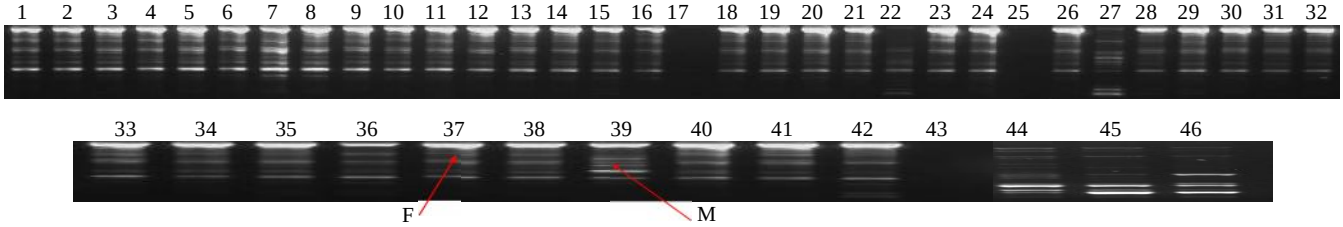

Fig. 3: RAPD profile of tomato hybrid 2 male line for primer OPD18

for the amplification of genomic DNA samples which is shown in Fig. 4.

The (OPN12) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 5.

Tomato hybrid 2 (male and female) parental lines were screened using random decamer primers out of which primers were selected on the basis of robustness of the amplification, clarity and scorability of bands and hence employed for genetic purity analysis. Amplicon were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence. A pair wise similarity matrix was determined using Jaccard's co-efficient and UPGMA cluster analysis was performed to develop a dendrograms. All three lines were analyzed using NTSys software and based on the dendrograms obtained the following conclusion was derived. This is shown in Fig. 5.

Hybrid-2 (Male) (Samples-46) :

Tomato hybrid 2 male parental lines were analyzed using NTSys software and based on the dendrograms obtained that tomato hybrid 2 male parental lines consisted of 46 plants were divided into one major group and two minor groups. The major group consisted of 25 plants (1, 2, 3, 44, 43, 7, 40, 39, 38, 34, 32, 30, 29, 28, 24, 22, 31, 23,

