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Analysis of genetic similarity and variability of selected varieties and F4 hybrids in Sesame (*Sesamum indicum* L.) by using RAPD method

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Abstract
Sesame is one of the oldest oil crops known and used by humankind because of its ease of extraction, great stability, and drought resistance. Sesame seed flour has high protein content with high level of the essential amino acids; methionine and tryptophan contain about 10 to 12 per cent of oil and has three times more calcium than milk. Nevertheless the Sesame productivity is low when compared to number of human population in India because of lack of potential varieties in Sesame genotype. In connection with that intraspecific hybridization is one of the easy and successful crossing methods to improve Sesame genotype. The present investigation deals with five varieties of Sesame (TMV-3, TMV-4, TMV-7, VRI-1 and VRI-2) were selected for analysis of genetic similarity and variability between varieties and its hybrids. The hybrids obtained from F4 research field population. Random amplified polymorphic DNA (RAPD) is a PCR based technique for identifying genetic variation and genetic similarities. It involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA products. So, the present research paper was undertaken to analysis of genetic similarity and variability of some selected varieties and its hybrids of Sesame (*Sesamum inducum* L.) by using RAPD method.

Keywords: genetic, similarity, variability, varieties, F4 hybrid, RAPD, dendrogram, *Sesamum inducum* l.

Introduction
Sesame (*Sesamum indicum L*) is one of the most ancient oil seed crop known and used by human kind mainly due to its ease of extraction, highly stability and resistance to drought (Iwo *et.al*., 2002 and weiss, 1971) [8, 13]. It is believed to have originated from tropical Africa (Bedigian and Harlan, 1986) [2]. However, India is held as the subcontinent where Sesame was first domesticated and then spread to other places in the World. Sesame plays an important role in human nutrition. Most of the Sesame seeds are used for Oil extraction and the rest are used for edible oil purpose (EI Khier *et al*., 2008) [4]. Sesame seed is rich in fat, protein, Carbohydrates, fiber and some minerals. The oil is renowned for its stability because it strongly resists oxidative rancidity even after long exposure to air (Global Agri systems, 2010; Kumar and Shanmugavalli, 2007 and Langham *et al*., 2007) [6, 3]. Sesame seed is approximately 50 percent oil and 45 percent meal (Lalpant luangi and Shah, 2018) [10]. In India, Sesame is cultivated on an area of 2.18 m ha with a production of 0.7. Million tons annually. However, the productivity is low in India (332 kg/ha) as compared to world's average (389 kg/ha) and it is far below compared to Egypt (1175 kg/ha) being the highest. This evidently indicates the potentiality of the crop for improvement in yields. The present sesame varieties under cultivation have limited yield potential. Hybridization technique is one of the best methods to evolve and release a potential variety in Sesame. However intraspecific hybridization is a well-known method to produce wide range of genetic modification in the species of *Sesamum indicum L*. Nevertheless, adequate genetic variability is not available in the existing germplasm for changing the plant type. Therefore the present investigation planned to work out the analysis of genetic variability with five sesame varieties and its F4 hybrids by using is RAPD molecular method. RAPD technique is one of the most commonly used molecular techniques to develop DNA markers and RAPD Profile. A target DNA sequence is exponentially amplified with the help of arbitrary primers, a thermostable DNA polymerase, dideoxy nucleotide tri - phosphates, magnesium and reaction buffer. The reaction involves repeated cycles, each consisting of a denaturation, a primer annealing and an elongation. Five sesame varieties (*Sesamum Indicum* L.) with the crossed F4 hybrids were subjected to analyze for identifying DNA Polymorphism. Hence, the present research paper aimed to deals with the analysis of genetic variability of some varieties in Sesame and its F4 hybrids.

Material and methods
Xpress DNA plant kit was purchased from Magenome technologies, India. Tris, EDTA, ETBr, Tris base, APS, TEMED, betamercaptoethanol, glycerol, Bromophenol blue, Acrylamide mix and Acetic acid were from Sigma

(USA), RAPD primer was from Xcelris Pvt. Ltd. India. PCR master mix kit (Applied Biosystems, CA, USA). EDTA, Sodium acetate was purchased from SRL, India.

Primers Used

Table 1

Gene	Sequence
RAPD	CCTGGCCTA

TE buffer
Stock 0.5 M Tris HCl (pH-8.0) - 2.0 mL (10 mM)
Stock 0.5 M EDTA (pH-8.0) - 0.2 mL (1 mM)
Made up the solution to 100 mL with distilled water, then autoclaved and stored at 4 °C.

TAE Buffer (50X – 1 Liter)
Trisbase - 242 g
Glacial acetic acid - 57.1 mL
EDTA - 100 mL (0.5 M, pH- 8)
242 g of Trisbase, 57.1 mL of glacial acetic acid mixed. 100 mL of 0.5 M EDTA, (pH 8.0) added and made up with distilled water to 1liter.

Acrylamide (30%)
Acrylamide - 29.2 g
Bis acrylamide - 0.8 g
Make up to 100 ml using distilled water

APS (10%)
100 mg in 1 ml water

SDS (10%)
10 g in 100 ml distilled water.

Sample buffer (2X)
100 mM Tris-HCL - 0.121 g
4% SDS - 0.4 g
0.2 % Bromophenol Blue - 0.02 g
20 % Glycerol - 2 ml
200 mM betamercaptoethanol - 0.156 ml
Total volume was made to 10 ml with distilled water.

Separating gel buffer
1.5 M Tris base - 18.15 g
Adjusted the pH to 8.8 using Hcl and made up to 100 ml

Gel composition

Table 2

Materials	Native PAGE gel 10% (10ml)
H2O	4.2 ml
Acrylamide 30%	3.3 ml
(Tris 1.5 M pH 8.8)	2.5 ml
APS 10%	50 µl
TEMED	5 µl

Procédure
Enough amount of Sesame leaf (15 days old plant) of F4 hybrids and parent samples (Parents: TMV-3, TMV-4, TMV-7, VRI-1 and VRI-2, Hybrids:
1. VRI-1 X VRI-2, 2. VRI-2 X TMV-3, 3. TMV-3X TMV-4, 4. TMV-4 X TMV-7, 5. TMV-7 X VR1-1) were placed in 1.5 mL tubes separately and then plant genomic DNA was extracted according to manufacture instruction using XpressDNA plant kit, Magenome technologies, India. The hybrids and parent plants samples obtained from F4 research field populations.

Quantification of DNA

Quantification of the extracted DNA was checked in UV spectrophotometer (SHIMADZHU, JAPAN) by taking the optical density (OD) at 260 nm and 280 nm. The quality was checked by measuring the ratio of absorbance at 260 nm and 280 nm (260/280). The value between 1.7 - 1.8 indicates good quality DNA without protein/RNA contamination. DNA quantification was done according to the following calculation: sample showing 1 OD at 260 nm is equivalent to 50 µg of DNA/mL. The OD of each DNA sample at 260 nm was measured and quantified accordingly.

PCR Amplification

The PCR amplification was carried out for the fungal DNA sample in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR condition

The PCR condition of RAPD was initial denaturation at 94°C for 7 min, followed by 40 cycles of denaturation 94°C for 1 min, Annealing at 37°C or 1 min and the extension temperature was for 72°C for 3 min and the final extension was 72°C for 10 min.

Preparation of native PAGE Gel

The two glass plates were clamped by the cassette holder. The components of the 10% native PAGE running gel were poured in between the glass plates. The comb was inserted in the gel layer without any bubbles. It was allowed to polymerize. After polymerization the comb was removed.

Genetic similarity of Sesam*e* variants

Data analysis procedure

Only distinct RAPD bands were recorded. The genetic variations between any two individuals were measured in terms of and Genetic Similarity (S).

$$S = 2\,N_{xy}/(N_x + N_y)$$

N*xy*: number of bands shared by both individuals, x and y N*x*: number of bands displayed by only individual x. N*y*: number of bands displayed by only individual y.