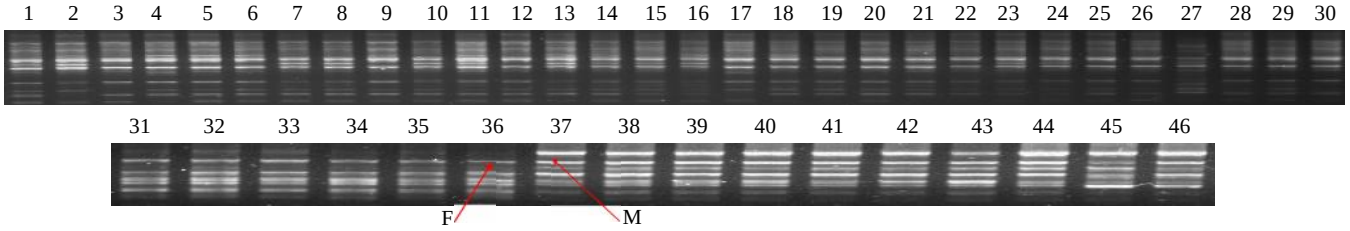

Fig. 4: RAPD profile of tomato hybrid 2 male line for primer OPC05

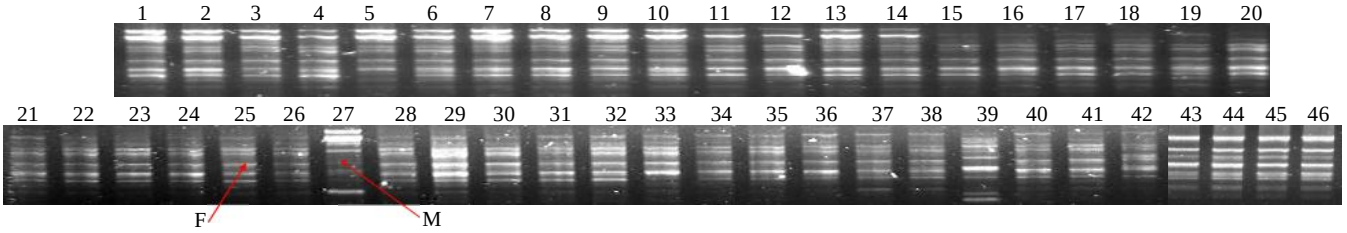

Fig. 5: RAPD profile of tomato hybrid 2 male line for primer OPN12

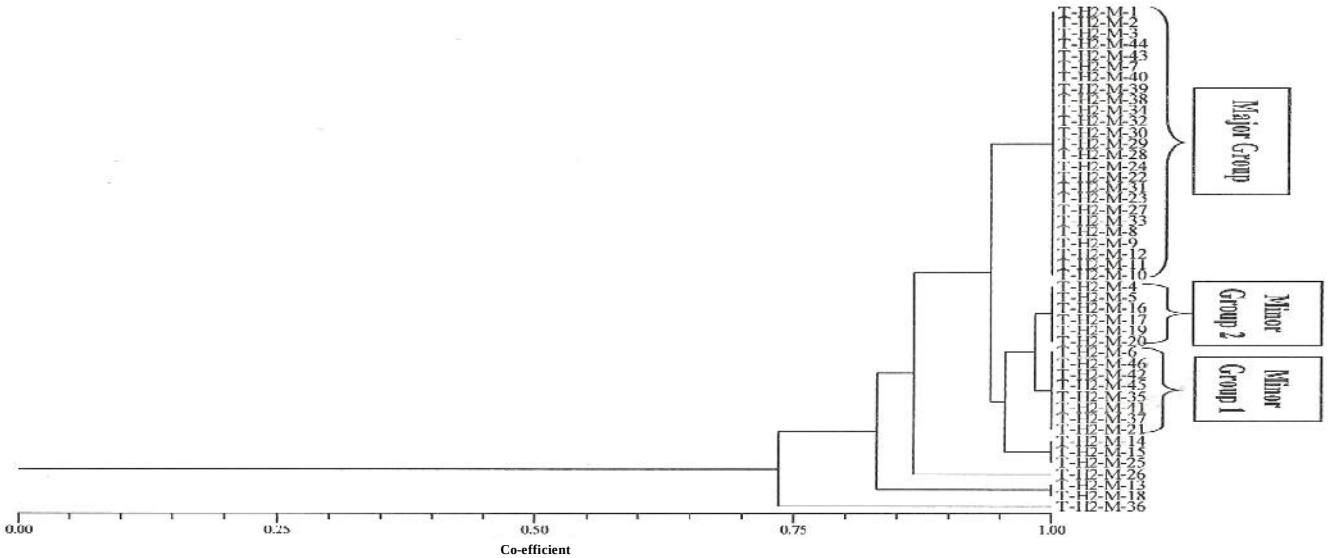

Fig. 6: Phylogenetic relationship between the tomato hybrid 2 male plants by RAPD analysis

27, 33, 8, 9, 12, 11, 10) showed 100 per cent similarity. The minor group 1 consisted of 8 plants (6, 46, 42, 45, 35, 41, 37, 21) and group 2 consisted of 6 plants (4, 5, 16, 17, 19, 20) showed 94 per cent similarity with respect to the major group. Remaining plants (14, 15, 25=94%, 26=87%, 13, 18=83%, 36=74%) which were not belonging to the major group are showing individual percentages. The overall similarity between the tomato hybrid 2 lines was found to be 74 per cent.

The DNA was isolated from tomato seedlings samples, using CTAB method. And sample underwent agarose gel electrophoresis for genomic DNA analysis. The gel documentation showed good concentration of genomic DNA in the observed sample, which is in Fig. 7. This isolated genomic DNA was used for MAS using PCR for evaluation.

Tomato hybrid 2 female parental lines:

The (OPC02) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 8.

The (OPD18) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 9.

The (OPC05) primer of RAPD marker was used for the amplification of genomic DNA samples which is

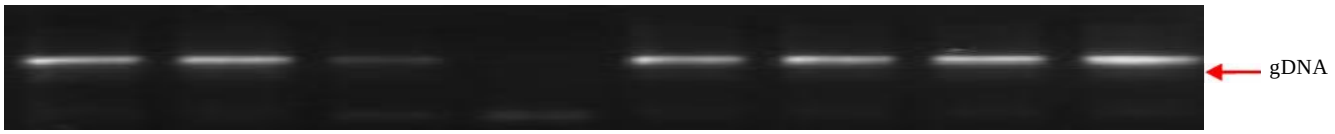

Fig. 7: Genomic DNA of tomato hybrid 2 lines isolated was viewed on 0.8 per cent agarose gel