Experimental Results

The Sesame (*Sesamum indicum* L.) variants such as VRI-1, VRI-2, TMV-3, TMV-4, TMV-7, and its F4 hybrids of VRI-1 X VRI-2, VRI-2 X TMV-3, TMV-3X TMV-4, TMV-4 X

TMV-7 and TMV-7 X VR1-1 were analysed by using random amplified polymorphic DNA. The polymorphism banding pattern was observed on the resolving gel among the *Sesame* varieties and crossed hybrids. The RAPD results showed that the highest the genetic similarity of F4 hybrid was 77.77% between VRI-1 X VRI-2, when compared to VRI-1 and VRI-2 parents. The VRI-2 X TMV-3 showed that the genetic similarity of 68.18% with their VRI-2 and TMV-3 parents. Similarly, 48% of genetic similarity was observed with TMV-3XTMV-4, when compared with TMV-3 and TMV-4 individual varieties. The least genetic similarity was identified for TMV-4 X TMV-7 at 46% compared to TMV-4 and TMV-7 varieties and 52% of genetic similarity was identified in TMV-7 X VR1-1 cross variety when compared to TMV-7 and VR1-1 varieties. RAPD results showed that the genetic similarity was expressed only with F4 hybrids for identify the best cross combinations. But parental varieties genetic similarities values was reffered from existing research work. So, the present research work expressed that the genetic similarity of selected F4 hybrids of Sesame (Sesamum indicum L.) was presented in table-1.

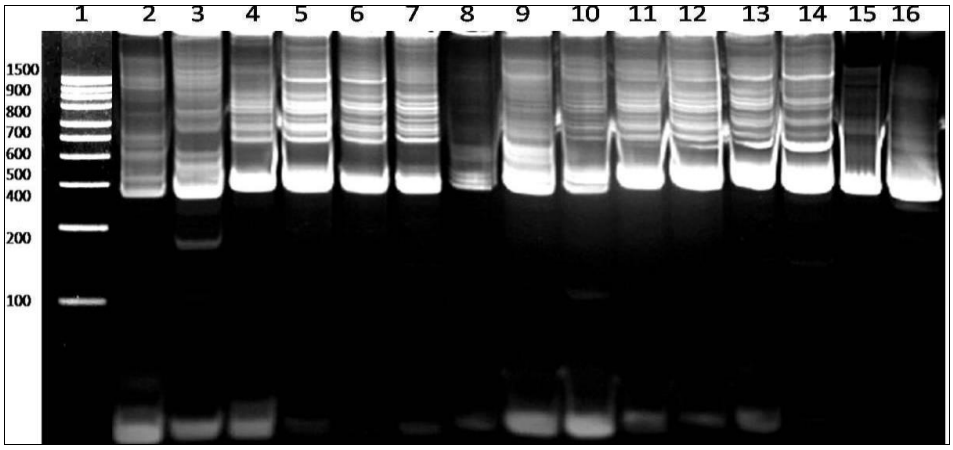


Fig 1: RAPD DNA banding pattern analysis results of Sesame samples of 10% native page

Parent varieties: Lane 1. DNA ladder, Lane 2. VRI-1 Lane 3. VRI-2 Lane 4. TMV-3, Lane 5. TMV-4, Lane 6. TMV-7,
F4 hybrid-Test-1: Lane 10. VRI-1 X VRI-2, Lane 11. VRI-2 X TMV-3, Lane 12. TMV-3X TMV-4, Lane 13. TMV-4 X TMV-7, Lane 14. TMV-7 X VR1-1.
F4-hybrid – test-2: Lane 7. VRI-1 X VRI-2, Lane 8. VRI-2 X TMV-3, Lane 9. TMV-3X TMV-4, Lane 15. TMV-4 X TMV-7 Lane 16. TMV-7 X VR1-1.