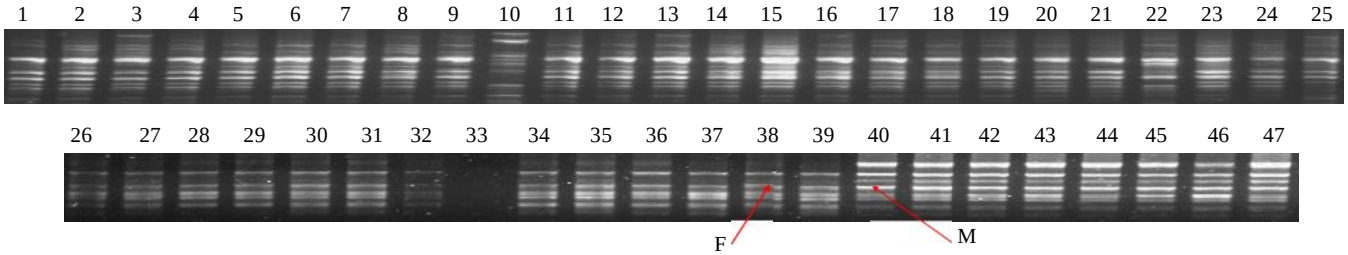

Fig. 8: RAPD profile of tomato hybrid 2 line for primer OPC02

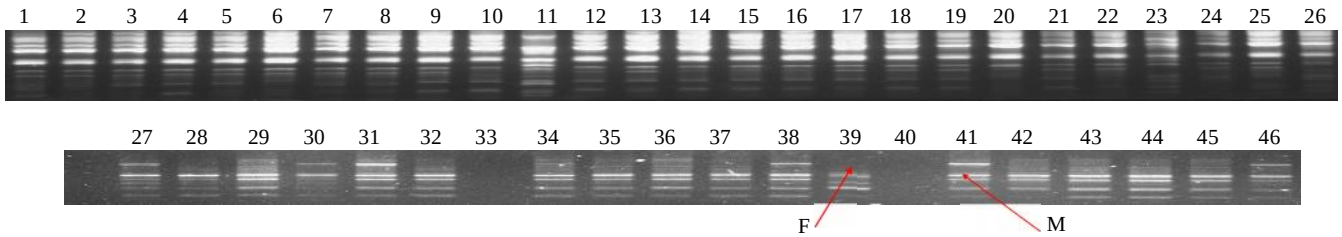

Fig. 9: RAPD profile of tomato hybrid 2 line for primer OPD18

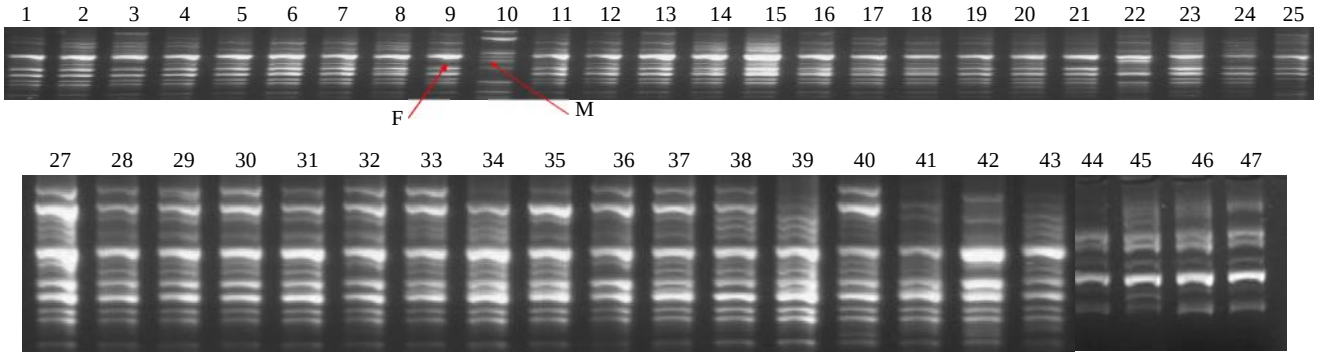

Fig. 10: RAPD profile of tomato hybrid 2 line for primer OPC05

shown in Fig. 10.

The (OPN12) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 11.

Tomato hybrid 2 female parental lines were screened using random decamer primers out of which primers were selected on the basis of robustness of the amplification, clarity and scorability of bands and hence, employed for genetic purity analysis. Amplicon were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence. A pair wise similarity matrix was determined using Jaccard's co-efficient and UPGMA cluster analysis was performed to develop a dendrograms. All three lines were analyzed using NTSys software and based on the dendrograms obtained the following conclusion was derived. This is shown in Fig. 12.