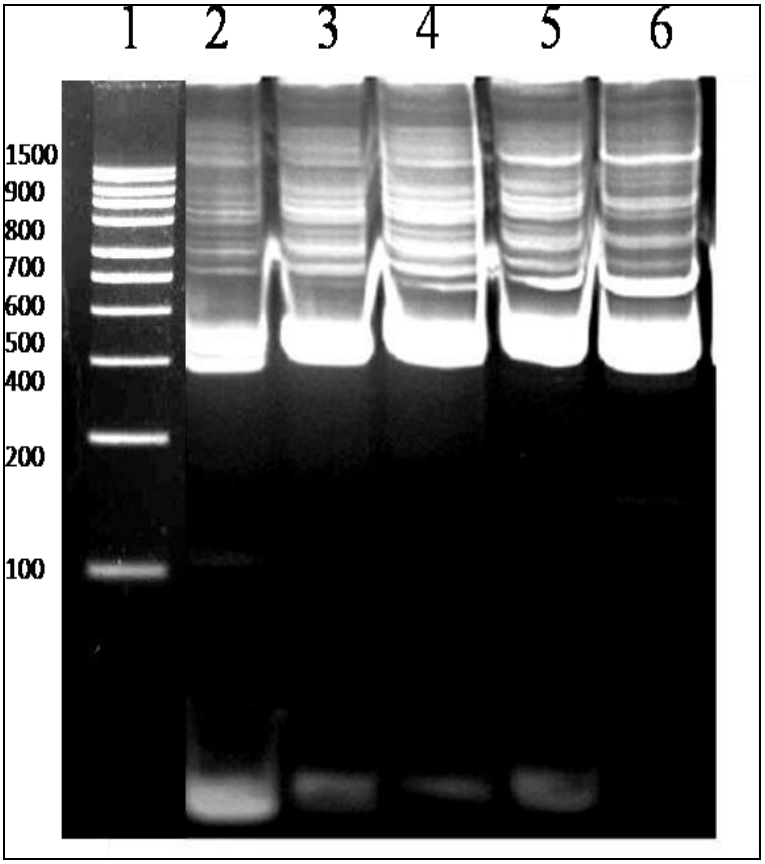
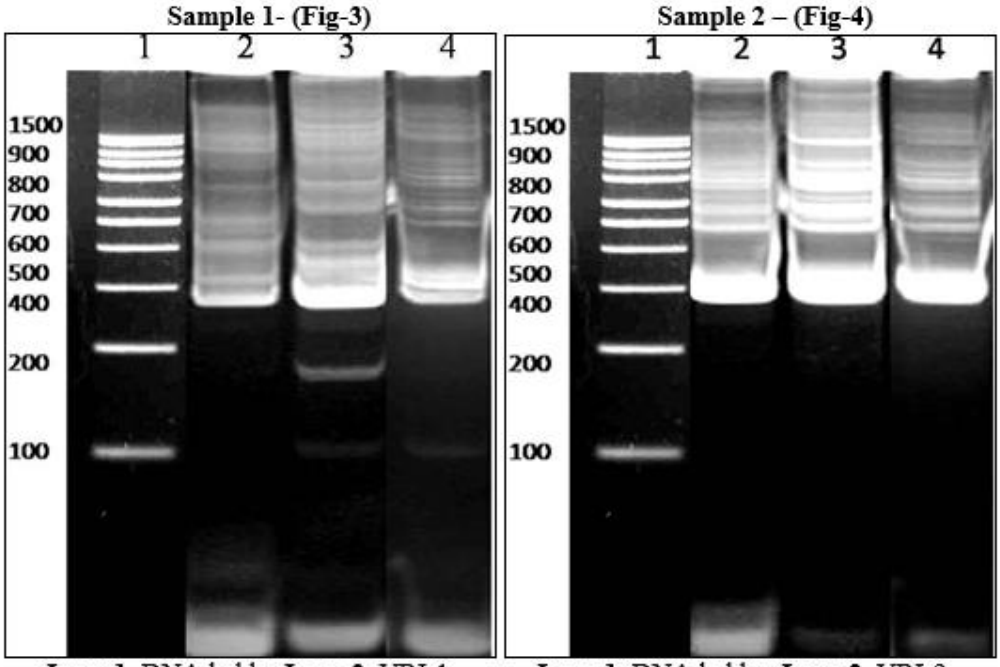
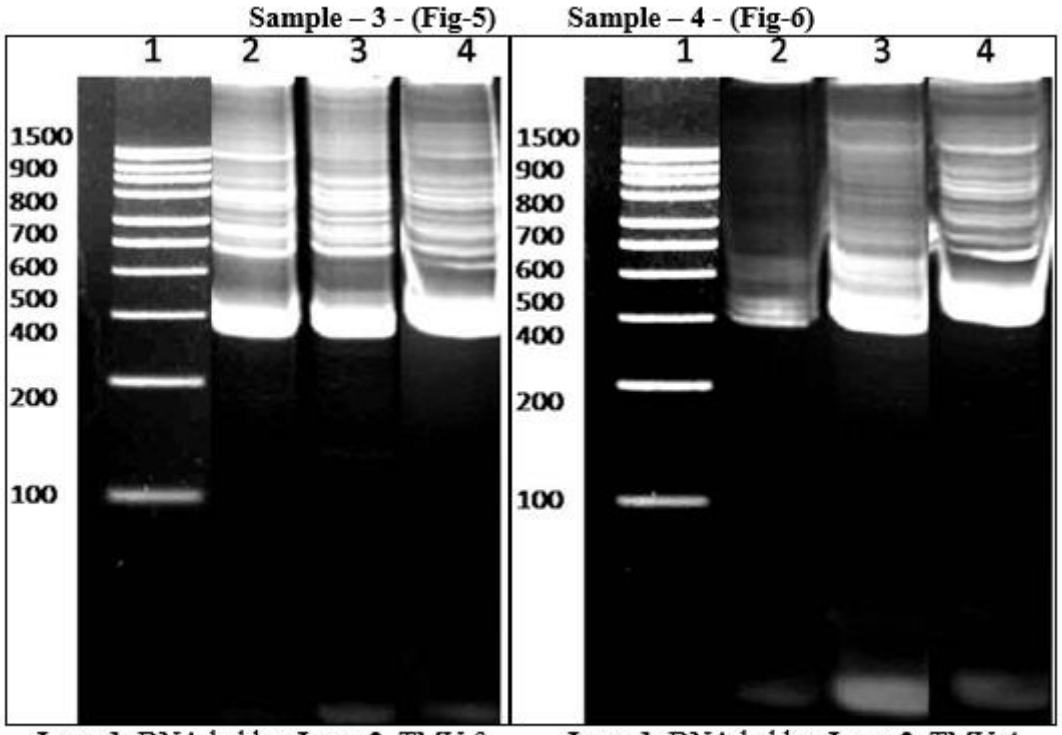