Hybrid-2 (Female) (Samples-47) :

Tomato hybrid 2 female parental lines, were analysed using NTsys software and based on the dendrogram obtained that tomato hybrid 2 female parental lines consisted of 47 plants which were divided into one major group and two minor groups. The major group consisted of 37 plants (1, 2, 3, 4, 45, 5, 6, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 46, 7, 29, 28, 27, 26, 25, 24, 8, 22, 21, 10, 19, 18, 17, 16, 15, 11, 13, 12) showed 100 per cent similarity. Remaining plants (9, 31= 90%, 23, 42 = 90%, 43 = 90%, 30 = 83%, 14 = 77%, 20 = 77%, 44 = 75%) which were not beloning to the major group are showing individual percentages. The overall similarity between the tomato hybrid 2 lines was found to be 75 per cent shown in Fig. 12. The DNA was isolated from tomato seedlings samples, using CTAB method then sample underwent agarose gel electrophoresis for genomic DNA analysis. The gel documentation showed good concentration of genomic DNA in the observed sample, which is shown in Fig. 13.This isolated genomic DNA was used for MAS using PCR for evaluation.

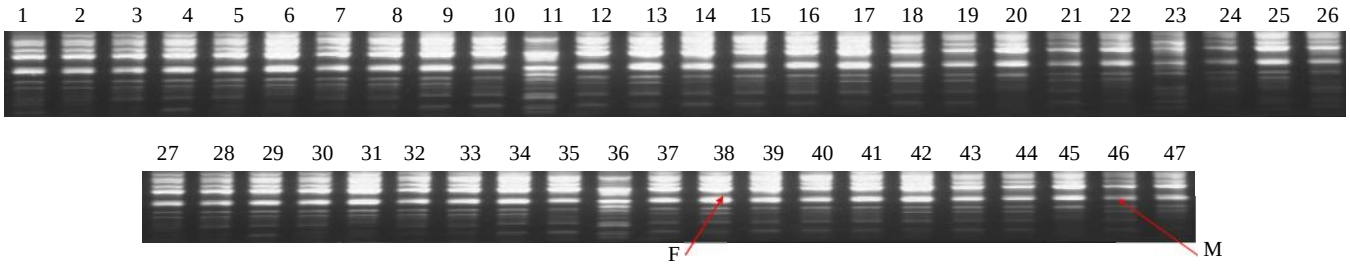

Fig. 11: RAPD profile of tomato hybrid 2 line for primer OPN12

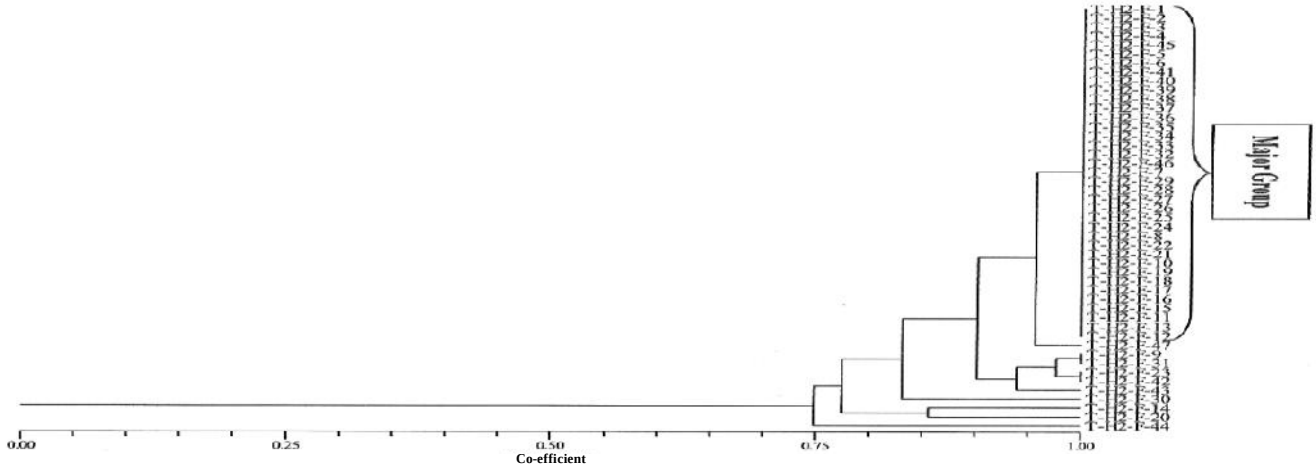

Fig. 12: Phylogenetic relationship between the tomato hybrid 2 female plants by RAPD analysis

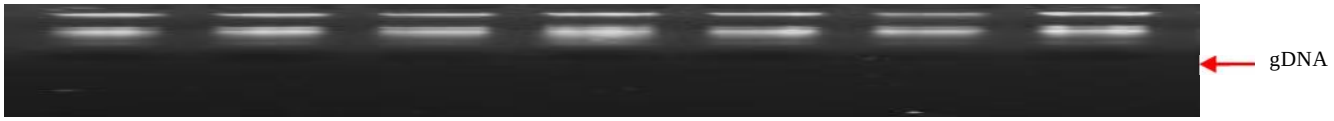

Fig. 13: Genomic DNA extraction of tomato 2 lines isolated was viewed on 0.8 per cent run on agarose gel

Tomato hybrid 2 female parental lines (Hybrid-2):

The (OPC02) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 14.

The (OPD18) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 15.

The (OPC05) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 16.

The (OPN12) primer of RAPD marker was used for the amplification of genomic DNA samples which is shown in Fig. 17.

Tomato hybrid 2 female parental lines were screened using random decamer primers out of which primers were selected on the basis of robustness of the amplification, clarity and scorability of bands and hence employed for genetic purity analysis. Amplicon were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence. A pair wise similarity matrix

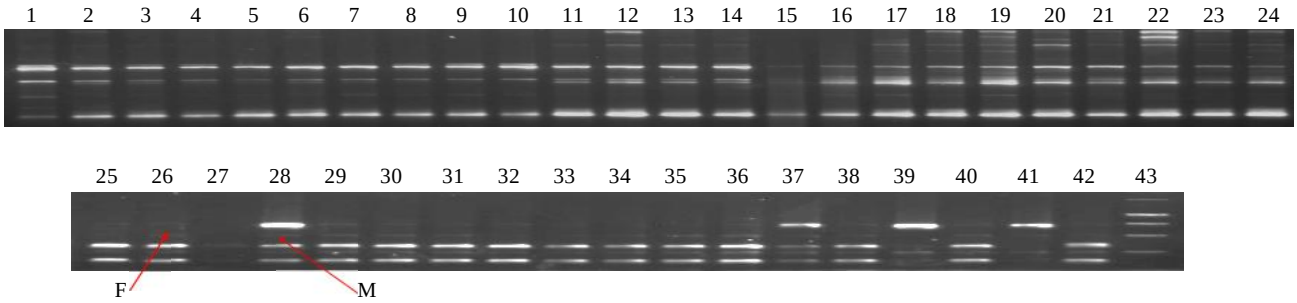

Fig. 14: M RAPD profile of tomato hybrid 2 line for primer OPC02

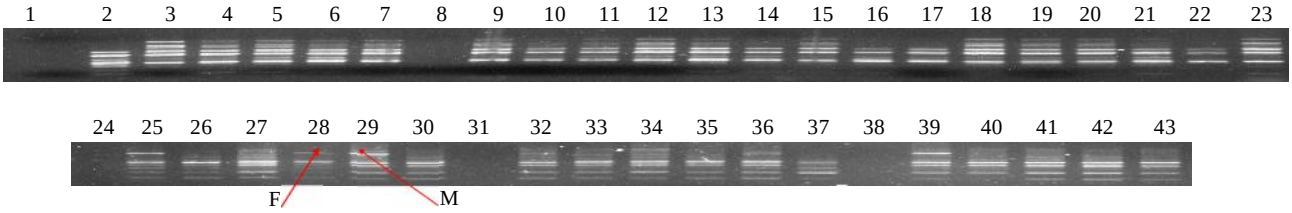

Fig. 15 : RAPD profile of tomato 2 line for primer 0PD18

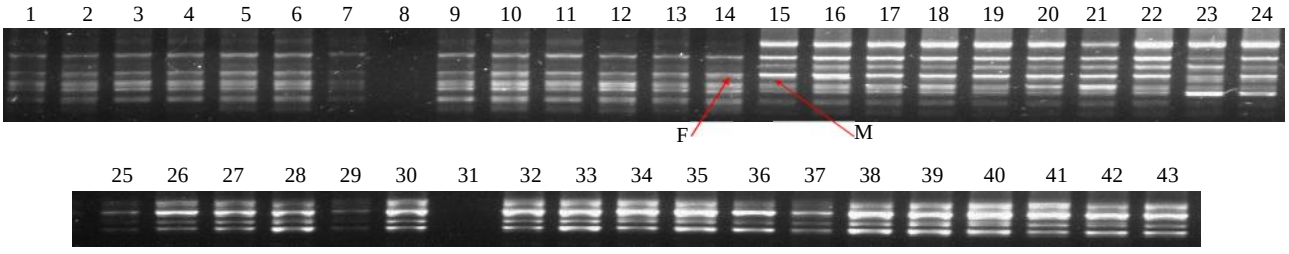

Fig. 16: RAPD profile of tomato hybrid 2 line for primer OPC05

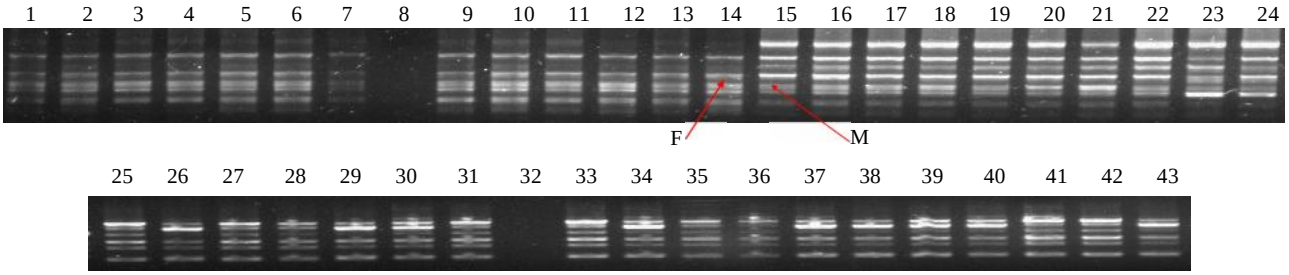

Fig.17: RAPD profile of tomato hybrid 2 line for primer OPN12