Fig 2: RAPD banding pattern analysis result of Sesame samples at 10% native page Lane 1. 100 bp ladder, Lane 2. VRI-1 X VRI-2, Lane 3. VRI-2 X TMV-3, Lane 4. TMV-3X TMV-4, Lane 5. TMV-4 X TMV-7, Lane 6. TMV-7 X VR1-1.



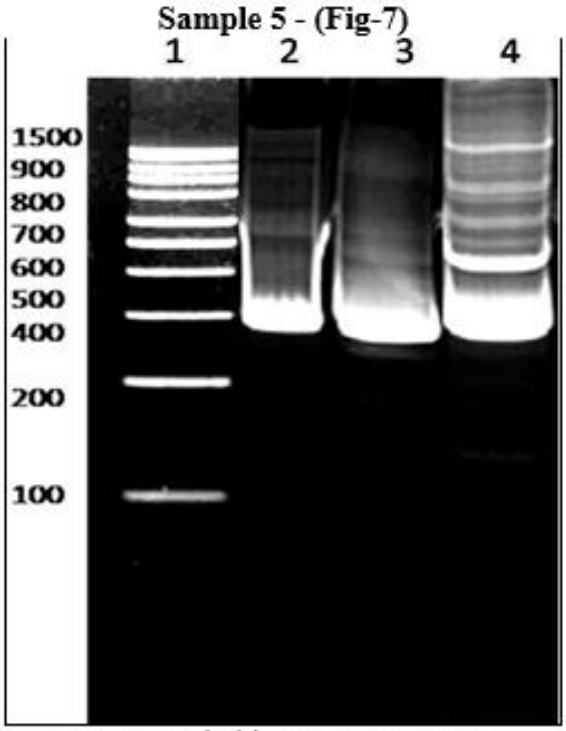
Lane 1. DNA ladder **Lane 2.** VRI-1 **Lane 3.** VRI-2 **Lane 4.** VR-I X VRI-2