was determined using Jaccard's co-efficient and UPGMA cluster analysis was performed to develop a dendrograms. All three lines were analyzed using NTSys software and based on the dendrograms obtained the following conclusion was derived. This is shown in Fig. 18.

Hybrid-2 (Samples- 43) :

Tomato hybrid 2 lines were analysed using Ntsys software and based on the dendrogram obtained that tomato hybrid 2 lines consusted of 43 plants which were divided into one major group and three minor groups. The major group consisted of 33 plants (1, 3, 4, 43, 41, 39, 37, 5, 33, 31, 30, 36, 32, 27, 6, 25, 38, 23, 22, 21, 20, 19, 18,17, 7, 15, 14, 13, 12, 11, 10, 9, 42) showing 100 per cent similarity. The minor group 1 consisted of 5 plants (2, 8, 29, 34, 40) showing 91 per cent similarity with respect to the major group. Remaining plants (16, 24=92%, 26, 28=69%, 35=69%) which were not belonging to the major group are showing individual percentages. The overall similarity between the tomato hybrid 2 lines was found to be 69 per cent which is shown in Fig. 18.

The (OPA09) Primer of RAPD Marker was used for the amplification of genomic DNA samples which is shown in Fig. 19.

Total 18 primers were screened in the tomato (*Lycopersicum esculentum* L.). Out of 18 primers only one primers showed polymorphic bands. OPA09 showed highest number of polymorphic bands produced (male