Lane 1. DNA ladder, **Lane 2.** VRI-2, **Lane 3.** TMV-3, **Lane 4.** VRI-2 X TMV-3,



Lane 1. DNA ladder, **Lane 2.** TMV-3, **Lane 3.** TMV-4, **Lane 4.** TMV-3X TMV-4,

Lane 1. DNA ladder, **Lane 2.** TMV-4, **Lane 3.** TMV-7, **Lane 4.** TMV-4 X TMV-7,



Lane 1. DNA ladder, **Lane 2.** TMV-7, **Lane 3.** VR1-1, **Lane 4.** TMV-7 X VR1-1.

Fig 3-7

Table 3: Genetic similarity of Sesame (*Sesamum indicum* L.*)* variants

S. No	Sample Name	Genetic similarity (%)
1.	VRI-1 X VRI-2	77.77%
2.	VRI-2 X TMV-3	68.18%
3.	TMV-3X TMV-4	48%
4.	TMV-4 X TMV-7	46%
5.	TMV-7 X VR1-1	52%

Table 4: Analysis of RAPD banding pattern in Sesame (*Sesamum indicum* L.) for F4 generation hybrids.

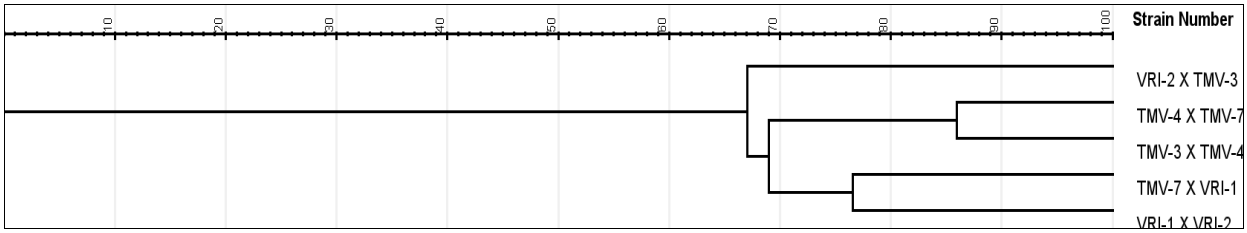
S. No	Sample Name	Total No. of Bands	No. of Polymorphic Bands	No. of Monomorphic Bands	Percentage of Polymorphism
1	VRI-1 X VRI-2	12	9	3	75 %
2	VRI-2 X TMV-3	11	7	4	63 %
3	TMV-3X TMV-4	12	5	7	41 %
4	TMV-4 X TMV-7	11	5	6	45 %
5	TMV-7 X VR1-1	14	8	6	57 %

Table 5: Distance matrix of Sesame (*Sesamum indicum* L.)

Strain Number	VRI-1 X VRI-2	VRI-2 X TMV-3	TMV-3 X TMV-4	TMV-4 X TMV-7	TMV-7 X VRI-1
VRI-1	0	0.405405	0.473684	0.578947	0.35
VRI-2	0.405405	0	0.513514	0.513514	0.538462
TMV-3	0.473684	0.513514	0	0.210526	0.35
TMV-4	0.578947	0.513514	0.210526	0	0.45
TMV-7	0.35	0.538462	0.35	0.45	0

The dentogram constructed by Unweighted Pair Group Method with Arithmetic mean (UPGMA) protocol using Gelj software.

Dendrogram A.



Dendrogram B.