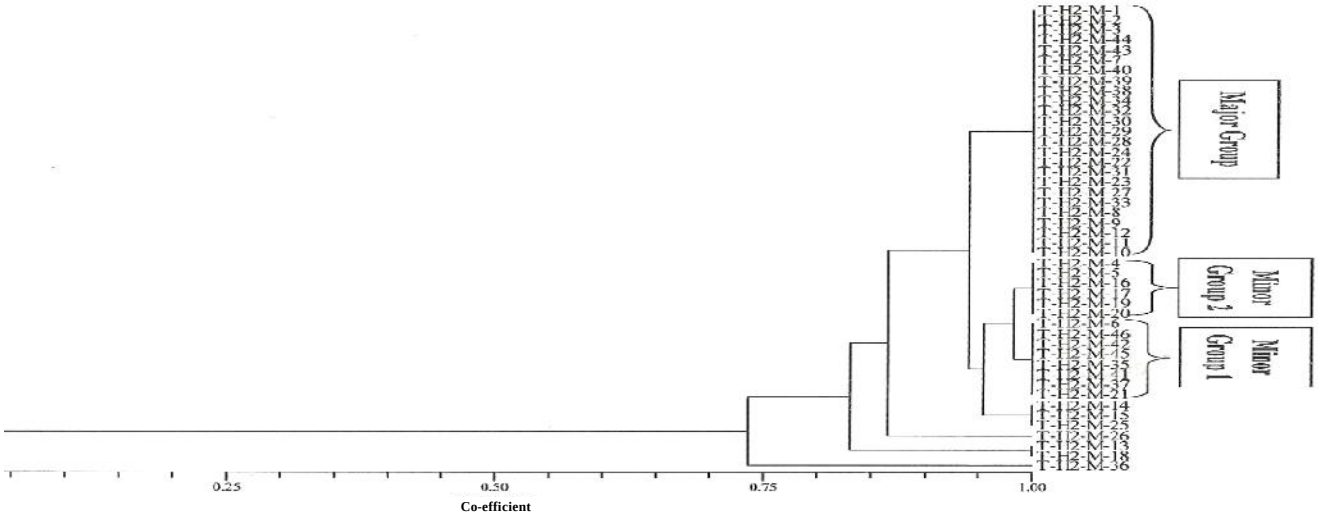

Fig. 18: Phylogenetic relationship between the tomato hybrid 2 plants by RAPD analysis

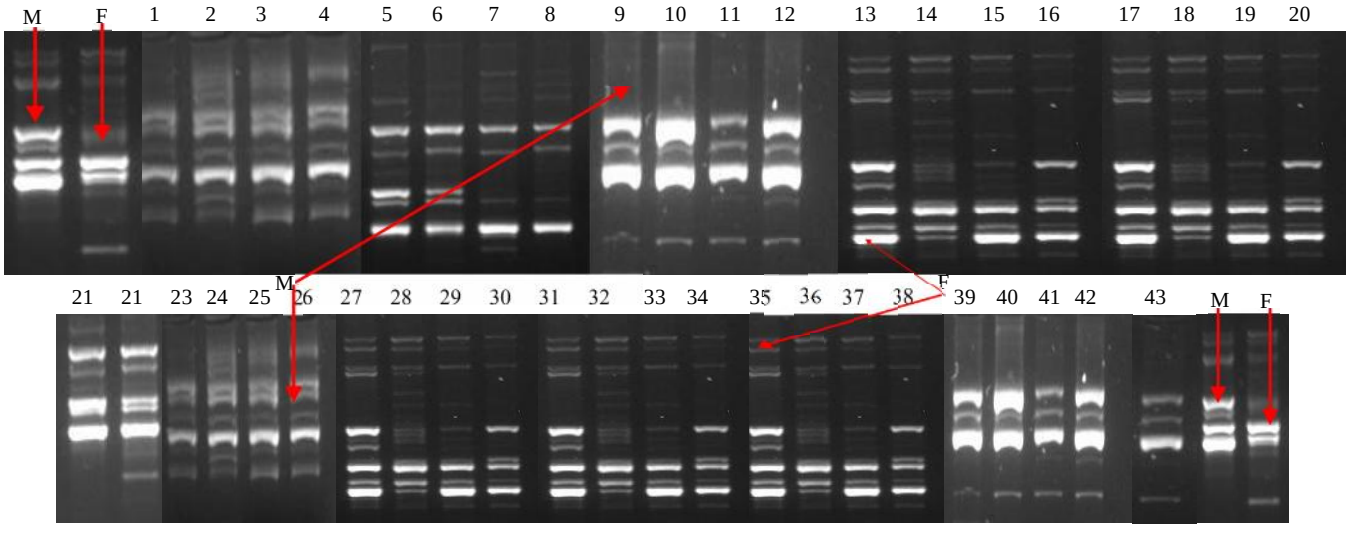

Fig. 19: Hybrid DNA showing male and female parental line specific RAPD bands. Polymorphism test in tomato hybrid 2 lines

and female) parental line, between Hyb-9 and Hyb-26 showed male characteristics and Hyb-13 and Hyb-35 showed female characteristics tomato samples.Thus, (OPA09) primer can be further used to analyze the difference between hybrid and parental sample of tomato, further these primers can also be used to confirm that the hybrid is of its respective parents. And thus can be used efficiently for genetic purity testing, which is shown in Fig. 19.

SSR analysis :

The OPN12 primer of SSR marker was used for the amplification of genomic DNA samples which is shown in Fig. 20.

The TGS100 primer of SSR marker was used for the amplification of genomic DNA samples which is shown in Fig. 21.

The above gel documentation shown the result of hybrid markers which were used to identify the male and female parental line, hybrids by using SSR marker by the help of agarose gel electrophoresis with using standard ladder sequence. Out of 42 hybrids, three hybrids samples (29, 40 and 42) shown female characteristics and three hybrid gel samples (28,37and 39), shown male characteristics with respective to standard ladder which is shown in Fig. 21. and it was successfully reproduced in the hybrids.Thus, the present investigation shows the efficiency of SSR marker as a molecular tool for genetic purity analysis.

The TGS0054 primer of SSR marker was used for the amplification of genomic DNA samples which is shown in Fig. 22.

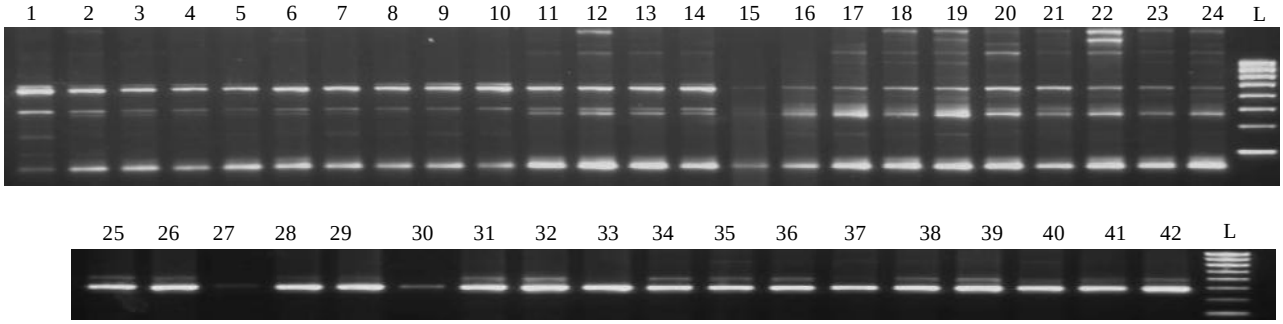

Fig. 20: SSR patterns of tomato parental line (male and female) DNA amplified with primer TGSOO93

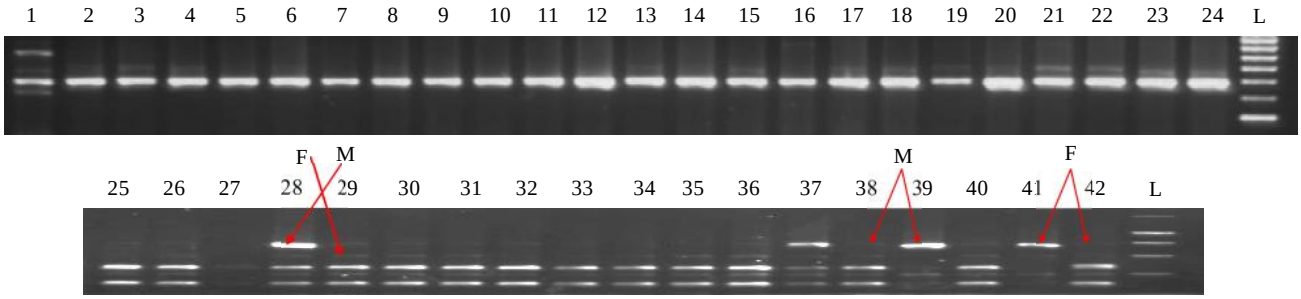

Fig. 21: SSR patterns of tomato parental line (male and female) DNA amplified with primer TGS0100

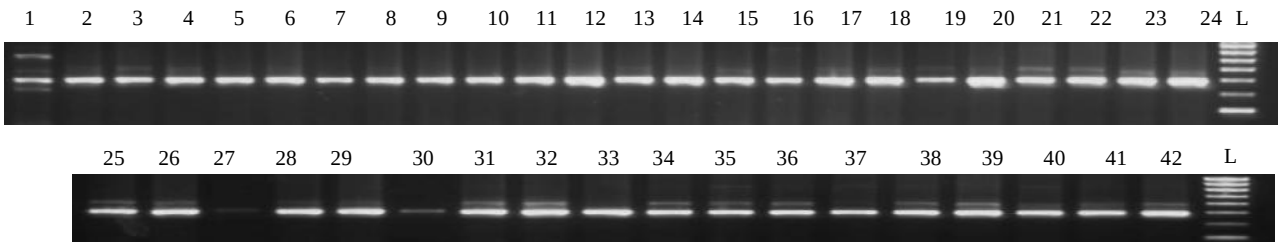

Fig. 22: SSR patterns of tomato parental line (male and female) DNA amplified with primer TGSOO54

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