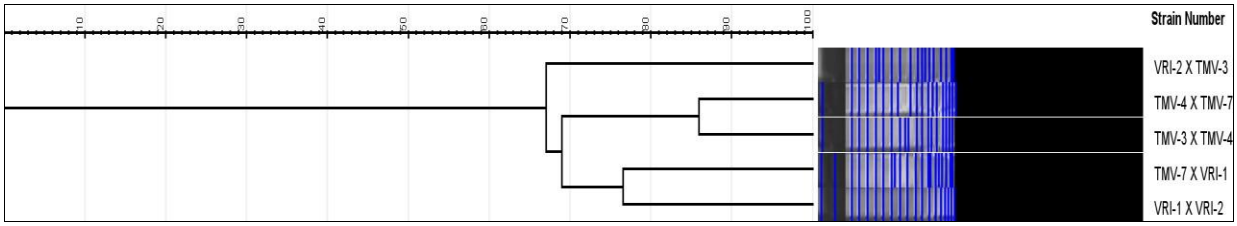


Fig 8(A&B): Dendrogram analysis of five F4 generation hybrids in Sesame (*Sesamum indicum* L.)

There are different number of DNA bands were (from 11 to 14) recorded in the hybrids of VRI-1 X VRI-2, VRI-2 X TMV-3, TMV-3X TMV-4, TMV-4 X TMV-7 and TMV-7 X VR1-
1 in the F4 Population (Fig-1, 2, 3, 4, 5, 6, and 7). The 11 number of DNA bands was observed in both the crosses of VRI-2XTMV-3 and TMV-4XTMV-7.The DNA band 12 was observed in both the crosses of VRI-1XVRI-2 and TMV-3XTMV-4. But in TMV-7XVRI-1 cross expressed that the number of DNA band was 14. The total number of polymorphic bands was 34 and monomorphic band was 26.The highest polymorphicDNA band was recordedat at VRI-1XVRI- 2(DNA bands -9) and highest monomorphic band was recorded at TMV-3XTMV-4(DNA band - 7). The highest pholymorphism percentage was recorded at VRI-1XVRI-2(75%) followed by VRI-2XTMV-3(63%). The lowermost polymorphism was recorded at TMV-3XTMV-4(41%) and results presented inTable-2. Further, the dendrogram constructed from pooled data clearly showed five major clusters namely, cluster 1,2,3,4 and 5. Cluster 1 was divided in to a VRI-2X
TMV-3 and cluster 2, 3, 4 and 5 was divided in to TMV-4XTMV-7, TMV-3X TMV-4, TMV-7X VRI-1 and VRI-1X VRI-2 (Table-3 and Fig-8[A&B]).

Discussion

RAPD based DNA analysis showed that the F4 best offspring of the 5 hybrids of Sesame with their parents. The hybrid F4 population like VRI-1XVRI-2, VRI-2XTMV-3, TMV-3X TMV-4 and TMV-4XTMV-7 and their related parents were analysed. The 60 number of DNA bands were totally studied for analysing genetic similarity with hybrids and their parents. Among these hybrids 34 DNA bands were polymorphic and 26 DNA bands were monomorphic. The highest polymorphic was identified at VRI-1X VRI-2 and the highest monomorphic was

recorded at TMV-3XTMV-4. The similar results were reported by Gulhan Ercan *et al*., (2004) [7] and Muez Berhe *et al* (2021) [11] in Sesame (*Sesamum indicum* L.). Genetic diversity is one of the best sources to produce a best hybrid in the oil crop breeding. In connection with that Basak and Uzun, 2019 [1] reported that the genetic diversity and population structure of the Mediterranean Sesame core collection with use of genome wide snp developed bhai double digest RAPD sequence. The dendrogram figure clearly showed that two groups based on their genetic similarities but it is divided into five individuals. Similar research report was published by Genesh and Thangavelu 1995 [5] and Tam *et al*., 2019 [12].

Conclusion

The present research work was concluded that the high level polymorphism was recorded in VRI-1X VRI-2 followed by VRI-2XTMV-3 of F4 hybrid populations through RAPD techniques and it may be a best hybrid to produce as new varieties. Further generation field studies need to get homozygosity and confirm the important yield and yield related genetic characters.

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